Investigating the Effect of Plant Amino Acid Transporters AtAAP1 and AtAAP2 on Aphid-Plant Interactions

By

Harley Kemp

A thesis submitted to
The University of Birmingham
For the degree of
DOCTOR OF PHILOSOPHY

School of Biosciences
The University of Birmingham
August 2011

Abstract

A model system of *Myzus persicae*, the peach-potato aphid (Homoptera:Aphididae), *Brevicoryne brassicae*, the mealy cabbage aphid (Homptera: Aphididae), and the plant *Arabidopsis thaliana* was used to investigate the effect of loss of function mutations in the plant amino acid transporter genes, At*AAP1* and At*AAP2.* Previously created, homozygous mutant lines for each transporter were screened for potential phenotypic changes. After day 50, rosette leaf width, rosette leaf number and mean silique numbers were reduced for both *aap1* and *aap2* plants in comparison to wildtype plants ($p < 0.05$). Individual seed weight was also significantly reduced in *aap2* plants ($p < 0.05$).

Aphid probing behaviour, measured in both aphid species using EPG, indicated both aphid species took significantly longer attempting to locate a sieve element and reach sustained E2 feeding when on *aap1* and *aap2* plants compared to control plants ($p < 0.05$). The rate of aphid feeding as determined using honeydew clock methods was also significantly slower for both aphid species feeding on *aap1* and *aap2* ($p < 0.000$).

Both *M. persicae* and *B. brassicae* feeding on *aap1* and *aap2* exhibited no change in aphid performance in terms of growth or reproductive output (r_m) when compared to aphids on control plants ($p > 0.05$).

Following the antibiotic elimination of aphid symbionts in both aphid species, aposymbiotic aphid performance in terms of growth rate was assessed again and both aphid species were found to grow significantly slower on *aap1* and *aap2* plants in comparison with the aposymbiotic aphids feeding on control plants ($p < 0.000$).

This investigation has suggested the ways in which amino acid transporters, AAP1 and AAP2, influence the complex relationship between aphid and plant. Evidence within this study suggests AAP1 and AAP2 both play a similarly significant role in the transport of amino acids within *A. thaliana*. These potential effects on amino acid transport were independently identified using the two aphid species, *M. persicae* and *B. brassicae*.

Acknowledgements

Firstly, I would like to thank my supervisors at the University of Birmingham, Jeremy Pritchard and Jeff Bale, who provided expert guidance and valuable support throughout the course of my project.

Secondly, I would like to thank the BBSRC for my funding without which this research would not have been possible and I would not have had this wonderful opportunity.

Acknowledgements and thanks to all members of the lab who have given me friendship, support and advice. Thanks also to Christine, Bob and Pauline for printer support, Lisa for proof-reading, Danielle and Claire for helping me through stressful times and my 'boys' for always making me smile.

Finally, I would like to give a special thanks to my parents, Deborah and Anthony, and my fiancé Kris. Without such a supportive and understanding team around me this achievement would not have been possible.

List of Figures

Chapter One

Chapter Two

sampled from *aap2* and control plants. Error Bars represent ± s.e. Data was analysed using a General Linear Model (GLM) ANOVA. The lower, middle and upper silique strata within the plant were identified and samples taken from these areas in order to allow for any developmental effects on silique seed weight to be accounted for. p 82

Figure 2.7 – Graph showing the mean dry weight of individual seeds from siliques sampled from the varying strata of plant development in *aap1* (n=33) and control plants (n=28). Sample size numbers refer to number of siliques sampled. Error bars represent ± s.e. Data was analysed using a GLM ANOVA. The lower, middle and upper silique strata within the plant were identified and samples taken from these areas in order to allow for any developmental effects on silique seed weight to be accounted for. p 83

Figure 2.8 – Graph showing the mean dry weight of individual seeds from siliques sampled from the varying strata of plant development in *aap2* (n=26) and control plants (n=30). Sample size numbers refer to the number of siliques sampled. Error bars represent ± s.e. Data was analysed using a GLM ANOVA. The lower, middle and upper silique strata within the plant were identified and samples taken from these areas in order to allow for any developmental effects on silique seed weight to be accounted for. p 84

Figure 2.9 – Graph showing the mean chlorophyll concentration of chlorophyll a, b and a+b in *aap1 mutants* (n=10) compared to wildtype plants (n=20). Error bars represent the ± s.e. Data was analysed using a two sample t-test. p 86

Figure 2.10 – Graph showing the mean chlorophyll concentration of chlorophyll a, b and a+b in *aap2* mutants (n=10) compared to wildtype plants (n=20). Error bars represent the ± s.e. Data was analysed using a two sample t-test. p 88

Table 2.11 – Table showing the mean plant parameters measured in *aap1* (n=39), *aap2* (n=40) and control plants (n=35) following standard and nitrogen supplemented watering regimes. Values in brackets refer to ± s.e. Data analysed using General Linear Model (GLM)

Chapter Three

over an 8 hour experimental period. Values shown are mean values (means are given in table 3.11(b) ± s.e.) *'s indicate statistically significant differences ($p < 0.05$) after analysis with a Mann-Whitney U test. Sample sizes are indicated as follows, wildtype (Col0) n=13, *aap1* n=14 and *aap2* n=15. p 142

Figure 3.12 – Mean Duration of time spent (s) by *M. persicae* (n=13) and *B. brassicae (n=13)* in EPG parameters during hour one (left graph) and hour two (right graph) of feeding on wildtype plants, Col0. Error bars represent ± s.e. * represents a statistically significant difference of mean time spent in 'pathway' between aphid species (analysis using a Mann-Whitney U-test, $p < 0.05$). p143

Figure 3.13 – Mean Duration of time spent (s) by *M. persicae* (n=14) and *B. brassicae* (n=14) in EPG parameters during hour one (left graph) and hour two (right graph) when feeding on *aap1* plants. Error bars represent ± s.e. Analysis using a Mann-Whitney U-test indicated no significant effect of aphid species ($p > 0.05$). p143

Figure 3.14 – Mean Duration of time spent (s) by *M. persicae* (n=15) and *B. brassicae (n=15)* in EPG parameters during hour one (left graph) and two (right graph) when feeding on *aap2* plants. Error bars represent ± s.e. Analysis using a Mann Whitney U-test indicated no significant effect of aphid species ($p > 0.05$). p144

Figure 3.15 – A graph representing the mean time *M. persicae* spent in E2 during the 8 hour experimental period when feeding on *aap1*(n=14) and control plants (n=13). Error bars represent the ± s.e. The Mann Whitney U test indicated a significant effect of genotype ($p <0.05$). p145

Figure 3.16 – A graph representing the mean time *M. persicae* spent in E2 during the 8 hour experimental period when feeding on *aap2*(n=15) and control plants (n=13). Error bars represent the ± s.e. The Mann Whitney U test indicated significant effect of genotype ($p < 0.05$). p146

Figure 3.17 – A graph representing the mean time *B. brassicae* spent in E2 during the 8 hour experimental period when feeding on *aap1*(n=14) and control plants (n=13). Error bars represent the ± s.e. The Mann Whitney U test indicated a significant effect of genotype ($p < 0.05$). p147

Figure 3.18 – A graph representing the mean time *B. brassicae* spent in E2 during the 8 hour experimental period when feeding on *aap2*(n=15) and control plants (n=13). Error bars represent the ± s.e. The Mann Whitney U test indicated a significant effect of genotype ($p < 0.05$). p148

Figure 3.19 - Rate of honeydew production by *M. persicae* feeding on *aap1 mutants* and wildtype plants over a 10 hr period. For wildtype plants each point is the mean of 12 determinations of droplet production rate ± s.e, for *aap1* plants each point is the mean of 16 determinations of droplet production rate ±s.e. A General Linear Model (GLM) ANOVA indicated a significant effect of genotype ($p < 0.000$). p149

Figure 3.20 - Rate of honeydew production by *B. brassicae* feeding on *aap1 mutants* and wildtype plants over a 10 hr period. For wildtype plants each point is the mean of 11 determinations of droplet production rate ± s.e , for *aap1* plants each point is the mean of 12 determinations of droplet production rate ±s.e. A General Linear Model (GLM) ANOVA indicated a significant effect of genotype ($p < 0.000$). p150

Figure 3.21 - Rate of honeydew production by *M. persicae* feeding on *aap2 mutants* and wildtype plants over a 10 hr period. For wildtype plants each point is the mean of 14 determinations of droplet production rate ± s.e, for *aap2* plants each point is the mean of 12 determinations of droplet production rate ± s.e. A General Linear Model (GLM) ANOVA indicated a significant effect of genotype ($p < 0.000$). p151

Figure 3.22 - Rate of honeydew production by *B brassicae* feeding on *aap2 mutants* and wildtype plants over a 10 hr period. For wildtype plants each point is the mean of 12

determinations of droplet production rate ± s.e, for *aap1* plants each point is the mean of 12 determinations of droplet production rate ± s.e. A General Linear Model (GLM) ANOVA indicated a significant effect of genotypes ($p < 0.000$). p152

Table 3.23 - A table of mean honeydew droplet volumes produced by *M. persicae* feeding on *aap1, aap2* and wildtype plants. Values in brackets refer to ± s.e. For each value n=30, with 10 droplets being measured from 3 aphids on three separate plants of each genotype. Data analysed using a one-way ANOVA indicated there was not a significant effect of genotype on the honeydew droplet volumes ($p = 0.156$). p153

Table 3.24 - A table of mean honeydew droplet volumes produced by *B. brassicae* feeding on *aap1, aap2* and wildtype plants. Values in brackets refer to ± s.e. For each value n=30, with 10 droplets being measured from 3 aphids on three separate plants of each genotype. Data analysed using a one-way ANOVA indicated there was a significant effect of genotype on the honeydew droplet volumes ($p = 0.003$). An asterisk indicates a statistically significant difference. P154

Figure 3.25 – Mean feeding rate of *M. persicae* on *aap1, aap2* and Col plant genotypes. Mean honeydew volume produced per hour by aphids feeding on *aap1* (n=12), *aap2* (n=12) and wildtype plants (n=30). Error bars refer to ± s.e. Data analysed using a one-way ANOVA indicated the a statistically significant effect of genotype ($p < 0.000$). p155

Figure 3.26 – Mean feeding rate of *B. brassicae* on *aap1, aap2* and Col plant genotypes. Mean honeydew volume produced per hour by aphids feeding on *aap1* (n=12), *aap2* (n=12) and wildtype plants (n=23). Error bars refer to ± s.e. Data analysed using a one-way ANOVA indicated a statistically significant effect of genotype ($p < 0.000$). p156

Chapter Four

Table 4.6 – A table showing the mean weight of *B. brassicae* on *aap1, aap2* and Co0 at three time points, 24, 72 and 120 hours. Data was analysed using a GLM ANOVA. Statistically significant differences are indicated with an asterisk (*). Values in brackets are ± s.e. p188

Figure 4.7 – Body Dimensions of *M. persicae* after 24 hours of feeding on *aap1* (n=16), *aap2* (n=16) and wildtype control plans, Col0 (n=21). Error bars represent ± s.e. Data analysed using a General Linear Model (GLM) ANOVA indicated no statistically significant differences ($p > 0.05$). (Refer to figure 4.1 for measurement descriptions). p190

Figure 4.8 – Body Dimensions of *M. persicae* after 72 hours of feeding on *aap1* (n=20), *aap2* (n=20) and wildtype control plants, Col0 (n=17). Error bars represent ± s.e. Data analysed using a General Linear Model (GLM) ANOVA indicated no statistically significant differences ($p > 0.05$). (Refer to figure 4.1 for measurement descriptions). p191

Figure 4.9 – Body Dimensions of *M. persicae* after 120 hours of feeding on *aap1* (n=20), *aap2* (n=20) and wildtype control plants, Col0 (n=17). Error bars represent ± s.e. Data analysed using a General Linear Model (GLM) ANOVA indicated no statistically significant differences ($p > 0.05$). (Refer to figure 4.1 for measurement descriptions). p191

Figure 4.10 – Body Dimensions of *B. brassicae* after 24 hours of feeding on *aap1* (n=19), *aap2* (n=20) and wildtype control plants, Col0 (n=20). Error bars represent ± s.e. Data analysed using a General Linear Model (GLM) ANOVA indicated no statistically significant differences ($p > 0.05$). (Refer to figure 4.1 for measurement descriptions). p193

Figure 4.11 – Body Dimensions of *B. brassicae* after 72 hours of feeding on *aap1* (n=20), *aap2* (n=20) and wildtype control plants, Col0 (n=21). Error bars represent ± s.e. Data

Chapter Five

Contents

Chapter 1

General Introduction

As a group, aphids are the single most important agricultural pest of temperate regions (Jones, 1974). These phloem feeding insects are of great economic importance and it is the complex aphid-plant relationship that is central to most ecological and agricultural situations. Aphid biology, ecology and plant phloem physiology are well studied, however this project aims to address the complex interactions between plant and aphid, focusing on the relationship between feeding aphid and its plant diet, in particular amino acid transport within the host plant.

1.1 Aphid Biology

1.1.1 Aphids as Pests

From the superfamily *Aphidoidea,* aphids are considered to be one of the most destructive and arguably the single most important agricultural pest of temperate regions (Jones and Jones, 1974). Measuring 1-10 mm in size these sap feeding insects (Auclair, 1963), Homoptera: Aphididae (Dixon, 1998), cause major economic losses in numerous crop types (Jones and Jones, 1974) either as direct pests, causal agents of sooty moulds or as vectors of numerous plant viruses (Schoonhoven et al., 1998). In the UK, there are

around 30 aphid species that exist as pests of a wide range of crops, causing substantial economic losses every year. Aphid infestations reduce the overall vitality of crops however this rarely occurs on a large enough scale to cause direct damage. The significant damage is caused by the transmission of viruses (Bradley, 1952; Nault and Gyrisco, 1966; Harrington and Gibson, 1986; Powell, 1991; Martin et al., 1997; Ponder et al., 2000; Powell and Hardie, 2000). One of the most widespread and important virus vectors is the peach-potato aphid, *Myzus persicae* (Sulzer) (Van Emden and Bashford, 1971). This generalist aphid efficiently transmits over 100 plant viruses (Van Emden 1971), namely among crucifers, including turnip mosaic (TMV), cucumber mosaic (CMV) and cauliflower mosaic viruses (CaMV). Its impact is particularly high in the seed potato and beet industry, spreading viruses such as Potato Leaf Roll Virus (PLRV), Potato Virus Y (PVY) and Beet Western Yellows Virus (BWYV) which decrease yields by up to 50-80% (Schoonhoven et al., 1998; Ponder et al., 2000; Powell and Hardie, 2000). Along with the aphid's ability to transmit viruses, this pest group is also able to increase its population numbers at a phenomenal rate. The aphids ability to reproduce parthenogenetically combined with the 'telescoping of generations' (a form of embryo development which means that embryos can be developing inside their mother while its mother is still embryonic herself) allows the aphid population to turn over rapidly, building in numbers at an alarming rate to the detriment of their hosts (Kennedy and Stroyan, 1959; Dadd and Mittler, 1966; Dixon, 1985; Dixon, 1992; Dixon, 1998). Due to global warming, climate change may exacerbate the effect aphids, like *M. persicae*, have on our crops. Changes to the UK climate over the previous

10-15 years have allowed the population of aphids to increase substantially. Increasingly mild winters have allowed aphid populations to arrive earlier in the year consequently increasing the damage caused on crops at their most vulnerable stages. Clearly the importance of these polyphagous insects as significant pests is increasing.

Existing control methods involve frequent applications of potentially hazardous pesticides throughout the cultivation process (Minks and Harrewijn 1987). The extensive use of these chemicals has led to widespread development of resistance in numerous aphid species, consequently this poses a potential problem for the future success of chemical control. The development of non-chemical control methods may lead to a significant reduction in the use of and dependence on insecticides and therefore must be considered to be of great importance. The development of the understanding of interactions between aphids and their host plants may help to inform new control strategies.

1.1.2 Aphid Life Cycles

As previously mentioned aphids are phloem feeding insects of the order Hemiptera that are considered to be one of the most devastating crop and ornamental plant pest of temperate regions. One of the reasons behind the aphids overwhelming success as a plant pest is their reproductive capacity.

Most aphid species are able to reproduce parthenogenetically, giving birth to live young, although some species also have a sexual phase of

reproduction (Stroyan, 1984). In parthenogenetic aphid reproduction the oocytes of a viviparous female aphid begin to develop immediately following ovulation. As mentioned previously, this form of embryo development means that embryos can be developing inside their mother while its mother is still embryonic herself, this is known as a 'telescoping of generations'. This reproductive ability, combined with a short nymphal development period allows the aphid population to rapidly increase (Kennedy and Stroyan, 1959; Dadd and Mittler, 1966; Dixon, 1985; Dixon, 1992; Dixon, 1998).

Aphid life cycles differ among different aphid species however the general principle is that throughout a year an aphid species will have several generations identified by a specific sequence of several morphs (Chinery, 1993). Some aphids possess complex life cycles with an alternation between sexual and asexual generations alongside alternation between host plants. This 'cyclic parthenogenesis', the alternation between a number of generations of asexual (parthenogenetic) reproduction with a solitary generation of sexual reproduction, can be found in 'holocyclic' aphid species (refer to figure 1.1). Species of aphid that produce only asexual morphs and lack any sexual generation are known as 'anholocyclic' aphid species. In holocyclic aphid life cycles the asexual and sexual morphs are produced at different times of the year, with the male morphs being produced at only one distinct time of the life cycle, usually in the autumn. Aphids are able to produce males at a certain time of the year only using their XO sex determining mechanism. Males are produced by elimination of

one X chromosome during meiosis II (Blackman and Hales, 1986). Consequently embryos with two sex chromosomes (XX) will develop into females, whilst embryos with only one sex chromosome (XO) will develop into males.

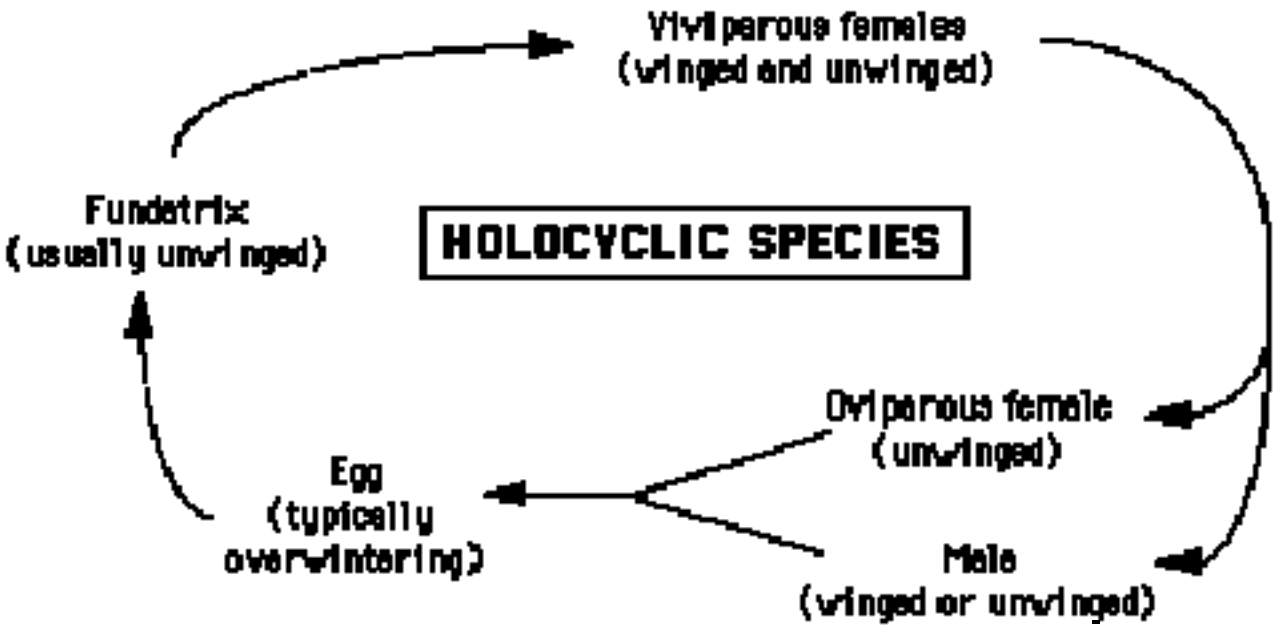

Figure 1.1 illustrates a typical holocyclic aphid life cycle with the characteristic morphs types.

Anholocyclic aphid species produce only asexual female morphs (refer to figure 1.2). Unlike the holocylic aphid species they do not produce any males, sexual females or the direct relations to these morphs, the eggs and the fundatrix (the morph which is specialised to feed on the primary host plants within the life cycle). Consequently the anholocylic life cycle is

somewhat simplified due to the loss of the fundatrix and therefore the primary host plants from the cycle. This simplification of this life-cycle is believed to offer aphid species an evolutionary advantage (Moran, 1988). By losing the ancestral fundatrix morph, certain aphid species over time have been able to remain all year round on their secondary herbaceous host. This ancestral life-cycle reduction consequently reduced the evolutionary constraints on the aphid species with regards to their need for over-specialisation in order to acquire a suitable host. This pattern of life cycle simplification is evident in some of the largest, most species-diverse groups of Aphididae.

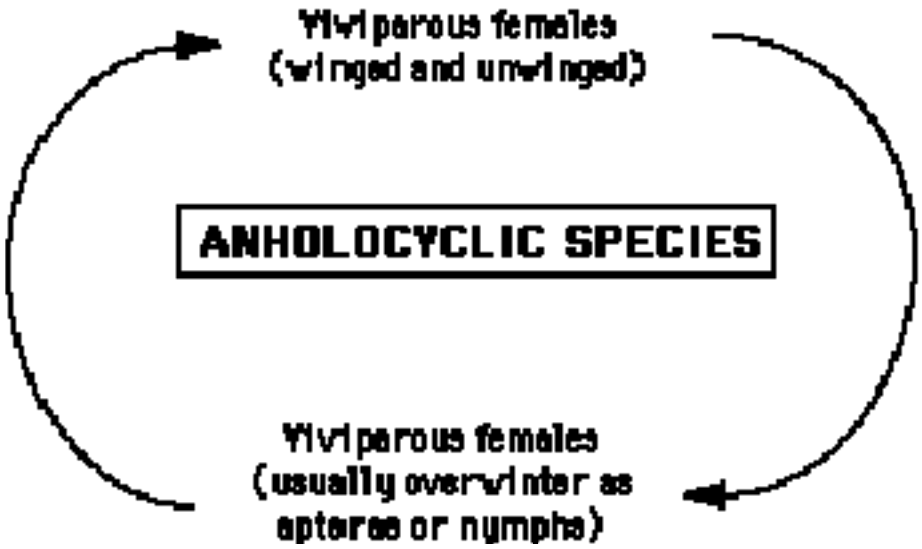

Figure 1.2 – This diagram illustrates a typical anholocyclic aphid life cycle with the characteristic morph types.

Although some aphid species can be described as holocyclic or anholocylic, other aphid species combine both holocyclic and anholocyclic clones within the same population. A large proportion of aphid species alternate between taxonomically different host plant species throughout the year. Aphid species that remain on host plants of the same species are known as 'monoecious' aphids. Whereas aphid species that alternate between different host plants are referred to as being 'heteroecious' aphids. Within those aphids which exhibit heteroecious host alternation, two distinct life cycle types exist. Aphid species of the Hormaphididae, Anoeciidae and Pemphigidae exhibit a type of heteroecious host alternation with gynoparae (also referred to as sexuparae) present (refer to figure 1.3). The second type of heteroecious aphid species do not present a life cycle with gynoparae, instead they possess alate sexual females and alate males.

Figure 1.3 represents a typical example of a heteroecious aphid species life cycle from the previously mentioned Hormaphididae, Anoeciidae, or Pemphigidae.

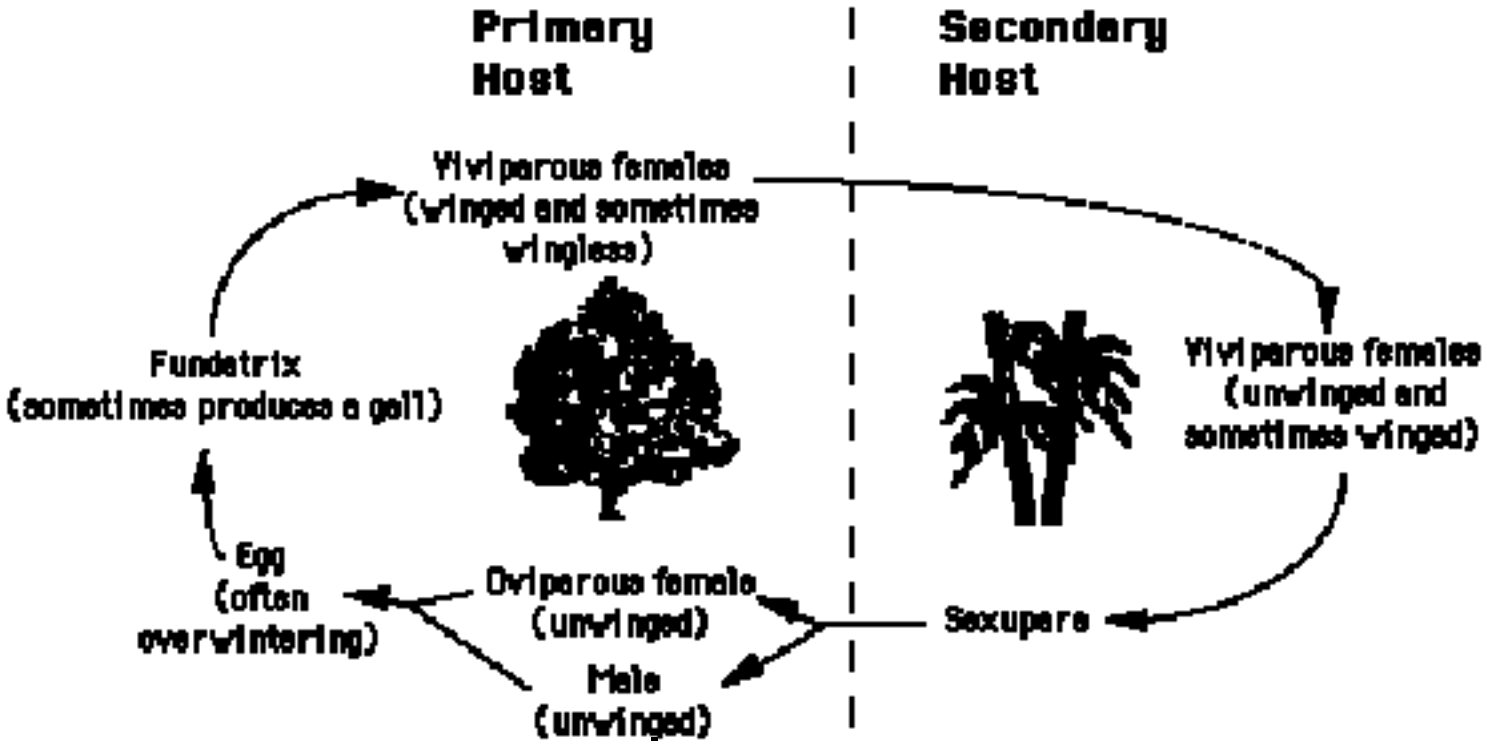

Figure 1.3 Illustrates an example of the type of host alternation occurring within a heteroecious aphids life cycle. This form of heteroecious life cycle is typical species of the Hormaphididae, Anoeciidae, or Pemphigidae, presenting a life cycle complete with gynoparae.

In both heteroecious and monoecious aphid species the primary host or 'ancestral host' is usually a tree or shrub, with the secondary host being a herbaceous plant, such as a crop plant. It is from this secondary host that the gynoparae flies to the primary host onto which it produces sexual male and female morphs. These morphs sexually reproduce, leaving the female to

deposit a single egg. This cold tolerant egg overwinters on the primary host and in the spring produces the fundatrix.

Aphid species of the Aphididae do not produce gynoparae, instead producing alate males and alate females which fly from the secondary host in order to locate and colonise the primary host. These alate females produce apterous oviparous female morphs that mate with the alate males to produce overwintering eggs (refer to figure 1.4).

Figure 1.4 represents another example of a heteroecious aphid life typical of aphid species of the Aphididae.

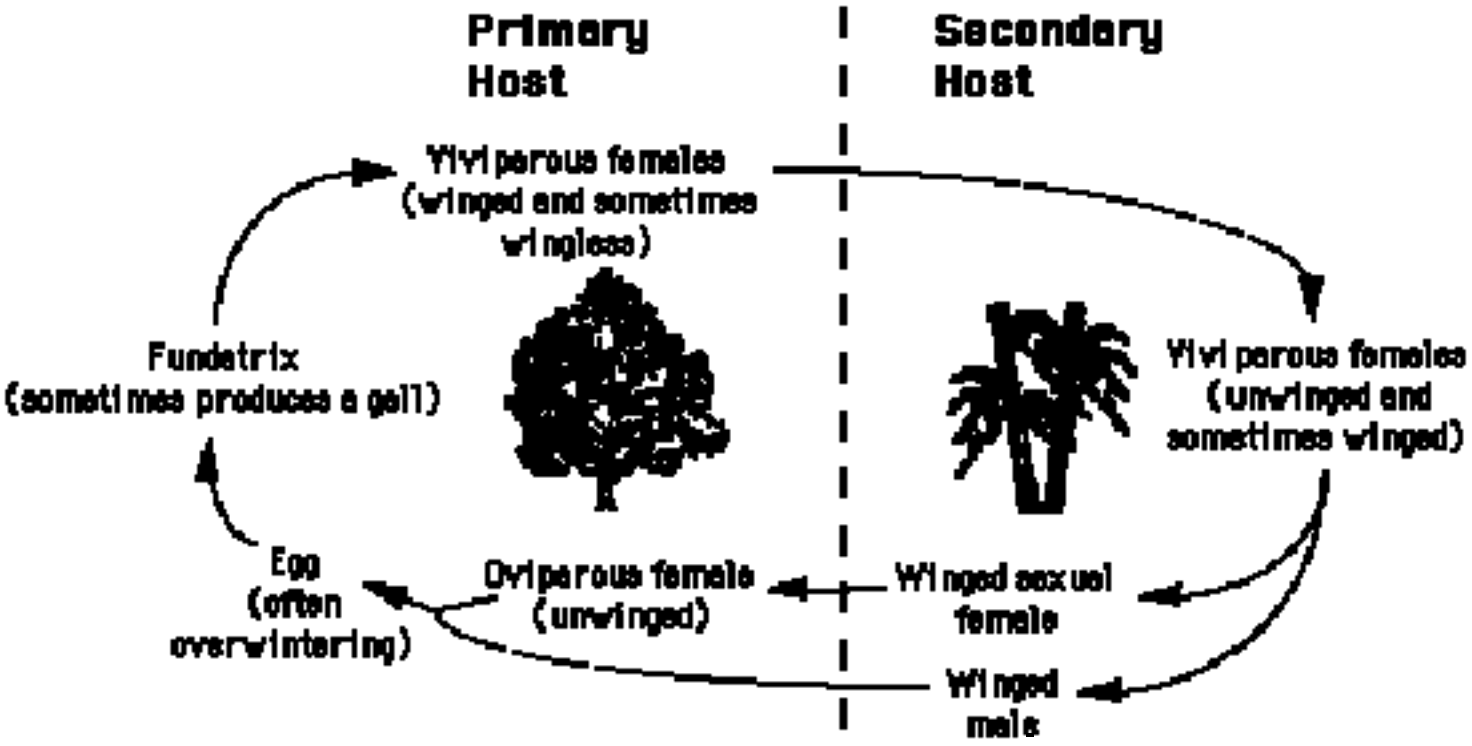

Figure 1.4 Illustrates a second example of the type of host alternation occurring within a heteroecious aphids life cycle. This form of heteroecious life cycle is typical of aphid species of the Aphididae, as they do not produce gynoparae, instead producing alate males and alate females which fly from the secondary host in order to locate and colonise the primary host.

1.1.3 Aphid Feeding

Aphids feed from sieve elements within the vascular bundle (Auclair, 1963). They tap into the vascular bundle of the host plant with their needle-like stylets (Bradley et al., 1962; Forbes, 1969; Forbes, 1977), gaining access to the sieve elements by piercing through the plant cuticle and passing through the mesophyll layer in search of a suitable sieve element. The stylets, which are contained within the proboscis when the aphid is not feeding, are very thin and can suffer damage while being pushed into the plant. Aphids therefore secrete a salivary fluid from the tips of their stylets which immediately starts to harden forming a hard, protective, lipoprotein sheath around their stylets and a flange which secures the stylet to the leaf surface (Cole, 1997; Miles, 1999). This protective sheath works by counteracting plant induced defence. Firstly the sheath works by polymerizing the plants apoplastic phenolics using its polyphenol oxidases, while also providing a track along which the stylet moves through the plant. These stylet sheath routes are multi-branched and highly complex pathways (Tjallingii, 2006) along which the aphids puncture and 'taste' the contents of virtually all mesophyll cells on route to the sieve element. This behaviour of continual tasting appears to orient the aphid's stylet towards the sieve element, providing gustatory cues and directionality to the stylets movement. The aphid is believed to effectively 'taste' the cell contents by imbibing and ingesting the vacuole or protoplast components (Mittler and Dadd, 1964; Wensler, 1974; Powell et al., 2006). Upon locating a suitable sieve element (Tjallingii, 1994) the feeding aphid uses its maxillary stylets to pierce

through the membrane and expel watery saliva into the sieve element sap stream (Wensler and Filshie, 1969; Schoonhoven, 1976; Dixon, 1998; Sauge, et al., 1998).

Figure 1.5 shows a diagrammatic representation of the aphid stylet bundle anatomy.

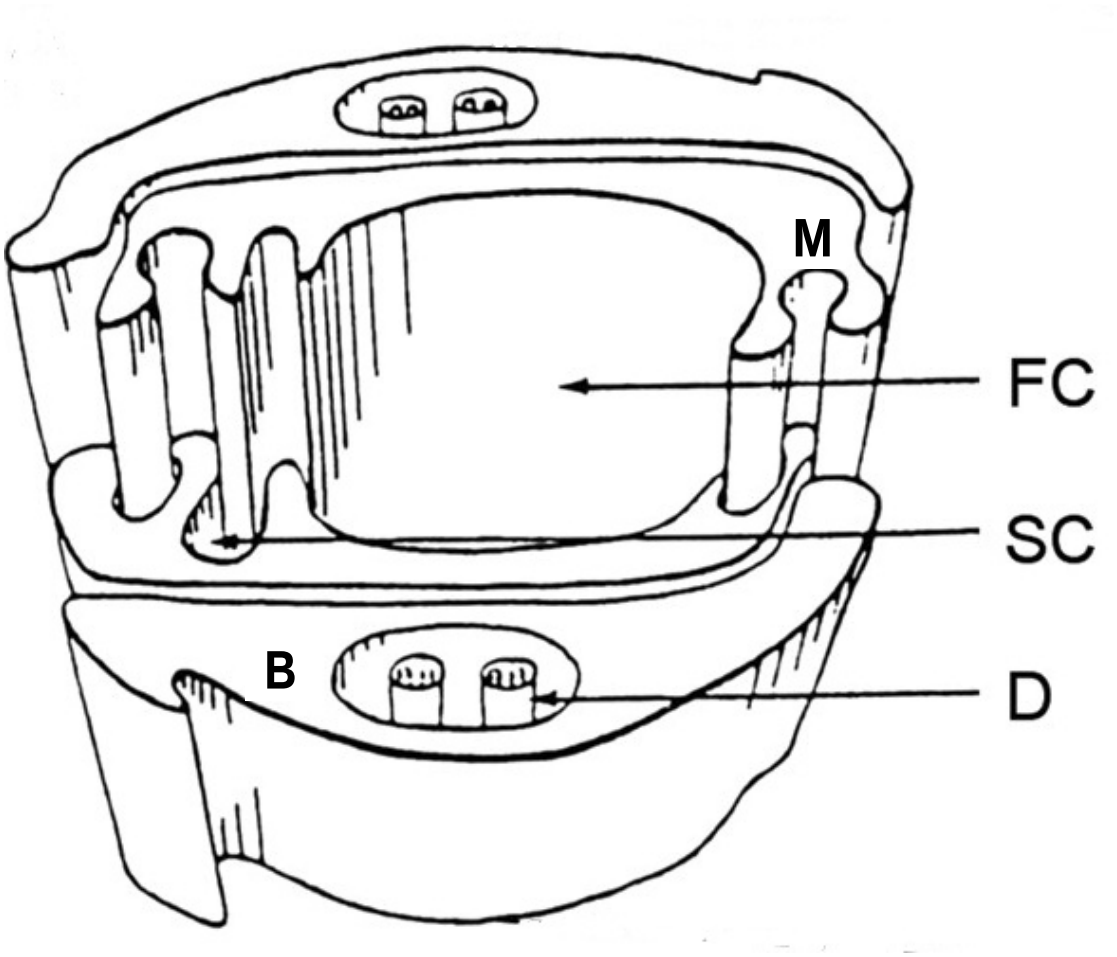

Figure 1.5 – This is a diagrammatic illustration of a transverse section through the stylet bundle of an aphid. FC: Food Canal, SC: Salivary channel, D: Mandibular canal with nerve axons, M: Maxillary stylet and B: Mandibular stylet. (Adapted from Uzest et al., 2007)

The watery saliva is believed to contain peroxidases that work to overcome plant defences, preventing feeding site closure thus allowing the aphid to proceed uninhibited in its feeding (Wensler and Filshie, 1969; Miles, 1999). Once the aphid has penetrated the sieve element the plant defences work quickly to seal the sieve element wound using callose and protein deposits (Will and van Bel, 2006). The feeding aphid works to modify these responses to their own benefit, preventing the influx of apoplastic Ca^{2+} by sealing the sieve element lesion with the gelling sheath saliva. Throughout sieve element feeding, the aphid manipulates the plant defences in order to make the host more suitable.

After settling to feed the aphid can continue to feed from the same sieve element for many hours and even days, a state of sustained feeding known in the electrical penetration analysis (EPG) as E2. The feeding process is often aided by the fact that the sieve element sap is under pressure, normally approximately 10-30 atmospheres of hydrostatic pressure (Dixon, 1998). This pressure simply allows the aphid to 'tap' into the sap stream, it effectively flows through the stylet and into the aphid alimentary tract. This pressurised flow is regulated by the cibarial pump. This pump allows the aphid to feed from a host plant under both positive (sieve element sap) and negative pressure (xylem sap). The dilator muscles of the pump relax and contract to allow the aphid to 'suck' the sap into their stylets and alimentary tract. It has been suggested that the aphid is able to detect harmful or deterring agents within the imbibed sap using their 'epipharyngeal organ' (Wensler, 1974) which lies within the dorsal wall of the cibarial pump, therefore when sap passes through this pump it also comes into contact with

the epipharyngeal organ. This organ is believed to play a gustatory role (Mittler, 1967; Harrewijn and Noordink, 1971; Wensler, 1974), detecting any harmful components of sap and in response is then able to shut off the pharyngeal valve to prevent any further ingestion of the sap.

A useful technique used to study the complex aphid feeding behaviour is the 'electrical penetration graph' (EPG) analysis (McLean and Kinsey, 1964; Tjallingii, 1978). An aphid is wired into a circuit with the insect connected to the input of an amplifier. The test plant that the aphid is allowed to feed on is then entered into the circuit by applying a small DC current into the soil via an electrode. The aphid's stylets act as microelectrodes and upon the aphid piercing the plant cuticle the circuit is closed, allowing current to flow through the fluid filled maxillary food and salivary canals and thus generating an electrical signal at the amplifier input (Tjallingii, 1987). Every time the aphid penetrates a cell membrane during the course of its feeding the recording software records the negative transmembrane potential as an abrupt drop in the recording signal (Tjallingii, 1985).

A series of characteristic waveforms are produced depending on the cell compartments being penetrated. These waveforms can be confirmed by sectioning the plant and aphid stylet in order to visualize the location of the stylet during the characteristic waveform being observed. It is these characteristic waveforms which form the electrical penetration graph, otherwise known as the 'EPG' (Tjallingii, 1985; Tjallingii, 1987).

1.2 Aphid Nutrition

1.2.1 Sieve Elements

Sieve element sap, the staple diet of aphids, is predominantly composed of nitrogen and carbohydrate components. Host plant selection and utilisation by feeding aphids are believed to be controlled by the relative differences in solute levels (Mittler and Dadd, 1964; Auclair, 1965; Van Emden, 1966; Mittler, 1967; Auclair, 1969; Weibull, 1987; Dixon, 1998), although it is not yet totally clear which solutes are specifically involved within this process.

1.2.2 Nitrogen

Nitrogen plays a central role in cellular structure, metabolic processes and genetic coding and for this reason nitrogen is vitally important to the growth of all living organisms. Although nitrogen makes up a large part of the earths atmosphere plant growth is often limited by this vitally important element. This scarcity is demonstrated in studies where additional nitrogen supplementation has elicited increased plant growth and fitness, suggesting that at normal levels within the environment nitrogen is usually a limiting factor. This limitation is predominantly due to the limited availability of atmospheric nitrogen suitable for biological use, thus leading to a relative scarcity in the nitrogen available for use in plant growth. In order to become useable nitrogen must be 'fixed' from the environment. This process sometimes occurs when lightning strikes the earth, however most fixation occurs when bacteria (symbiotic or free-living) use their

nitrogenease enzymes to combine gaseous nitrogen with hydrogen in order to produce ammonia. This ammonia is then further converted into organic compounds. A typical example of this occurs in the root nodules of leguminous plants, with the symbiotic relationship with the nitrogen fixing bacteria *Rhizobium*. These bacteria form a mutualistic relationship with the leguninous plants by producing useable nitrogen in the form of ammonia in exchange for carbohydrates produced by the plants. The chemical manufacture of ammonia also produces fixed nitrogen, contributing about 30% of the total fixed nitrogen present within the enviroment. Following the fixation of nitrogen, plants are then able to absorb the nitrate or ammonium ions from the rhizosphere and assimilate them for growth and development, and it is the abundance of this fixed form of nitrogen, or lack thereof, which determines the level of plant growth which can occur.

Following uptake and assimilation, the available nitrogen is distributed around the plant according to plant region requirements. Throughout different plant tissues nitrogen levels vary, with the highest levels of nitrogen being observed in the actively growing regions of the plant. During periods of rapid growth these areas are supported by the elevated nitrogen levels. After the period of rapid development, the nitrogen levels begin to fall until senescence commences and the nitrogen levels increase for a while before finally decreasing around the time of abcision. Large seasonal and between plant nitrogen variation is observed in the xylem and phloem, with variation up to 1-4 orders of magnitude. Generally sap from the phloem contains approximately 10-100 times more nitrogen than xylem sap and it is

this level of available nitrogen within the phloem sap which acts as a major determinant of 'host plant quality' for phloem sap feeding insects.

As discussed above, nitrogen is a limiting factor for plant growth, with levels varying seasonally, diurnally and from plant to plant. Nitrogen is also considered to be a major determinant of host plant quality for phytophagous insects (Mattson, 1980, Scriber and Slansky, 1981 and Awmack and Leather, 2002). As with plants, nitrogen is usually a limiting factor in the diet of herbivorous insects (see section 1.7). Unlike animals, plants utilise carbohydrates as their structural building blocks. Because of this predominance of carbon to nitrogen in plants, phytophagous insects are generally limited by nitrogen and not carbon. For many phytophagous insects nitrogen sequestering processes are generally only 50% efficient and as the average herbivore diet is inadequate or unbalanced most herbivores must eat excessive quantities in order to obtain their necessary nitrogen intake. However this compensation for inadequate diet cannot be maintained indefinitely and poses problems for the feeding insect. The level at which an insect can obtain nitrogen from its host plant therefore determines the performance of the feeding insect along with the whole population dynamics (McNeill and Southwood, 1978, Mattson, 1980 and Strong et al., 1984). In terms of insect performance, high levels of plant nitrogen have been linked with increased growth rates, improved survival (Myers and Post, 1981, Scriber and Slansky, 1981, Tabashnik, 1982, Minkenberg and Ottenheim, 1990, Wheeler, 2001, Wheeler, 2003) and

increased fecundity (Myers and Post, 1981, Minkenberg and Ottenheim, 1990, Heard and Winterton, 2000, Hinz and Müller-Schärer, 2000 and Awmack and Leather, 2002). At a population level, high levels of plant nitrogen have been found to increase population growth with high levels of intrinsic rate of increase (Room and Thomas, 1985, Minkenberg and Ottenheim, 1990 and Heard and Winterton, 2000). Over time, through evolution, insects have been known to develop strategies and preferences for host plants of high nutritional quality. An example of this type of strategy can be observed in insects that have developed ovipositional preferences to facilitate improved larval performance. Price (1991) showed that over time that many insects have evolved to lay eggs and nymphs in an area of high host plant quality.

For aphids, this relationship with host plant nitrogen quality is crucially important. Nitrogen is a central factor in all aspects of aphid biology, influencing host alternation, reproduction (Barker and Tauber, 1954; Banks and MacAuley, 1964), the production of alate and apterous aphid morphs (Mueller et al., 2001), the determination of sexual and parthenogenetic morphs (Kennedy and Stroyan, 1959; Klingauf, 1987; Mueller et al., 2001) and aphid settlement and development (Harrewijn, 1970; Van Emden and Bashford, 1971). A clear example of the influence of nitrogen in determining insect performance is that of aphid response to changes in phloem sap quality. *Drepanosiphum platanoidis,* the sycamore aphid, only feeds on sycamore trees, *Acer pseudoplatanus*. During periods of growth,

the sycamore phloem sap contains an increased level of amino acids and in response to this, the feeding sycamore aphids increase in size and display high levels of fecundity. Later in the season when growth has slowed considerably, the level of amino acids in the phloem sap has fallen, and in response the feeding aphids are found to be comparatively smaller in size with lower reproductive outputs. During senescence sieve element sap composition changes again from predominantly carbohydrate to predominantly amino acids (Taiz and Zeiger, 1991) and as the available amino acid levels increase within the phloem again, the feeding aphid size increases and aphid fecundity levels increase.

This close relationship between dietary nitrogen and aphid biology is demonstrated by their preference for young, actively growing vegetation when they migrate from their winter hosts to their summer hosts. The aphids colonise the young, summer hosts (usually crop plants) utilising the plentiful nitrogenous resources until their winter hosts (in some cases woody plants) begin to senesce and therefore once again become an attractive host due to the increased localised nitrogen levels. However the relationship between the nitrogen composition of the host and the relative performance of aphids feeding upon it is far from simple. Most of the work attempting to understand this relationship is carried out on whole tissue, not specifically phloem, this combined with inconsistency within the literature brings attention to the fact that the above relationship is a complex one (Ponder et al., 2000).

1.2.3 Sucrose

The presence of sucrose in the sieve element sap is a crucial factor in determining whether an aphid chooses to feed on a host or reject it. This is clearly demonstrated by the acceptance or rejection of artificial diets that have sucrose and those that do not (Mittler and Dadd, 1963; Mittler, 1967; Mittler et al., 1970; Srivastava, 1987). The substantial changes in sucrose levels that occur as a result of fluctuating levels of photosynthesis throughout the year may also be responsible for the variations in aphid behaviour, growth, reproductive capacity as well as their tendency to migrate from a particular host plant to another more suitable host. Areas of the host plant with the highest levels of carbohydrates such as growing regions are often preferentially targeted by feeding aphids (Auclair, 1969).

1.2.4 Aphid Metabolism and the Problems Faced by a Feeding aphid

Sieve element sap is primarily a concentrated solution of simple sugars with a low level of nitrogen present (Taiz and Zeiger, 1991). It is regarded as a difficult dietary source due to the low proportion of free essential amino acids present within sap (10-30%) (Ponder et al., 2000). This nutritionally inadequate diet coupled with the aphids own low bodily level of proteins (50% of essential amino acids) makes obtaining an optimal nutrient level inherently difficult.

A secondary problem with the diet of an aphid involves osmotic related difficulties. The sugary sieve element diet of the aphid is potentially fatal for a feeding aphid because of the inherent risk of dehydration. This is due to the osmotic pressure of the dietary sap being much higher than the aphids own bodily fluid. Consequently, after feeding on this sugar rich diet water movement into the gut is much more likely to occur leaving the aphid potentially dehydrated. The aphid therefore faces two major problems when feeding from a plant. Firstly the low level of free amino acids means the aphid must ingest a large quantity of poor nitrogen quality sap in order to meet its dietary 'quota' of nitrogen. Secondly, along with this large sap consumption comes the aphid's second problem, the risk of dehydrating due to the higher osmotic pressure of the sieve element sap. In order to survive and thrive on their host plants, which clearly many aphid species do, it has become necessary for aphids to develop certain 'strategies' with which to combat these issues.

Aphids quite often employ behavioural strategies in which to prevent the fore mentioned problems becoming an issue. These strategies involve feeding on the young, freshly growing and the old, senescing areas of the plant in order to target the readily mobilized free amino acids prevalent in these areas (Kennedy et al., 1950) and also regulating the level and speed of sap ingested by using the cibarial pump and by feeding sporadically from the xylem flow of the plant (Mittler and Dadd, 1962; Mittler and Dadd, 1963; Mittler, 1967).

However a major adaptation which provides the greatest aid to a feeding aphid are endosymbiotic bacteria. Within the digestive tract of an aphid lies mycetocytes, specialised insect cells which compartmentalize intracellular bacterial symbionts within their cytoplasm. It has been shown that the relationship between these endosymbionts and the aphids allows the aphid to thrive on poor quality, nutritionally inadequate sap diets. The symbionts, primarily *Buchnera*, work in the aphid's favour by upgrading the amino acids present in the ingested sap, releasing vitally important essential amino acids into the haemocoel, as well as providing a way of harnessing aphid waste ammonia to the benefit of the aphid. Thus these symbionts compensate for the nutritional deficit of the aphid's diet, allowing the aphids to perform better than would be expected from the composition of the dietary sap. Consequently studies have shown that the elimination of these bacterial symbionts using antibiotic treatments (Douglas, 1998; Douglas et al., 2001; Koga et al., 2007) rendered the aphid unable to reproduce and unable to maintain a normal level and rate of growth (Koga et al., 2007).

1.2.5 The Aphid Alimentary Tract

The 'mouth' of the aphid is situated at the junction with the cibarial pump and forms the start of the aphid alimentary tract. As previously discussed in section 1.1.3, aphids feed from sieve elements within the vascular bundle (Auclair, 1963). They tap into the vascular bundle of the host plant with their needle-like stylets (Bradley et al., 1962; Forbes, 1969; Forbes, 1977), gaining access to the sieve elements by piercing through the plant cuticle

and penetrating through mesophyll cells in search of a suitable sieve element. Upon the aphid reaching a suitable sieve element, sap enters through the stylet food canal under positive pressure and from here enters the 'mouth' of the aphid. As previously mentioned, the mouth of the aphid is situated at the junction with the cibarial pump and forms the start of the aphids alimentary tract. Extending from the mouth is the oesophagus which is separated from the stomach by the oesophageal valve. Leading from the stomach is the aphids long intestine, together forming the aphid midgut where nutrients from ingested sap are assimilated (Douglas, 2003). This process is facilitated by the specialized, immensely folded basolateral membranes of the intestinal epithelial cells and the presence of microvilli. The midgut region is also believed to be the site of enzyme production. These digestive enzymes are delivered into the lumen of the intestine following apocrine secretion and the budding of epithelial cell cytoplasm (Ponsen, 1987).

The excretory processes in the aphid alimentary tract differ from other insects in that aphids do not have any malpighian tubules (Dixon, 1987). These tubules are primarily involved in excretion and osmoregulation and are usually found in the posterior regions of arthropod alimentary canals. Aphids however accumulate their excreta known as honeydew in their hindgut prior to being expelled from the rectum of the aphid one drop at a time, usually at around one drop per 15-45 minutes although rates differ according to various environmental factors. The honeydew of aphids is rich in carbohydrates and the waste products of the digestive process, however

it is iso-osmotic with aphid haemolymph. Some waste ammonia is recycled into essential amino acids via symbiont action however most waste ammonia is excreted via the honeydew (Sasaki et al., 1990). Upon elimination of *Buchnera* from the aphid, the content within the honeydew droplets is infact greatly increased supporting the evidence of symbiont recycling (Wilkinson and Douglas, 1995).

1.2.6 Amino Acid Assimilation During Aphid Digestion

Amino acids are required by aphids for a variety of functions. Most amino acids assimilated during digestion are incorporated into proteins. The supply of amino acids is essential for the growth and reproductive requirements of the aphid. An example of this is the amino acid tyrosine. This amino acid is vital for aphid growth and forms a precursor for the synthesis of the aphid cuticle. Aphids also require amino acids for the purpose of cell signaling. Certain amino acids act as neurotransmitters, neurohormones or act as precursors of these forms of cell signaling molecules. An example of such an amino acid is tyrosine which is a precursor to dopamine, a catecholamine neurotransmitter found in both vertebrates and invertebrates (Douglas, 2003).

Another role of assimilated amino acids lies in the process of osmoregulation, utilised within the haemolymph. In order to utilise the amino acids in this manner the aphid transports amino acids across the gut

into the haemolymph using what is thought to be cation coupled midgut transporters (Wolfersburg, 2000).

With approximately 40 proteins characterized within sieve element sap these would appear to be another potential source of dietary nitrogen for the aphid. Early work had suggested that the digestion of proteins within the aphid gut is not possible due to the lack of general gut proteases, however recent studies of *Sitobion avenae* have now shown that aphid gut extracts do show significant levels of proteolytic activity (Pyati et al., 2011). Although proteolytic activity has been observed in the gut of the cereal aphid, *S. avenae*, it is believed that the ability to digest proteins within the gut, offers no significant nutritional benefit to the phloem feeding aphid (Pyati et al., 2011).

1.3 The Host Plant

1.3.1 Physiology of the Phloem

The phloem is the conduit that allows for the redistribution of photoassimilates, water and other sap components around the plant system (Taiz and Zeiger, 1991). This transport system is the dominant food source of aphids. The phloem is made up of companion cells, sieve elements, parenchyma cells and in some plants, sclereids and laticifers (Taiz and Zeiger,1991).

1.3.2 Sieve Elements

Sieve elements are living, highly specialised cells that are uniquely involved in direct translocations of sap (Taiz and Zeiger, 1991). Mature sieve elements typically measure 20-30 micrometers in diameter and 100-500 micrometers long (Tjallingii and Hogan Esch, 1993), and lack common cell structures including a nucleus, tonoplast (both lost during development) microtubules, microfilaments, golgi bodies and ribosomes. However membrane anchored mitochondria, plastids and smooth endoplasmic reticulum are all retained in the mature cell, their position thought to prevent them being swept along the translocation stream (Tjallingii and Hogan Esch, 1993). Their un-obstructed central lumen facilitates the flow of the translocation stream when the sieve elements are linearly arranged end-to-end. The end walls of the sieve tubes (sieve plates) have pores which allow the unobstructed flow of sap between adjacent vessels. The sieve element is often described as a 'living tube' through which sieve element sap is able to flow (Zimmermann, 1960; Tjallingii and Hogan Esch, 1993).

1.3.3 Companion Cells

Companion cells provide essential support for their associated sieve element cell, (Zimmermann, 1960) taking over the sieve element's redundant functions that are lost during development, for instance protein synthesis. A complex between each sieve element and its associated companion cell is

formed and connected by plasmodesmata. The pore connection allows for the metabolically dependent sieve element to achieve an intimate relationship, facilitating sap transport between the two associated cell types (Tjallingii and Hogan Esch, 1993). To prevent detrimental leakage from the transport system the companion cells otherwise remain isolated (Taiz and Zeiger, 1991).

1.4 Sieve Element Sap Composition

Sieve element sap forms the main diet of aphids. The sap consists of mainly water, nitrogenous compounds, and carbohydrates, however other components including inorganic solutes have also been found.

1.4.1 Water

Within sieve element sap water has been identified as being the most abundant component (Taiz and Zeiger, 1991) acting as a solvent for approximately 10-25% of sieve element exudates (Zimmermann, 1960).

1.4.2 Miscellaneous Compounds

Inorganic components have been detected within sieve element sap, with dominant components including, potassium (50-150mM) and phosphate (10-50mM (Gould et al., 2004). Plant hormones have also been found and

identified, they include auxins, gibberellins, cytokinins and abscisic acid, all of which are transported within the sieve element. Nucleotide phosphates and enzymes have also been discovered within the sieve element sap (Taiz and Zeiger, 1991).

1.4.3 Carbohydrates

Within sieve element sap carbohydrates exist mainly in the form of sugars. The three main sugars which have been found to be translocated within the phloem are sucrose, raffinose-family oligosaccharides (RFOs – raffinose, stachyose, verbascose and ajugose) and polyols (sorbitol and mannitol). In many plant species, including *Arabidopsis*, sucrose is the most commonly transported sugar, comprising 90% of all dissolved solutes (Zimmermann, 1960). Sucrose can be found at concentrations of 0.3-1.5 M (or 10-25% w/v) dependent on the species of plant, the plant section being analysed and the time of day and season (Zimmermann, 1960; Auclair, 1963; Smith et al., 1980; Srivastava, 1987). Other sugars including raffinose, galactinol and maltose are also detected in *Arabidopsis* with trace amounts of glucose and fructose and a high ratio of sucrose to hexose.

1.4.4 Nitrogenous Compounds

Nitrogenous compounds are abundant within sieve element sap, second only to sucrose (Rentsch et al., 1998). Most essential and non-essential amino

acids can be detected within the sieve element sap although it is usual for one of the amino acids to predominate, usually glutamate, glutamine, aspartate, or asparagine (Van Emden, 1969; Marschner, 1989; Taiz and Zeiger, 1991). Relative concentrations of the amino acids, histidine (His), tryptophan (Try), threonine (Thr) and valine (Val) also varies in different plant species.

Total concentrations of amino acids vary between 50-800 mM / 4.5-26.7% w/v (Weibull 1987), with essential amino acids being consistently low and accounting for 10-30% of total amino acids (Zimmermann, 1960; Auclair, 1963). Variations to the composition of the sieve element sap amino acid profile can be induced by abiotic and biotic stress. Droughting has been observed to create proline synthesis with large accumulations of proline observed in the actively growing regions of the primary root (Boggess and Stewart, 1975). Biotic stress such as that of an aphid infestation has been seen in wheat plants to increase the amino acid concentration of particular amino acids, including the essential amino acids, leucine, methionine, phenylalanine, histidine, isoleucine, trptophan and Valine. This form of reaction to an infestation could be considered as being particularly beneficial for the feeding aphid.

Inorganic compounds such as ammonium and nitrate however, tend to be present in very low concentrations and are often undetected during sap analysis (Douglas, 2003). The poor detection of ammonium and nitrate may be due to the rapid assimilation of ammonium following its uptake into the

roots, along with the fact that nitrate is translocated in the xylem (Fischer, 2002). Sieve element sap also contains approximately 150-200 different phloem proteins present in extremely low concentrations. These phloem proteins are believed to have several roles, mainly defence against insect and microbial attack (Hayashi et al., 2000). Although generally low, comprising <1% of SE exudate (Zimmermann, 1960; Auclair, 1963), levels of nitrogenous compounds are increased during senescence (Zimmermann, 1960; Taiz and Zeiger, 1991) and through the transfer of nitrogenous compounds from the xylem.

1.5 Plant Nitrogen Acquisition

Nitrogen is the element which is required by the plant in the greatest abundance. Nitrogen is an integral component within plants occurring within vital structures including, nucleic acids, chlorophyll and some plant hormones (Taiz and Zeiger, 1991).

Nitrogen is essential for normal plant growth and development. Without sufficient nitrogen a plant is not able to thrive, depletion will lead to stunted growth and development. Continuous nitrogen deficiency will eventually lead to the chlorosis and abcision of leaves. Clearly the plants ability to obtain and distribute nitrogen within its tissues is essential to the plants survival and overall success (Fischer et al., 1998).

1.5.1 Nitrogen Uptake

Nitrogen is acquired by plants via the roots from the soil (von Wiren et al., 1997). It is taken up from the rhizosphere in a variety of different forms, mainly nitrate and ammonium. These inorganic forms are then assimilated into organic nitrogen within the roots (ammonium) or the roots and leaf tissue (nitrate) (Fischer et al., 1998). Following the acquisition and assimilation of various nitrogenous compounds, nitrogen can be incorporated into amino acids for transport around the plant or utilised in protein synthesis or in other nitrogenous compound synthesis within plant tissues. However previous studies have indicated that organic forms of nitrogen, such as amino acids, are also obtained from the soil (Schobert and Komor, 1989; Chapin et al. 1993; Kielland, 1994; Raab et al., 1996; Fischer, 1998; Schmidt and Stewart, 1999).

The process of amino acid uptake by the roots may occur passively by bulk flow and diffusion or actively via transport mechanisms involving proton-coupled amino acid transporters (Bush, 1993, 1999; Delrot et al., 2001; Fischer et al., 2002). Following uptake the amino acids may be transported symplasmically or apoplastically along the cells wall. In the case of apoplastic transport, the amino acids would require loading into the symplasm before reaching the Casparian strip of the endodermis. After the endodermis the amino acids are transported to the vascular cylinder whereupon they are translocated in the xylem to the shoots (Schobert and Komor, 1990). With symplasmic transport, the uptake of amino acids may

take place directly into the root epidermis or into the root cortex following an apoplastic transport route (Dolan, 2001; Dolan et al. 1993). Barlow (2002) also suggests that amino acid transport in the roots may occurr via the root-cap cells.

For some plants nitrogen acquisition is accomplished by nitrogen fixing bacteria within roots 'nodules'. These bacteria essentially provide the plant with nitrogen in the form of ammonia. Other species however are nourished by associations with ectomycorrhizal fungi which absorb nitrogenous compounds directly from the soil interface transforming them into forms of nitrogen which are more readily used by the plant (Raven et al., 1999). Translocation of nitrogen occurs to regions that cannot manufacture nitrogen independently. These regions actively require nitrogen with which to grow and develop, for example young, developing leaves and roots (Ortiz-Lopez et al., 2000).

1.5.2 Mechanisms of Translocation

Nitrogen is translocated from the supplying 'source' regions of the plant (commonly a fully grown, photosynthesising leaf) to 'sink' regions (regions of the plant that cannot produce the necessary levels of photosynthetic products needed for their growth, development and storage purposes.) Such regions include tubers, roots, maturing fruits and young leaves. At these sink regions translocated nitrogen will either be used for growth or stored as

a supply. This 'source to sink transport' of nitrogen occurs via the phloem in the sieve element transport system (Zimmermann, 1960). Photoassimilates produced at the source regions are translocated to the sieve elements of the phloem by a process known as 'loading', a term which implies energy is needed to drive the movement of sugar against a concentration gradient (Loomis, 1955). The product solutes can be translocated by either of the two types of phloem loading, apoplastic or symplastic. In apoplastic loading, the transport of the sugar occurs actively from the extracellular space into the sieve element – companion cell complex (SE-CC) via membrane transporters located within the SE-CC complex plasma membrane. In the case of symplastic loading, the photoassimilates are loaded directly into the SE-CC complex via plasmodesmata (Turgeon 1996). Most herbaceous plant species of temperate regions, including economically important species of crop plants, have relatively few plasmodesmatal connections and thus are putative apoplastic loaders (Gamalei 1991, Oparka and Turgeon 1999). Exceptions to this observation include the predominantly symplastic phloem loader *Coleus blumei* (Turgeon and Gowan, 1990).

The process of loading and unloading into the sieve element generates an 'osmotically generated pressure gradient' which is believed to sustain 'bulk flow' of sieve element sap and therefore solute translocation (Gould et al., 2004). At the source region active loading of solutes (such as nitrogen or sucrose) into the companion cells and sieve elements generates an increase in osmotic pressure. This accumulation of solutes induces water to move into the sieve element via osmosis. Consequently turgor pressure builds up

within these source regions and generates movement of water and solutes from these regions of greater osmotic pressure to regions of lower osmotic pressures, the sink regions. At these regions solutes are unloaded into cortex cells. This now widely accepted pressure-flow theory of translocation was formulated by Münch in 1930 (Münch, 1930).

This process of unloading can occur either via an apoplastic or symplastic route, varying between species of plant, however unlike phloem loading it is thought that the process of unloading occurs via leakage along a facilitated diffusion gradient maintained by either the conversion of sucrose to storage molecules or the process of cleavage and storage in the vacuole (Kuhn et al., 1999; Lalonde et al., 2003; Gould et al., 2004). A switch between symplastic and apoplastic unloading can also occur within the same plant due to developmental and spatial changes to the sink tissues (Turgeon, 1989). The movement of the sieve element sap can occur in either direction within the plant depending on the requirements of the 'hydraulically connected' sink and source tissues (Taiz and Zeiger, 1991; Gould et al., 2004).

The movement of amino acids through the plants, starting at the roots and moving to the leaves, sink organs and finally the embryo cells, is facilitated by plasma membrane transporters. These transporters are essential for the movement of amino acids throughout the plant tissue with large number of transporters being identified in plants, some with overlapping functions. However, although there are many of these transporters present in plants, not much is known of their physiological function (Lalonde et al., 2003; Liu

and Bush, 2006; Tegeder and Weber, 2006; Rentsch et al., 2007). An example of an amino acid transporter is AAP1. AAP1 or 'amino acid permease 1' is a member of the amino acid transporter superfamily (ATF) superfamily. This transporter is known to function as a low to moderate affinity amino acid transporter, transporting neutral amino acids and glutamate in co-transport with protons across membranes (Boorer et al., 1996; Fischer et al., 2002). Localisation studies have suggested a function for AAP1 in amino acid uptake in root cells and also in supplying resources to developing seeds (Sanders et al., 2009).

1.5.3 Nitrogen Trafficking Affecting Overall Plant Fitness

As previously discussed, plants are able to use nitrogen following a process involving several steps, firstly uptake and assimilation by the plant followed by translocation around the plant and finally remobilisation. The major sink in the mobilisation of nitrogen are the developing seeds. It is this final stage of mobilisation that is particularly important to a plant's overall fitness.

Plant fitness in terms of seed yields relies on primary nitrogen uptake for initial reproductive organ growth, but also the later stages of nitrogen processing during the remobilisation process during seed formation and maturation (Kichey et al., 2007). The yield of a plant in terms of seed number and viability can be thought of as a good measure of the plants fitness and ability to produce offspring successfully.

The process of the seed sink receiving nitrogen through loading is usually regulated by the efficiency of the phloem pathway, the trafficking processes located in the source leaves and also the strength of the seed as a sink.
The concentration of free amino acids within sieve element sap is dependent upon the loading rate into the phloem from the source. It is this loading from source to phloem which is dependent upon the presence and efficiency of the amino acid transporters which are situated in the sieve element/companion cell complexes (Tilsner et al., 2005).

The phloem loading process is a pivotal step for the remobilisation of nitrogen with studies indicating that the over expression of certain amino acid permease genes have lead to increases in individual seed size, in some cases by up to 30% (Rolletschek et al., 2005). Other studies have shown that amino acid transporters expressed within seeds are thought to be involved with the nitrogen loading or 'filling' of the endosperm during the process of embryogenesis (Rentsch et al., 2007; Tsay et al., 2007). The absence of these transporter proteins can have a significant effect on the overall number of seeds produced by the plant (Schmidt et al., 2007).

1.6 Aphid Plant Interactions

1.6.1 Host Plant Attraction - Pre-Alighting Behaviour

Plants are generally colonised by alate aphids (Dixon et al., 1993; Dixon 1998). Alate flight generally occurs at low speeds with little direction or

control over landings (Klingauf 1987; Dixon et al., 1993; Kobayashi and Ishikawa, 1993; Dixon, 1998). Directed landings can only be made at the last moment by the aphid once it enters the calm air directly above the area of vegetation. This 'boundary layer' of air allows the aphid to 'home in' on a plant of their choice. The choices made once the aphid has established its limited flight control are primarily 'phototactic' responses to plant reflected wavelengths (Kennedy et al., 1961; Hardie, 1989). The basis for colour acting as an attractant to the aphid originates from the fact that the colour is usually a reliable indicator of plant status in terms of its nutrient resources. Aphids are particularly attracted to the colour yellow, the reasoning behind this being that the more nutritious tissues of a plant tend to be yellow in colour, for example both young, developing tissue and senescing tissue (Kennedy et al., 1961; Hodgson and Elbakhiet, 1985; Dixon et al., 1993; Dixon, 1998).

Other evidence within the literature has suggested that as well as colour, the shape of the potential host plant target may also play a role in aphid attraction (Kennedy et al., 1961; Hodgson and Elbakhiet, 1985). Landing responses are also thought to be aided by the presence of volatiles (Chapman et al., 1981; Nottingham and Hardie, 1993) which are sensitively detected by the antennal olfactory sensilla (Visser et al., 1996; Park and Hardie, 2004). Although the above factors all act as primary cues for locating a suitable host plant only a very small proportion of alates will be successful in doing so (Ward et al., 1998).

1.6.2 Primary Plant Contact and Initial Assessment of Surface Cues Prior to Stylet Penetration

Aphids will actively assess the surface cues present upon the host plant after landing. Antennation behaviour, involving antennae waving, allows for the detection of odours that lie close to the plant surface (Storer et al., 1996; Powell et al., 2006) while at the same time detecting specific cues using the chemosensory hairs upon the antennal tips (Powell et al., 2006). These cues include epicuticular wax composition (Klingauf, 1987), trichome exudates (Neal et al., 1990), surface topology (Ibbotson and Kennedy, 1959), and host colour (Pelletier, 1990). All of these cues may influence the aphid's behaviour prior to stylet insertion, however these cues are known to be ultimately less important than internal plant factors as at this stage once tarsal contact with a hard substratum has been made the aphid will attempt penetration independent of any cues (Powell and Hardie, 2000). This even occurs upon the presence of repellent cues (Griffiths et al., 1982; Phelan and Miller, 1982) however the apparent absence of any recognisable cue generally creates a restless aphid that will not settle (Visser et al., 1996).

1.6.3 Plant Penetration - Probing the Epidermis

Probing of the host plant follows tarsal contact (Ibbotson and Kennedy, 1959; Powell et al., 2006). Initial penetrations remain within the epidermis lasting for relatively brief periods (Sauge et al., 1998). Upon arrival on a plant and following antennation behaviour an alate will often probe perhaps

only once or twice before flying away (Nault and Styer, 1972; Powell, 1991; Caillaud and Via, 2000) or moving to another feeding site, for instance the abaxial leaf surface (Wensler, 1962; Calabrese and Edwards, 1976). Although initial probing can determine acceptance or rejection, surprisingly both the stylets and labium are thought to lack any external 'contact chemoreceptors' (Wensler, 1974; Wensler, 1977; Tjallingii, 1978).

1.6.4 Stylet Pathway Penetration

Penetration into the stylet 'pathway' within the mesophyll and parenchyma tissues (Caillaud and Via, 2000) usually occurs in longer more sustained stylet penetrations (approximately 30 s to 1 min). Upon stylet penetration the aphid recognises specific cues within the 'pathway' (epidermal, mesophyll, and phloem tissues). This process of chemoreception is believed to be the most important stage in determining whether or not the aphid accepts or rejects the plant (Powell et al., 2006). Evidence within the literature also suggests that deterrent agents may act within the pathway to deter or hinder the aphid from accepting the plant and then feeding from it (Sauge et al., 1998). Once the aphid has successfully penetrated into the pathway, the tissue between the epidermis and the sieve element, the aphid will periodically puncture a cell to imbibe and, using its epipharyngeal organ, effectively taste the cell contents (Wensler and Filshie, 1969). The aphid will test for suitability and the presence of any harmful allelochemicals (Schoonhoven et al., 1976). Previous studies have also indicated that the physical and chemical composition of plant tissues

themselves may act to deter the aphid from accepting a particular host (Srivastava and Auclair, 1975; Campbell and Dreyer, 1985; Dreyer et al., 1985; Klingauf, 1988). If the plant tissue composition encountered on route to the sieve element along with the sieve element sap itself are deemed suitable the aphid will continue probing, eventually feeding and reproducing. If after probing through the pathway tissues and locating the sieve element the aphid does not deem the plant suitable in terms of nutritional quality, or if harmful allelochemicals are present the plant will be rejected. At the time of penetration plant defences may be initiated (Walling, 2000; Moran and Thomas, 2001), possibly inducing the production of chemical signals involved in the plant's defence pathways. These chemical signals may act as cues although they are generally considered to be less important in host selection due to the fact that the transcription of the pathway genes would occur over many hours, whereas selection usually occurs over a much more rapid timescale. Again these chemical compounds would be detected sensitively by the aphid's gustatory organ present in the epipharynx thus allowing the aphid to avoid any significant intoxication (Mittler and Dadd, 1964; Wensler and Filshie, 1969; Prado and Tjallingii, 1994).

Overall, host plant acceptance by a feeding aphid can be considered a complex process. The presence of pathway tissue cues or deterrents, pathway tissue attributes, and the palatability of the sieve element sap all contribute to the overall decision of acceptance or rejection made by the aphid.

1.6.5 Sieve Element Factors

Upon sieve element penetration the aphid's relative ability to overcome the plants effective wound reactions will greatly influence the aphid's decision whether to accept or reject the plant (Wensler and Filshie, 1969; Powell et al., 2006). This decision is also influenced by the aphid's ability to counteract the wound defence processes with its use of salivary secretions (Wensler and Filshie, 1969; Powell et al., 2006). Enzymatic activity within these secretions may lessen the plants ability to defend itself against aphid feeding. However studies also suggest that salivary secretions have a dual role in effectively conditioning the sieve element sap (Miles, 1999). The aphid's relative ability to detoxify potential secondary metabolites that may be present within the sieve element sap can also act as a determinant of acceptance or rejection. However it is ultimately the nutritional quality of the sieve element sap (Douglas, 1993), sampled from the plant during sieve element penetration, that determines the acceptance or rejection of that plant (Auclair, 1963; Auclair, 1965; Auclair, 1969; Klingauf 1987). Not only does this nutritional quality dictate optimal feeding sites within the plant but it also overall determines the feeding aphid's relative level of performance (Awmack and Leather, 2002).

In general, aphids prefer to feed on diets that have the optimum nutrient composition that in turn will allow them to thrive and perform in terms of their growth and development and also their reproductive output (Auclair, 1965; Awmack and Leather, 2002). However, there are cases where aphids

may feed on sub-optimal diets to avoid predation or high levels of competition for resources (Heisswolf et al., 2005). Host plant selection, however, is a complex process which involves a wide array of mechanisms, both sensory and behavioural (Powell, 2000). Of all the factors involved with plant-aphid interactions the nutritional requirements of the feeding aphid are clearly fundamental.

1.7 Sieve Element Nitrogen Composition and its Effect on Aphid Feeding Behaviour and Performance

According to previous studies, manipulation of sieve element nitrogen composition, more specifically amino acid concentration, is thought to dictate whether or not a plant is accepted by a probing aphid as well as potentially determining the overall performance of the aphid in terms of growth and reproductive output (Awmack and Leather, 2002). The fact that nitrogen content within the aphid diet influences aphid feeding behaviour and performance has been demonstrated both in plants and in artificial diets (Mittler and Dadd, 1962; Mittler and Dadd, 1963; Dadd and Mittler, 1965; Dadd and Mittler, 1966; Van Emden, 1966; Woolridge amd Harrison, 1968; Srivastava and Auclair, 1974; Weibull, 1987). Experiments using environmentally manipulated levels of nitrogen supplied in nutrient solutions have also seen both positive and negative effects of increased and decreased levels of nitrogen respectively. Increasing levels of nitrogen

supplied to *Brassica oleracea* increased the performance of the aphid *M. persicae* (Van Emden, 1966), whereas other studies using nitrogen deficient barley saw a decrease in the performance of the aphid *Rhopalosiphum padi* (Ponder et al., 2000).

Previous studies have indicated that nitrogen is fundamental to the performance of feeding aphids, with increased nitrogen levels eliciting increased aphid performance. However as mentioned previously, the relationship between host plant nitrogen status and relative aphid performance is a complex one. Within the sieve element sap it is the 'quality' rather than the 'quantity' of the nitrogen which determines the level of aphid performance, with the amino acid profile determining the nutritional quality of the host plant rather than the total nitrogen amount. The nitrogenous compounds that are key to an aphid's optimum diet are the nine essential amino acids, histidine, threonine, tryptophan, methionine, valine, isoleucine, leucine, lysine and phenylalanine (Mittler, 1967). Sieve element sap is considered to be of poor quality due to the low concentrations of these essential amino acids. For individual amino acids, an inadequate concentration would be enough to significantly impede the growth and performance of the feeding aphid. These amino acids cannot be synthesised by the aphid itself and therefore must either be ingested from the food source or internally synthesised by the aphid's symbiotic bacteria *Buchnera* (Douglas, 1998; Douglas et al., 2001). These bacterial symbionts modify the quality of the ingested host plant sieve element sap by upgrading the nonessential amino acids into the vitally important essential amino

acids. This process allows the aphids to maintain high levels of reproductive output whilst feeding on a diet of low nitrogen quality. A symbiotic diet modification is not unique to aphids, however no other association is able to upgrade nonessential amino acids into essential amino acids. 'Aposymbiotic aphids' or aphids lacking their symbionts show poor growth and very low, if any reproductive output. This therefore suggests that without the diet quality upgrade, feeding aphids would struggle to survive on their normal host plants. Some evidence suggests that different aphid species show varying sensitivities (Cole, 1997) to lowered essential amino acid concentrations, for instance *M. persicae* is believed to be more sensitive to the specific amino acid composition than the cabbage aphid *Brevicoryne brassicae* (Van Emden, 1969).

1.8 Manipulating Sieve Element Nitrogen Content – A Functional Genomics Approach

Over recent years, developments in knowledge regarding genomic sequencing and the study of gene function has been immense. New and improved molecular methods (Baldwin et al., 2001) have facilitated researchers to explore new opportunities, the study and application of 'functional genomics' is one such method. Functional genomics is generally defined as the 'study of gene function' (Bouchez and Höfte, 1998). Through the use of processes such as insertional mutagenesis (commonly referred to as 'gene knockouts') and large scale sequencing programmes (TAGI, 2000;

Bouché and Bouchez, 2001) functional genomics provides an in situ approach to assessing gene function at either the basal biochemical level, the cellular level or even the structural level.

1.8.1 Insertional Mutagenesis

Insertional mutagenesis or 'gene knockout' involves the inactivation of a gene's activity in situ within the organism being studied (Bouchez and Höfte, 1998). The inactivation of a particular gene of interest is achieved by the aimed insertion of foreign DNA, either in the form of transposable elements or, now more commonly used, transfer DNA (T-DNA) (Krysan et al., 1999). By inserting foreign DNA into a gene of interest the expression of the gene is affected (Bouchez and Höfte, 1998) so that the potential target gene product is altered or even not produced (Bouché and Bouchez, 2001). This insertion of foreign DNA is achieved using a method known as the 'floral dip method' (Clough and Bent, 1998). The floral tissues of a wildtype plant with numerous immature floral buds and only few siliques are simply dipped into a solution containing *Agrobacterium tumefaciens*, 5% sucrose and 500 microliters per litre of a surfactant known as Silwet L-77. This dipping can be repeated to improve transformation rates and increase the yield of the transformants. This methodology facilitates high-throughput transformation of *A. thaliana.*

The mutation can be identified by the foreign DNA which serves a dual purpose as a tag for the insertion into the target gene (Krysan et al., 1999).

The most common form of foreign DNA used as an insertional mutagen in this form of genetic study is that of T-DNA.

1.8.2 Transfer DNA (T-DNA)

Transfer DNA occurs in nature within *Agrobacterium tumefaciens*, the causal bacterial pathogen of crown gall. *A. tumefaciens* transfers T-DNA from its tumour inducing (Ti) plasmid to plants cells that it has invaded within the roots. It is here that this short length of DNA integrates into the host plants genome, instructing the host plant to provide nutrients for the resident bacteria and develop a gall or tumorous growth. By exploiting *A. tumefaciens* T-DNA of several kilobases (kb) in length can be successfully used as an insertional mutagen within genes of interest in a lab based environment (Krysan et al., 1999). Antibiotic resistance markers have now taken the place of former tumour promoting and biosynthetic genes on the T-DNA. By using a marker in this way transformed plants can be selected for efficiently (Azpiroz-Leehan and Feldmann, 1997). T-DNA is also particularly suited for its use in insertional mutagenesis as it has a low copy number (average number being 1.5 independent inserts per diploid genome, 57% of transformants possessing a single insert and 25% of transformants containing two inserts) and upon intergration into the host's genome remains stable (Feldmann, 1991). *A. thaliana* carrying insertional mutations within almost any desired gene of interest have been created by researchers through the use of insertional mutagenesis with T-DNA. Information regarding gene function and putative gene function is stored in public databases. Seeds

containing these mutant lines are held within 'seed stock centres', for example, The Nottingham Arabidopsis Stock Centre, or NASC as they are known.

1.8.3 The Model Plant; *Arabidopsis thaliana*

Arabidopsis thaliana, also known more commonly as mouse eared cress or thale cress, is a flowering crucifer (family *Brassicaceae*) that has been used for many years in molecular studies of angiosperms as a model organism. *A. thaliana* is ideally suited to this role as a model research plant because of the following qualities; small size, short generation time, small genome (TAGI, 2000; Bevan et al., 2001), prolific seed production and high gene density (Parinov and Sundaresan, 2000). It also belongs to the same family as many agriculturally important crop plant crucifers, for example cabbage and oilseed rape.

Like its brassicaceous relatives, *A. thaliana* it is also a target for many phytophagous pests, many of which are able to complete their life cycle on *A. thaliana*, for example *Myzus persicae* (Cole, 1997). These characteristics along with the availability of its fully completed genomic sequence and gene predictions make *A. thaliana* an ideal candidate for a model plant in the study of aphid plant interactions.

1.9 Plant Nitrogen Transporters as Targets for Insertional Mutagenesis

1.9.1 Amino Acid Transporters

Amino acid transporters are thought to play an integral role in resource allocation around the plant since organic nitrogen is predominantly transported in the form of amino acids (Ortiz-Lopez et al., 2000). For this reason amino acid transporters are thought to be essential to a plant's development and survival (Ortiz-Lopez et al., 2000; Lalonde et al., 2003). Amino acids are also the major component of an aphid's dietary nitrogen intake therefore from this perspective amino acid transport within the host plant is also of major interest. Amino acids are loaded at source regions and translocated around the plant via the phloem (Boorer et al., 1996). The amino acids are then unloaded at sink regions of the plant. This loading and unloading of amino acids at the source and sink regions of a plant require transporters that are specific to amino acids (Williams and Miller, 2001). The presence of these amino acid transporters is therefore essential to controlling the amino acid composition of the sieve element sap, the food source of the aphid. The feeding aphid can therefore be exploited as a tool to study manipulations in sieve element amino acid composition.

1.9.2 Selection of Genes for Insertional Mutagenesis

As previously mentioned, evidence suggests that sieve element amino acid content affects the feeding behaviour and relative performance of feeding aphids (Mittler and Dadd, 1963; Mittler, 1967; Awmack and Leather, 2002). Therefore the genes allocated for this study were those which had a possible role in affecting the sieve element composition of *A. thaliana*. The plant amino acid transporter genes, At*AAP1* and At*AAP2* were previously selected for this study in order to focus on the broad specificity transport of amino acids (Frommer et al., 1993).

1.9.3 Amino Acid Transporters

Amino acid transporters currently identified have been grouped into two superfamilies; the amino acid, polyamine and choline transporter superfamily (APC) and the amino acid transporter superfamily (ATF) (Fischer 1995). Of both superfamilies members of the ATF family have been studied most intensely. The subclass attracting the most attention is that of the amino acid permeases (AAPs). This subclass has received the most attention out of all of the long distance amino acid transporters (Fischer et al., 1995; Fischer et al., 1998).

1.9.4 Amino Acid Permeases - AAPs

Amino acid permeases are amino acid transporters that are thought to play

an integral role in transporting amino acids across plant cell membranes. Evidence within the literature suggests that some of these transporters are specific to the phloem (Fischer et al., 1995; Fischer et al., 1998; Williams and Miller, 2001). For this reason AAPs are generally considered to be ideal candidates in the search for genes that may potentially control the sieve element amino acid composition (Hunt 2005). AAPs most probably function as proton (H+) - amino acid co-transporters (Fischer et al., 1995; Boorer et al., 1996; Fischer et al., 1998; Williams and Miller, 2001; Fischer et al., 2002) being able to recognise a wide range of amino acids. The coupling of the co-transporter with the proton-pumping plasma membrane H+ - ATPase transport system allows for the generation and maintenance of the proton electrochemical gradient across the plasma membrane (Rentsch et al., 1998). This is necessary in some cases where the process of amino acid loading into the phloem requires energy.

AAPs can generally be classified under the three subfamily titles based on their activities towards amino acids; 1) broad specificity transporters that recognise acidic and neutral amino acids (AAP1, AAP2, AAP4 and AAP6), 2) general amino acid transporters, that recognise acidic, neutral and also basic amino acids (AAP3 and AAP5) (Fischer et al., 1995) and 3) preferential acidic amino acid transporters (AAP8 and AAP6). Functional redundancy of the numerous amino acid transporters could be assumed however, their expression within a wide range of tissues along with instances of regulation occurring dismiss this idea (Fischer et al., 1995; Williams and Miller, 2001). The transporters AAP1, AAP2 and AAP8 are found to be highly expressed

within developing siliques of *A. thaliana*. These transporters are presumed to be involved with supplying amino acids to the immature seeds as well as remobilising the nitrogen stored from within the developing seedlings (Fischer, 1998). The transcripts of the transporter AAP3 transcripts have been found solely within the roots. This would suggest that this specific transporter was involved in the uptake of amino acids from the soil rhizosphere (Fischer et al., 1995). AAP2 is also found to be expressed within stems along with AAP4 and AAP5 where it is believed that these transporters essentially recover amino acids from the translocation stream (Fischer et al., 1995) Serving a dual function in maturing leaves, AAP4 and AAP5 are believed to load amino acids into the translocation stream where upon they are delivered to sink regions of the plant. The concentration of free amino acids within sieve element sap is dependent upon the loading rate into the phloem from the source. It is this loading from source to phloem which is dependent upon the presence and efficiency of the amino acid transporters which are situated in the sieve element/companion cell complexes (Tilsner et al., 2005).

The phloem loading process is a pivotal rate determining step for the remobilisation of nitrogen with studies indicating that the overexpression of certain amino acid permease genes have lead to increases in individual seed size, in some cases by up to 30% (Rolletschek et al., 2005). Other studies have shown that amino acid transporters expressed within seeds are thought to be involved with the nitrogen loading or 'filling' of the endosperm during the process of embryogenesis (Rentsch et al., 1998; Rentsch et al., 2007;

Tsay et al., 2007). The absence of these transporter proteins can have a significant effect on the overall number of seeds produced by the plant, a mutation of AtAAP8 caused a 50% reduction in seed number per individual silique sampled (Schmidt et al., 2007).

1.10 Project Aims

This study aims to use a functional genomics approach in order to investigate plant aphid interactions in *Arabidopsis thaliana*. The primary aim of this functional genomics investigation is to study the effect of insertional mutations within amino acid transporters of *A. thaliana* on aphid–plant interactions in two comparatively different aphid species, *Myzus persicae* and *Brevicoryne brassicae*. This study of aphid plant interactions between two aphid species and two amino acid transporters of interest are to be studied in terms of whole plant phenotype, aphid feeding behaviour and aphid reproductive performance.

Previously, insertional mutations were created within the model plant *A. thaliana* (Hunt, 2005). These mutations were created within the plant's Amino Acid Transporter superfamily (ATF), more specifically within the subclass of Amino Acid Permeases (AAPs). In this study two transporters are to be studied in reference to their interaction with aphids, these transporters being AtAAP1 (Accession number: At1g58360) and AtAAP2 (Accession number: At5g09220). As discussed in the molecular section of the

project background, the *AAP1* and *AAP2* gene in these mutant plants were previously disrupted so that the function of AAP1 and AAP2 within the *aap1* and *aap2* 'knockout' plants was lost. The preliminary aim of this study is therefore to investigate if this loss of AAP1 and AAP2 function affects the whole plant fitness status, the aphid feeding behaviour and also aphid reproductive performance in either aphid species studied. In order to compare the effects of the *AAP1* and *AAP2* gene knockout, Col0 wildtype plants will be used to act as controls. Both *M. persicae* (a generalist feeding aphid) and *B. brassicae* (a specialist feeding aphid) will be used as the aphid species within the model system on *A. thaliana*. Generalist aphid species are those that are able to thrive on a wide variety of host plants compared to specialists who are limited to a particular range of host plants. Previous studies have shown that the process of acceptance or rejection of a host plant differed between generalist and specialist aphid species, with specialists exhibiting faster, more direct searching and acceptance behaviours when compared to generalist species (Bernays and Funk, 1999, Vargas et al., 2004). Due to the comparatively different levels of host plant commitment and behavioural patterns of host acceptance observed within generalist and specialist aphid species, it is hoped that any subtle effect occurring within the aphid-plant interaction can be detected by using both *M. persicae* and *B. brassicae.*

Past studies have suggested the growth, development and reproductive performance of an aphid is closely correlated to the host plant quality (Awmack and Leather, 2002), in terms of the sieve element nitrogen

composition. As this study will be initially investigating the effect of AAP1 and AAP2 insertional loss of function mutants on aphid-plant interactions, it can be hypothesised that if AAP1 and AAP2 play a significant role in the transport of amino acids throughout the phloem of the plant, then their disruption within the *aap1* and *aap2* mutant plants may directly affect aphid feeding behaviour and performance. Although gene redundancy within the subclass of AAPs may be expected due to the transporters overlapping functions, previous studies have indicated that similar insertional loss of function mutants have exhibited measurable differences with regards to whole plant physiology and their interactions with aphids (Hunt, 2005).

Overall, this study aims to compare the effects of AAP1 and AAP2 on aphid-plant interactions whilst also allowing for a general comparison of aphid feeding behaviours and performance between two ecologically distinct and economically important aphid species, *M. persicae* and *B. brassicae.*

Chapter 2

What Effect do AAP1 and AAP2 Transporter Mutations have on Whole Plant Growth, Nutrient Status and Reproductive Plant Fitness?

2.1 Abstract

Experiments were carried out to determine whether the loss of function mutations in AAP1 and AAP2 amino acid transporters affects the growth, morphology, and/or reproductive fitness of the plant.

Prior to day 50 there were no significant differences between whole plant growth of *aap1* plants and the control plants in any of the characteristics measured ($p > 0.05$). Similarly, prior to day 50 there were no observed differences (for all characteristics measured) between *aap2* plants and their control wildtype comparison plants ($p > 0.05$).

At day 50 the plants measured exhibited several characteristics that were significantly altered in *aap1* plants compared to control plants. A significant reduction in rosette leaf number ($p < 0.000$), reduction in plant height ($p = 0.013$) and lowered silique number ($p < 0.000$) were observed in *aap1* plants compared to control plants. All other parameters measured including rosette leaf width and above root plant mass were not significantly affected by the disruption to AAP1.

At day 50 several characteristics were also significantly altered in *aap2* plants compared to wildtype plants. A significant reduction in rosette leaf number ($p < 0.000$), rosette leaf width ($p = 0.003$) and plant height ($p < 0.000$) was observed in *aap2* plants when compared to wildtype, control plants. Above root plant mass was not significantly affected by the disruption to AAP2.

Plant fitness in terms of the number of siliques produced was significantly decreased in *aap1* plants ($p < 0.005$) compared to control plants. Further analysis showed that the mean dry seed mass of *Ataap1* siliques was significantly lower than those of control plants ($p = 0.001$). The individual seed weights for *aap1* plants were observed to be consistently lower than wildtype seed weights at two of the three strata positions however when analysed with a GLM statistical test the mean individual seed weights of the *aap1* plants were not found to be significantly lower ($p = 0.066$).

Plant fitness in terms of the number of siliques produced was also significantly decreased in *aap2* plants ($p < 0.005$) compared to control plants. Further analysis showed that the mean dry seed mass of *Ataap2* siliques was significantly lower than those of control plants ($p < 0.000$). The mean individual seed weights of the *aap2* plants were indeed significantly lower than those of wildtype plants ($p < 0.001$).

Concentrations of chlorophyll measured in *aap1* were determined not to be altered for chlorophyll a ($p = 0.840$), chlorophyll b ($p = 0.023$) and chlorophyll a + b ($p = 0.435$) in comparison to wildtype plants. Similarly the concentrations of chlorophyll measured in *aap2* were also determined not to be altered for

chlorophyll a ($p = 0.508$), chlorophyll b ($p = 0.014$) and chlorophyll a + b ($p = 0.074$) when compared to wildtype plants.

When assessing plant characteristics under nitrogen supplementation the *aap1* plants that were watered with a nitrogen supplemented solution did not show significantly altered growth in any of the plant characteristics measured when compared to plants of the same genotype that were watered with a standard water regime ($p > 0.05$). Similarly the *aap2* plants that were watered with a nitrogen supplemented solution did not show significantly altered growth in any of the plant characteristics measured when compared to plants of the same genotype that were watered with a standard water regime ($p > 0.05$). Wildtype plants that were watered with a nitrogen supplemented solution did show significantly increased growth in some of the characteristics measured when compared to wildtype plants on the standard watering regime. Rosette leaf number ($p < 0.000$) and rosette leaf width ($p < 0.000$) were significantly increased in wildtype plants supplemented with nitrogen. Above root plant mass ($p = 0.105$), plant height ($p = 0.058$) and silique number ($p = 0.818$) were not significantly affected by the nitrogen supplementation. When nitrogen supplemented *aap1* and *aap2* plants were compared with nitrogen supplemented wildtype (Col0) plants statistically significant differences were observed for all characteristics measured ($p < 0.05$).

When assessing plant characteristics following aphid infestation the *aap1*, *aap2* and wildtype control plants that were purposefully infected with aphid infestations did not show any aphid induced significant effects on the plant characteristics measured ($p > 0.05$).

2.2 Introduction

2.2.1 The Amino Acid Permease Genes AtAAP1 and AtAAP2

Previous work focusing on plant-aphid interactions have indicated that the Amino Acid Permease (AAP) ATF superfamily should be seriously considered as a gene family of interest because of this close link between host plant amino acid status and aphid feeding behaviour and performance. This link between host amino acid status and consequent aphid feeding behaviour and performance is particularly evident from previous work by Hunt (2005) which investigated the closely related amino acid transporter, AAP6. This study suggests an interesting link which can be investigated further using other members of the Amino Acid Permease family and other aphid species.

This chapter aims to utilise a functional genomics approach in order to investigate how the transporters AAP1 and AAP2 affect whole plant phenotype, nutrient status and reproductive fitness. Using the technique of insertional mutagenesis the function of these two amino acid transporters can be proposed with regards to their role in affecting various aspects of plant growth.

The process of insertional mutagenesis, also known as 'gene knockout', involves inserting a foreign piece of DNA (usually transfer or T-DNA from *Agrobacterium tumefacien*) into the identified gene of interest in order to prevent gene function (Krysan et al., 1999; Parinov and Sundaresan, 2000). In preventing a functional

gene product being produced the effect on the whole organism can be determined and studied in a functional manner (Bouché and Bouchez, 2001).

The *Arabidopsis thaliana* Genome Initiative (TAGI, 2000) is an example of a large scale sequencing project that has utilised the method of insertional mutagenesis as a method of studying gene function. This initiative and other organisations have made available large collections of *A. thaliana* mutants, allowing those interested in researching a particular gene of interest to search a database and find a seed stock centre providing a homozygous line for that particular insertional mutant. A database search was performed for each of the amino acid transporters of interest, AtAAP1 and AtAAP2. By requesting the homozygous seed lines for this work it was possible to omit the time consuming genetic characterisation and isolation of seed lines possessing a mutation in the AAP1 and AAP2 transporters.

2.2.1.1 AtAAP1 and AtAAP2

AAP1 (Amino Acid Permease 1, also known as NAT1) was the first amino acid transporter of its kind to be studied and isolated within plants (Frommer et al., 1993; Hsu et al., 1993). Early studies indicated the amino acid specificity of AAP1 to be neutral and acidic amino acids (Frommer et al., 1993; Hsu et al., 1993) with later studies showing AAP1 to predominantly transport neutral amino acids with short side chains and glutamate (Boorer et al., 1996; Fischer et al., 2002). AAP1 has been previously shown to be a plasma membrane transporter functioning to uptake amino acids into the root epidermal cells (including throughout the root tip

and root hair cells of the main and lateral roots (see figure 2.1). AAP1 has also been localised to developing seeds where it is assumed to be involved in the transport of amino acids for development and the storage of proteins (Fischer et al., 1995; Frommer et al., 1993; Hirner et al., 1998; Kwart et al., 1993). Previous work has shown that AAP1 transporters play the initial role in amino acid acquisition within the roots, allowing for subsequent amino acid translocation to shoots. AAP1 inhibition work in potato plants demonstrated a reduction in the levels of free amino acids within the potato tubers (Koch et al., 2003) whereas overexpression experiments performed in leguminous plants showed increases in the storage proteins within seeds. Work involving the successful growth of At*aap1* plants on toxic levels of amino acids indicates these *aap1* mutants have a reduced ability to uptake a broad spectrum of amino acids (Lee et al., 2003). This demonstrates AAP1's function as a transporter of a broad spectrum of amino acids. AAP2 has previously been shown to be expressed within the vascular strands of the funiculi within siliques (Hirner et al., 1998). It is thought that AtAAP2 might play an integral role in the long distance translocation of amino acids (Hirner et al., 1998; Ortiz-Lopez et al., 2000).

Figure 2.1 represents a model of amino acid transport occurring within the roots featuring both the AAP1 and AAP2 transporters (Taken from Lee et al., 2007)

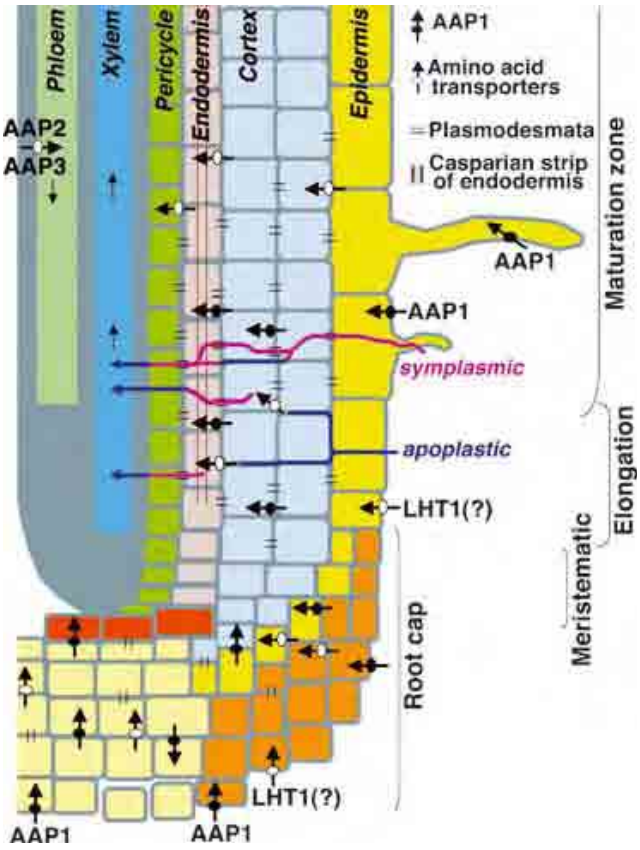

Figure 2.1 represents amino acid transport occurring within roots and the role which AAP1 and AAP2 play within this transport process. The process of amino acid transport within the roots may occur apoplastically (from cell wall to cell wall, not entering the cytoplasm at any point) until the casparian strip within the endodermis prevents further passage. At this point the amino acids are loaded into the symplast via amino acid transporters such as AAP1, located in the root cortex/endodermis cell membrane. Upon entering the endodermis the amino acids are transported within the vascular cylinder, being translocated into the xylem where via transpiration the amino acids eventually reach the shoots. AAP2 are believed to be responsible for loading amino acids into the phloem, supplying the roots tips with nitrogen needed for growth. (Hirner et al., 1998; Okumoto et al., 2004). Image taken from Lee et al., 2003.

2.2.2 Growth scoring techniques as a means of studying whole plant morphology

As discussed in chapter one, *A. thaliana* is a flowering crucifer (family *Brassicaceae*) that is ideally suited to its role as a model research plant. Small in size with a small genome and high gene density, *A. thaliana* is capable of producing high seed yield over a short generation time. *A. thaliana* has a rapid lifespan reaching mature seed from germination in approximately 6 weeks. The growth stages of wildtype *A. thaliana* have been studied and characterised in great detail (Boyes et al., 2001) allowing for comparative observations or 'scoring' to be made against mutant *A. thaliana* genotypes. The process of growth scoring to 'screen' plant morphological characteristics is a technique often used to investigate mutant genotypes. This technique is extremely valuable when used for retrieving information regarding the function of the mutated gene (Boyes et al., 2001). Plant growth in terms of morphology and rate of development determines the level of seed production and ultimately a plant's fitness. The overall morphology of a plant determines the level at which the plant is able to function normally while carrying out processes such as respiration and photosynthesis. Changes to the standard morphology of a plant may be disadvantageous to the plant in terms of its ability to function optimally. Likewise, the development of a plant determines the speed at which it can begin to successfully function and compete for resources.

Another important variable is the plant's ability to utilise the nutrient resources at its disposal. The nutrient status of a plant will clearly determine how the plant

develops, its morphology and consequently its overall fitness. In order to allow correct growth and development along with sustaining fundamental metabolic processes, a plant must be able to absorb and utilise the correct levels of certain nutrients usually present within the surrounding soil (Raven et al., 1999). Deficiencies in these nutrients or the plants inability to absorb and utilise nutrients could lead to negative effects on plant health and in prolonged cases even plant death (Taiz and Zeiger, 1991). By monitoring the morphology of the mutant and wildtype plant genotypes using growth scoring techniques it is possible to determine the whole plant quality and overall plant fitness. Growth scoring characteristics that may assist in determining whole plant quality and overall plant fitness may include, leaf morphology (size, shape and abundance), plant height, silique number, seed characteristics and whole plant mass.

2.2.3 Focused techniques of investigating plant phenotype using silique analysis, seed analysis, and chlorophyll analysis

Silique and Seed Analysis

Nitrogen is translocated from the supplying 'source' regions of the plant (commonly a fully grown, photosynthesising leaf) to 'sink' regions (regions of the plant that cannot produce the necessary levels of photosynthetic products needed for their growth, development and storage purposes). Such regions include tubers, roots, maturing fruits and young leaves. At these sink regions translocated nitrogen will either be used for growth or stored as a supply. This 'source to sink transport' of

nitrogen occurs via the phloem in the sieve element transport system (Zimmermann, 1960) with the developing seeds being the major sink. This final stage of mobilization into the seed is of major importance to the overall fitness of the plant. A reliable measure of overall plant fitness and ability to produce offspring successfully is a plant's seed yield (in terms of seed number and viability). This yield relies on the primary nitrogen uptake and the later stages of nitrogen processing during resource remobilization (Kichey et al., 2007).

The process of the seed sink receiving nitrogen through loading is usually regulated by the efficiency of the phloem pathway, the trafficking processes located in the source leaves and also the sheer strength of the seed sink. The concentration of free amino acids within sieve element sap is dependent upon the loading rate into the phloem from the source. It is this loading from source to phloem which is dependent upon the presence and efficiency of the amino acid transporters which are situated in the sieve element/companion cell complexes (Tilsner et al., 2005).

The phloem loading process is a pivotal rate determining step for the remobilisation of nitrogen with studies indicating that the over expression of certain amino acid permease genes have lead to increases in individual seed size, in some cases by up to 30% (Rolletschek et al., 2005). Other studies have shown that amino acid transporters expressed within seeds are thought to be involved with the nitrogen loading or 'filling' of the endosperm during the process of embryogenesis (Rentsch et al., 2007; Tsay et al., 2007). The absence of these transporter proteins can have a significant effect on the overall number of seeds produced by the plant (Schmidt et al., 2007). A recent study demonstrated that

the synthesis of seed protein and the seed weight itself and overall seed yield is dependant on AAP1 mediated amino acid uptake (Sanders et al., 2009).

Chlorophyll Analysis

Chlorophyll molecules are located in and around the photosystems of plants. These photosystems are embedded within the thylakoid membranes of the chloroplasts forming a complex which, in the vast majority of chlorohpyll, serves to absorb light and transfer this energy to the reaction centre of the photosystem, eventually facilitating the production of sugars. Chlorophyll *a* is essential for the release of chemical energy in most photsynthetic organisms. Alongside chlorophyll a, accessory pigments, such as chlorophyll b are also used during photosynthesis for the production of sugars (see figure 2.2 and table 2.3).

The level at which a plant is able to photosynthesise and produce these sugars can potentially affect the interaction between the feeding aphid. The presence of sugars within the sieve element sap is a crucial factor in determining whether an aphid chooses to feed on a host or reject it. This is clearly demonstrated by the acceptance or rejection of artificial diets that have sucrose and those that do not (Mittler and Dadd, 1963; Mittler, 1967; Mittler, 1970; Srivastava, 1987). This is supported by the observation that areas of a host plant with the highest levels of carbohydrates are often preferentially targeted, for example regions of growth in tips and shoots (Auclair, 1969). The substantial changes in sucrose levels that occur as a result of fluctuating levels of photosynthesis throughout the year are also thought to be responsible for the variations in aphid behaviour, growth,

reproductive capacity as well as their tendency to migrate from a particular host plant to another more suitable host. Variations in the levels of carbohydrates also occur daily due to the diurnal pattern of photosynthesis. Early studies demonstrated the immediate decrease of sucrose in source leaves upon the onset of darkness (Fondy and Geiger, 1982). However, these diurnal fluctuations of carbohydrates such as sucrose do not appear to significantly alter aphid feeding. Previous studies investigating the diurnal feeding pattern of cotton aphid (*Aphis gossypii* G.) populations found no significant patterns when analysing honeydew production (Gomez et al., 2004).

	Chlorophyll a	Chlorophyll b
Molecular formula	$C_{55}H_{72}O_5N_4Mg$	$C_{55}H_{70}O_6N_4Mg$
C2 group	$-CH_3$	$-CH_3$
C3 group	$-CH=CH_2$	$-CH=CH_2$
C7 group	$-CH_3$	-CHO
C8 group	$-CH_2CH_3$	$-CH_2CH_3$
C17 group	$-CH_2CH_2COO$-Phytyl	$-CH_2CH_2COO$-Phytyl
C17-C18 bond	Single	Single
Occurrence	Universal	Mostly plants

Table 2.2 – Table showing the molecular descriptions of Chlorophyll a and Chlorophyll b

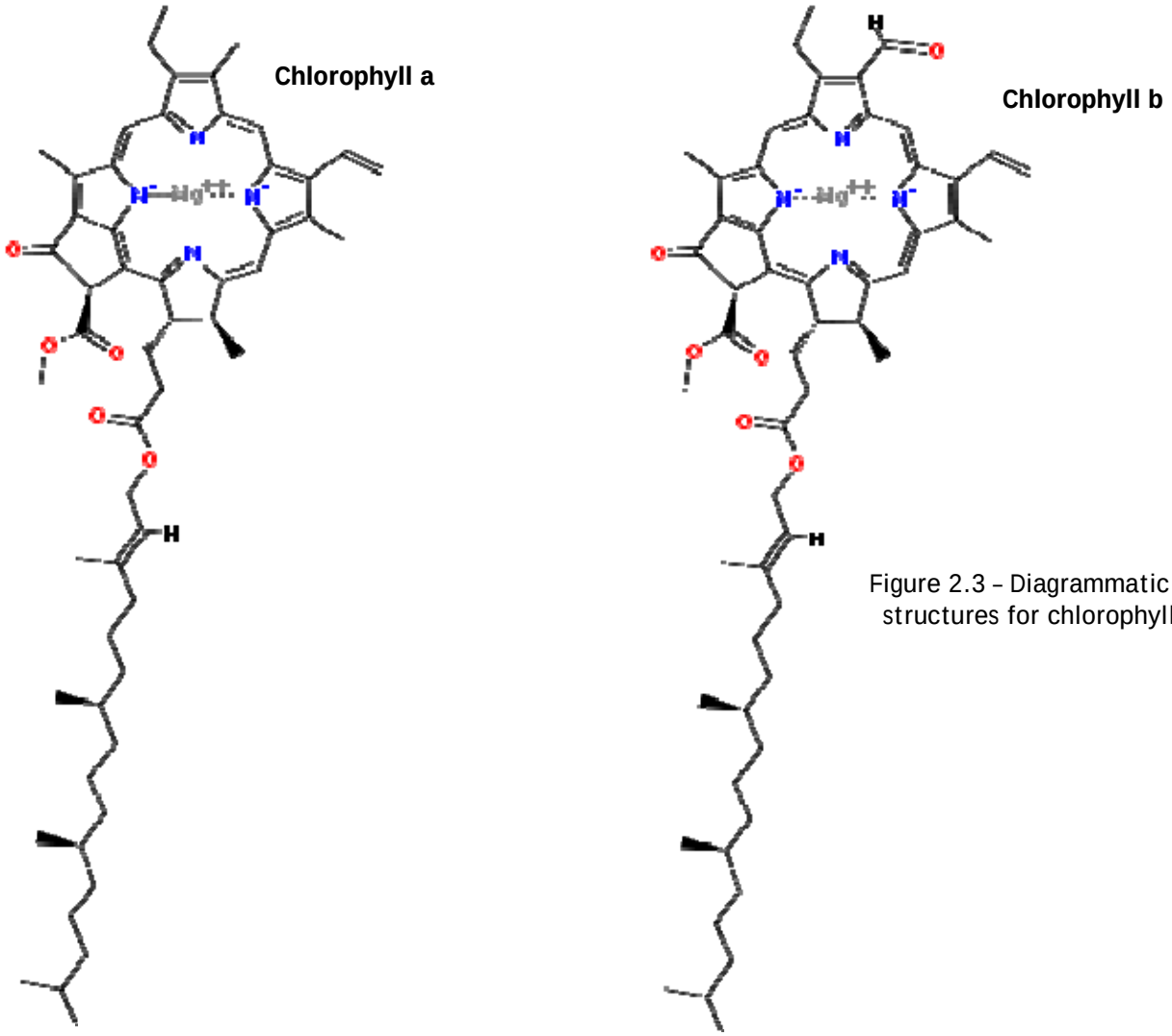

Figure 2.3 – Diagrammatic molecular structures for chlorophyll a and b.

2.3 Chapter Aims

The aim of this chapter was to investigate the effects of insertional mutations in genes for the amino acid transporters AtAAP1 and AtAAP2 on whole plant phenotype. This involved comparing whole plant morphology of *aap1, aap2* and wildtype plants along with comparing measures of plant fitness and plant phenology.

Primarily the phenology of *aap1, aap2* and wildtype plants were monitored for various morphological characteristics in order to determine potential affects of 'knocking out' At*AAP1* and At*AAP2* on whole plant phenotype. Secondly to this, the same morphological characteristics were to be monitored in *aap1, aap2* and wildtype plants after being cultivated under two different nitrogen treatments. The aim of this approach was to determine whether *aap1* and *aap2* mutants had altered amino acid uptake and assimilation abilities when compared to wildtype plants. Thirdly, the same morphological characteristics were to be monitored in *aap1, aap2* and wildtype plants after being cultivated under stress conditions, in this case aphid infestations of *Myzus persicae* and *Brevicoryne brassicae.* The aim was to investigate how plant growth was affected in *aap1* and *aap2* plants under the levels of aphid induced stress of both aphid species.

2.4 Materials and Methods

2.4.1 Cultivating Transgenic *Arabidopsis thaliana*

Compost consisting of 4 parts John Innes loam-based compost N^{o} 2, 4 parts peat-based compost, 1 part silverperl and osmocote slow release fertiliser was frozen for 48 h at –20 ºC and left to defrost before filling plant pots (6 cm in diameter) to within 1 cm of the top. Filled pots were sprayed with water to moisten and level the compost before evenly placing four *A. thaliana* seeds on the surface using a fine, dampened paintbrush. This was repeated for each genotype being planted, *aap1* (Accession number: At1g58360) and *aap2 (*Accession number: At5g09220*)* plants (insertional loss of function mutants also referred to as 'knockout' or KO plants) and Columbia0 wildtype plants (also referred to as control plants) with a total of twenty replicate pots for each genotype. All plant pots were labelled and arranged in a randomised design within large trays. These trays were kept within a growth room maintained at 20-22ºC with an 18-6 light:dark regime. Water was poured into the trays on a daily basis to ensure plants had a constant supply of water. Upon the germination of the seeds, three out of the four seedlings were removed at random from each pot to leave one seedling per pot. This seedling was then grown to the required stage (Boyes et al., 2001).

2.4.2 Phenotype Characterisation

2.4.2.1 Growth scoring

Each plant genotype being grown was observed and scored using a standard growth scoring index. Plants were observed on a daily basis and scored at day twenty, thirty, thirty five and also at day 50. The parameters to be scored include; time to germination, leaf number on day 20, rosette width on day 20 (cm), height on day 30 (cm), height on day 35 (cm), height at day 50 (cm), rosette leaf number at day 50, rosette width at day 50 (cm) and total number of siliques at day 50.

2.4.2.2 Statistical Analysis

Data was collected in growth scoring sheets before being tested for normality using an Anderson-Darling test. As the data was normal, it was analysed using a General Linear Model (GLM) ANOVA with a one-way ANOVA being used to investigate specific data interactions. See appendix 1.1 for details of the statistical analysis.

2.4.3 Whole Silique Analysis

2.4.3.1 Plant Material

Both *aap1, app2* and wildtype plants were cultivated according to the methods featured in section 2.4.1. These plants were selected for use in the whole silique analysis when at their mature stage of growth (50 days old from sowing).

2.4.3.2 Silique Analysis

Individual siliques were removed from the three clearly distinct developmental strata of the *aap1, aap2* and control plants, referred to as bottom, middle and upper strata. This sampling method was used in order to rule out any developmental effect of the siliques and their contents. Care was taken not to rupture the siliques during collection to ensure all seed contents remained intact within the silique for later analysis. After collection the siliques were dissected individually with a razor blade and the seed contents from each silique were extracted and stored individually in separate 1ml Microfuge tubes. The seeds from each silique were then weighed as a group using a Sartorious microbalance (sensitive to 10µg).

2.4.3.3 Statistical Analysis

Data was collected in growth scoring sheets before being tested for normality using an Anderson-Darling test. As the data was found to be normal it was then analysed using a General Linear Model (GLM) ANOVA with a one-way ANOVA being used to investigate specific data interactions. See appendix 1.2 for details of the statistical analysis.

2.4.4 Seed Analysis

The individually sampled seed contents sampled using the techniques of method 2.4.3.2 were analysed further under microscope in order to determine the number

of seeds produced in all developmental stages of the *aap1, aap2* and control plants.

After seed counts had been made for each silique sampled, an average seed weight for each individual silique was attained using the following equation;

$$\text{Individual Seed Weight} = \frac{\text{Whole Silique Seed Content Weight}}{\text{Total Number of Seeds within the Sampled Silique}}$$

2.4.4.1 Statistical Analysis

Data was collected in growth scoring sheets before being tested for normality using an Anderson-Darling test. As the data was found to be normal it was analysed using a General Linear Model (GLM) ANOVA with a one-way ANOVA being used to investigate specific data interactions. See appendix 1.3 for details of the statistical analysis.

2.4.5 Leaf Chlorophyll Analysis

2.4.5.1 Plant Material

Both *aap1, aap2* and wildtype plants were cultivated according to the methods featured in section 2.4.1. These plants were selected for use in the chlorophyll analysis when at their optimum stage of growth (25-28 days old).

2.4.5.2 Chlorophyll Analysis

Leaves from both plant genotypes were collected and halved then weighed in pre-weighed 1.5 ml microfuge tubes to ascertain the leaves 'fresh weights'. After this the leaves were dried overnight within a drying oven in order to remove all water before being weighed to ascertain the 'dry weight'. At this stage these dry leaf samples still in individual microfuge tubes were suspended individually within 1ml of 10% acetone solution and refrigerated overnight in order to extract the leaf chlorophyll components. The following morning the leaf samples were removed from the labelled microfuge tubes and the solutions were poured off one at a time into a quartz cuvette whereupon the optical density for each sample was measured at two different wavelengths, 665nm and 645nm. These measurements were taken using a spectrophotometer previously calibrated with a 'blank' solution of the acetone extraction buffer.

In order to calculate the chlorophyll concentrations from the measurements taken for each sample at each wavelength the following formulae were used (Jeffrey et al., 1975),

$$\text{Chl a } (\mu g/ml) = 13.7\ OD_{665nm} - 5.76\ OD_{645nm}$$

$$\text{Chl b } (\mu g/ml) = 25.8\ OD_{645nm} - 7.6\ OD_{665nm}$$

$$\text{Chl a + Chl b } (\mu g/ml) = 6.1\ OD_{665nm} + 20.04\ OD_{645nm}$$

2.4.5.3 Statistical Analysis

Data was collected in growth scoring sheets before being tested for normality using an Anderson-Darling test. As the data was found to be normal it was then analysed using two-sample t-tests. See appendix 1.4 for details of the statistical analysis.

2.4.6 Analysis of Plant Growth Under Varying Nitrogen Conditions

2.4.6.1 Plant Material

Both *aap1, app2* and wildtype plants were cultivated according to the methods featured in section 2.4.1 apart from a difference to the watering regime. For each genotype plants were cultivated up to germination and the presence of the initial, primary leaves. At this point the 80 plants for each genotype were separated into two groups. One group of plants (40 *aap1, aap2* and wildtype plants) were to be watered with just water and the other group were to be watered with water and a 10% nitrogen supplement (ammonium nitrate solution (NH_4NO_3)). One litre of water and the 10 % nitrogen supplemented solution were poured into the trays on a daily basis to ensure plants had a constant supply. These two separate trays of plants would continue to be treated with their different watering regimes, until they were growth scored and analysed at their mature stage of growth (50 days old from sowing).

2.4.6.2 Assessment of Plant Growth Under Varying Nitrogen Treatments

Each plant genotype being grown under both watering regimes was observed and scored using a standard growth scoring index. Plants were observed on a daily basis and scored at day 50. The parameters scored included; rosette leaf number, rosette width (cm), height (cm) and number of siliques.

2.4.6.3 Statistical Analysis

Data was collected in growth scoring sheets before being tested for normality using an Anderson-Darling test. As the data was normal it was analysed using a General Linear Model (GLM) ANOVA and one-way ANOVA's for analysis of specific interactions. See appendix 1.5 for details of the statistical analysis.

2.4.7 Analysis of Plant Growth Under Aphid Infestation

2.4.7.1 Plant Material

The *aap1, aap2* and wildtype plants were cultivated according to the methods featured in section 2.4.1. These plants were selected for use in the plant performance analysis when 30 days old from sowing.

2.4.7.2 Aphid Culture

The *B. brassicae* and *M. persicae* used in the plant performance analysis were produced and used under the methods in section 3.4.1.

2.4.7.3 Assessment of Plant Growth under Aphid Infestation

Upon previous analyses of the level of infestation achieved by each species on plants of differing genotypes it was decided to assess the level of plant performance of the respective genotypes upon infestation by the two different aphid species.

Both *aap1, aap2* and wildtype control plants aged 30 days old were selected for use in this experiment (with multiples of two for each genotype) and the following parameters measured prior to infestation;

- Height of plant (cm)
- Number of rosette leaves
- Width of rosette leaves (cm)
- Above root plant mass
- Number of siliques

The 'above root plant mass' measurement was obtained from duplicate plants that allowed for a 'destructive sampling' method.

Upon these parameters being measured on all of the plants, ten adult aphids of each species (previously synchronised as adult aphids in Austin tube cultures) were applied to each of the 'treated' plants of differing genotypes. The remaining half of the plants (a mirrored set of the genotypes that were treated with aphids) were caged as control plants with no aphids infesting them. The ten aphids on each treated plant were applied using a fine paintbrush to the upper surface of the largest rosette leaf on each plant. The cages were then sealed with masking tape and placed to stand in a tray of water for 3 weeks. After this duration of time both the treated and control plants were removed from their cages individually and the following parameters measured:

- Height of plant (cm)
- Number of rosette leaves
- Width of rosette leaves (cm)
- Number of siliques
- Above root plant mass

2.4.7.4 Statistical Analysis

Data was collected in growth scoring sheets before being tested for normality using an Anderson-Darling test. As the data was found to be normal it was then analysed using a General Linear Model (GLM) ANOVA. See appendix 1.6 for details of the statistical analysis.

2.5 Results

2.5.1 Phenotype Characterisation

Plant scoring data up until day 50 showed no significant differences between the *aap1* plants and the control plants, and the *aap2* and control plants in all characteristics measured ($p > 0.05$). Refer to section 2.4.2.1 for characteristics measured.

Measurements taken at day 50 did however exhibit several characteristics that were significantly altered in *aap1* plants compared to control plants. A significant reduction in rosette leaf number ($p < 0.000$), reduction in plant height ($p = 0.013$) and lowered silique number ($p < 0.000$) were observed in *aap1* plants compared to control plants. All other parameters measured including rosette leaf width and above root plant mass were not significantly affected by the disruption to AAP1. See table 2.4.

At day 50 several characteristics were also significantly altered in *aap2* plants compared to wildtype plants. A significant reduction in rosette leaf number ($p < 0.000$), rosette leaf width ($p = 0.003$), plant height ($p < 0.000$) and silique number ($p < 0.000$) was observed in *aap2* plants when compared to wildtype, control plants. Above root plant mass was not significantly affected by the disruption to AAP2. See table 2.4.

Mean Plant Characteristics (at day 50 after sowing)	*aap1*	*aap2*	**Col0**
Plant Height (cm)	**34.2*** **s.e 0.804** **(n=39)** **p=0.013**	**32.2*** **s.e 0.828** **(n=40)** **p<0.000**	**37.2** **s.e 0.90** **(n=35)**
Rosette Leaf Number	**14.3*** **s.e 0.381** **(n=39)** **p<0.000**	**13.7*** **s.e 0.245** **(n=40)** **p<0.000**	**17.6** **s.e 0.494** **(n=35)**
Rosette Leaf Width (cm)	**8.5** **s.e 0.365** **(n=39)** **p=0.128**	**7.76*** **s.e 0.308** **(n=40)** **p=0.003**	**9.2** **s.e 0.264** **(n=35)**
Above Soil Plant Mass (g)	**0.49** **s.e 0.040** **(n=39)** **p=0.298**	**0.49** **s.e 0.033** **(n=40)** **p=0.262**	**0.55** **s.e 0.037** **(n=35)**
Total Number of Siliques	**72.8*** **s.e 5.77** **(n=34)** **p<0.000**	**71.5*** **s.e 5.30** **(n=34)** **p<0.000**	**102.5** **s.e 4.87** **(n=34)**

Table 2.4 – Table showing the mean plant parameters measured in *aap1* (n=39), *aap2* (n=40) and control plants (n=35) at day 50 after sowing (silique analysis n=34). Data was analysed using one-way ANOVAs.* denotes a statistically significant difference in comparison to wildtype control plants.

2.5.2 Whole Silique Analysis

2.5.2.1 Whole silique analysis of *aap1*

The mean dry mass of seeds sampled from individual siliques was found to be significantly less ($p = 0.001$) in At*aap1* plants compared to control plants (see figure 2.5). Statistical analysis using a General Linear Model (GLM) ANOVA indicates a significant effect of genotype and also strata position on the plant ($p < 0.005$). Performing one-way ANOVAs for each strata position also revealed a significant difference in the mean dry mass of seeds of *aap1* and Col0 sampled from the middle strata ($p < 0.003$).

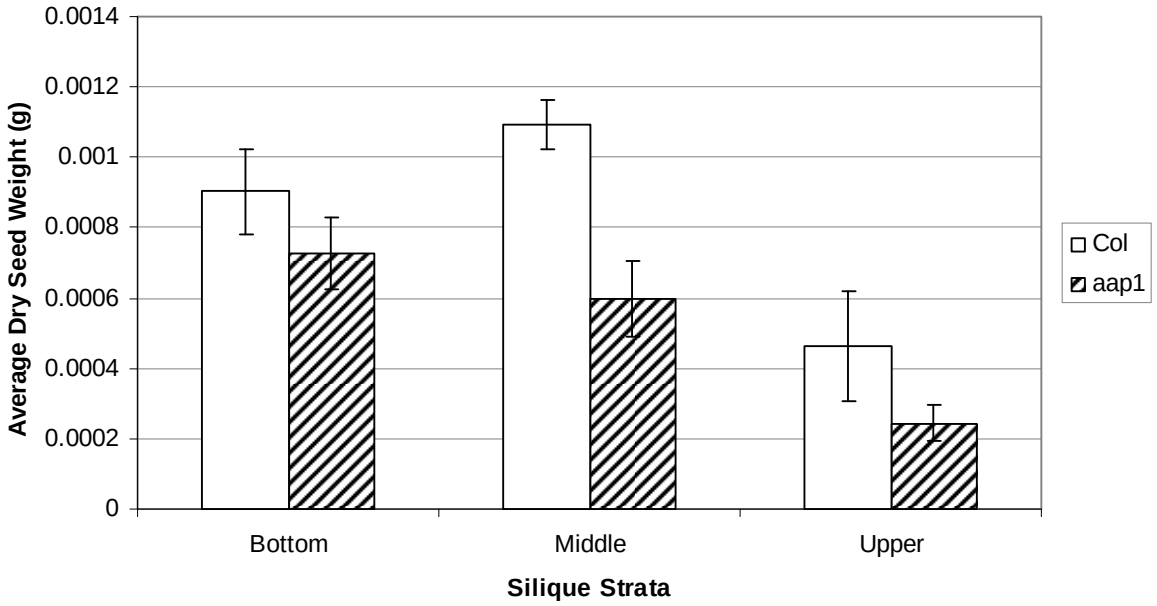

Figure 2.5 – Graph showing the mean dry weights of total seed matter extracted from individual siliques sampled from the varying strata of plant development in *aap1* (n=18) and control plants (n=16). Sample size numbers, n=18 and n=16, refer to the number of siliques sampled from *aap1* and control plants. Error bars represent ± s.e. Data was analysed using a General Linear Model (GLM) ANOVA. The lower, middle and upper silique strata within the plant were identified and samples taken from these areas in order to allow for any developmental effects on silique seed weight to be accounted for.

2.5.2.2 Whole silique analysis of *aap2*

The mean dry mass of silique seeds sampled from individual siliques was found to be significantly less ($p < 0.000$) in At*aap2* plants compared to control plants (see figure 2.6). Statistical testing using a General Linear Model (GLM) ANOVA indicated a significant effect of genotype on whole silique seed mass.

Performing one-way ANOVAs for each strata position also revealed a significant difference in the mean dry mass of seeds of *aap2* and Col0 sampled from the middle and upper strata ($p < 0.003$ and $p < 0.013$ respectively).

When a comparison was made between the two mutant genotypes the dry mass of seeds from individual siliques of *aap2* plants did not differ significantly from the dry mass of seeds sampled from *aap1* mutants ($p = 0.102$). There was however a significant effect of position ($p < 0.005$).

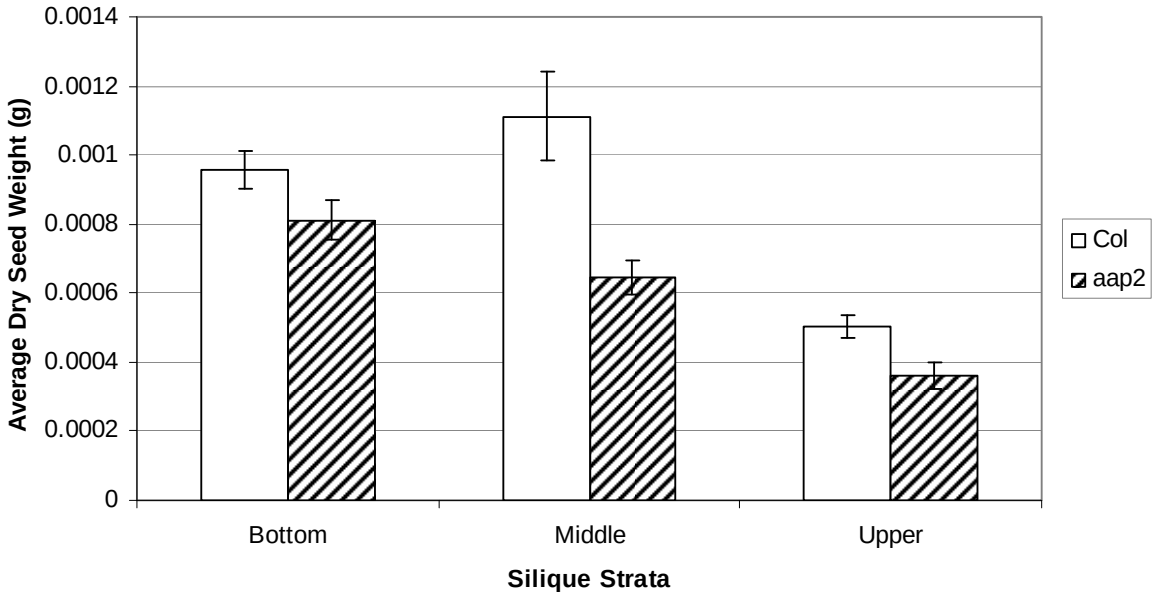

Figure 2.6 – Graph showing the mean dry weight of total seed matter extracted from individual siliques sampled from the varying strata of plant development in *aap2* (n=23) and control plants (n=22). Sample size numbers, n=23 and n=22, refer to the number of siliques sampled from *aap2* and control plants. Error Bars represent ± s.e. Data was analysed using a General Linear Model (GLM) ANOVA. The lower, middle and upper silique strata within the plant were identified and samples taken from these areas in order to allow for any developmental effects on silique seed weight to be accounted for.

2.5.3 Seed Analysis

2.5.3.1 Seed analysis of *aap1* plants

The mean individual seed weights were determined for *aap1* (n=33) and their wildtype control plants (n=28). The individual seed weights for *aap1* plants were observed to be consistently lower than wildtype seed weights at two of the three strata positions however when analysed with a General Linear Model (GLM) ANOVA the mean individual seed weights of the *aap1* plants were not found to be significantly lower ($p = 0.066$). Refer to figure 2.7. An effect of position was observed to be significant ($p < 0.000$).

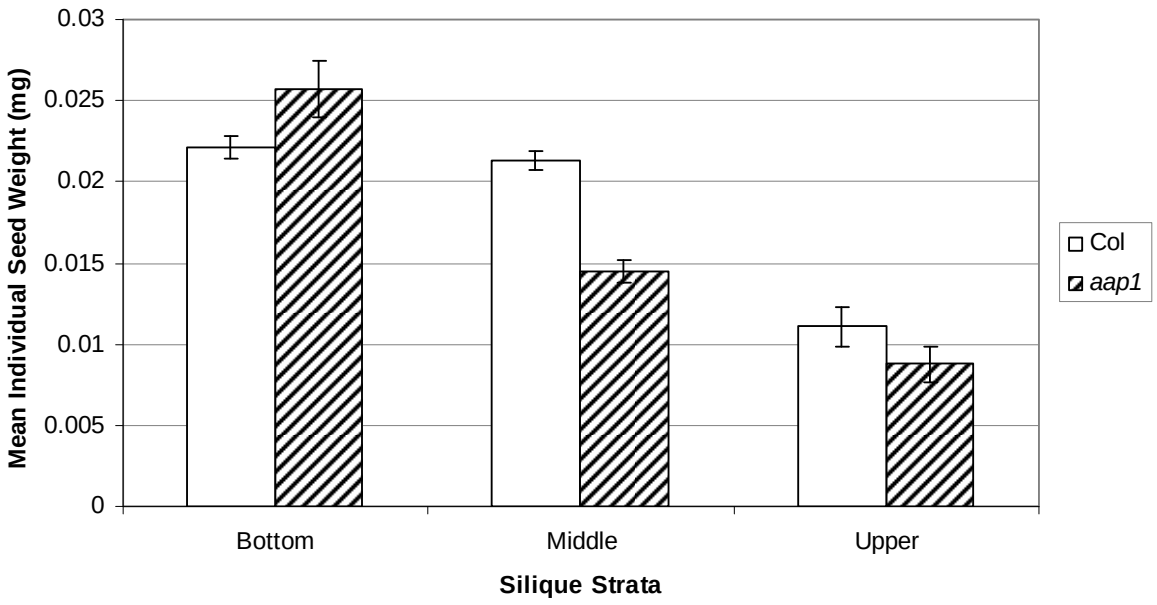

Figure 2.7 – Graph showing the mean dry weight of individual seeds from siliques sampled from the varying strata of plant development in *aap1* (n=33) and control plants (n=28). Sample size numbers refer to number of siliques sampled. Error bars represent ± s.e. Data was analysed using a GLM ANOVA. The lower, middle and upper silique strata within the plant were identified and samples taken from these areas in order to allow for any developmental effects on silique seed weight to be accounted for.

2.5.3.2 Seed analysis of *aap2* plants

The mean individual seed weights were determined for *aap2* (n=26) and their wildtype control plants (n=30). At each of the plant strata positions (bottom, middle and upper strata) the individual seed weights for *aap2* plants were observed to be lower than wildtype seed weights. Analysis with a General Linear Model (GLM) ANOVA confirmed the mean individual seed weights of *aap2* plants were indeed significantly lower than those of wildtype plants ($p = 0.001$). Refer to figure 2.8. An effect of position was also observed to be significant ($p < 0.000$).

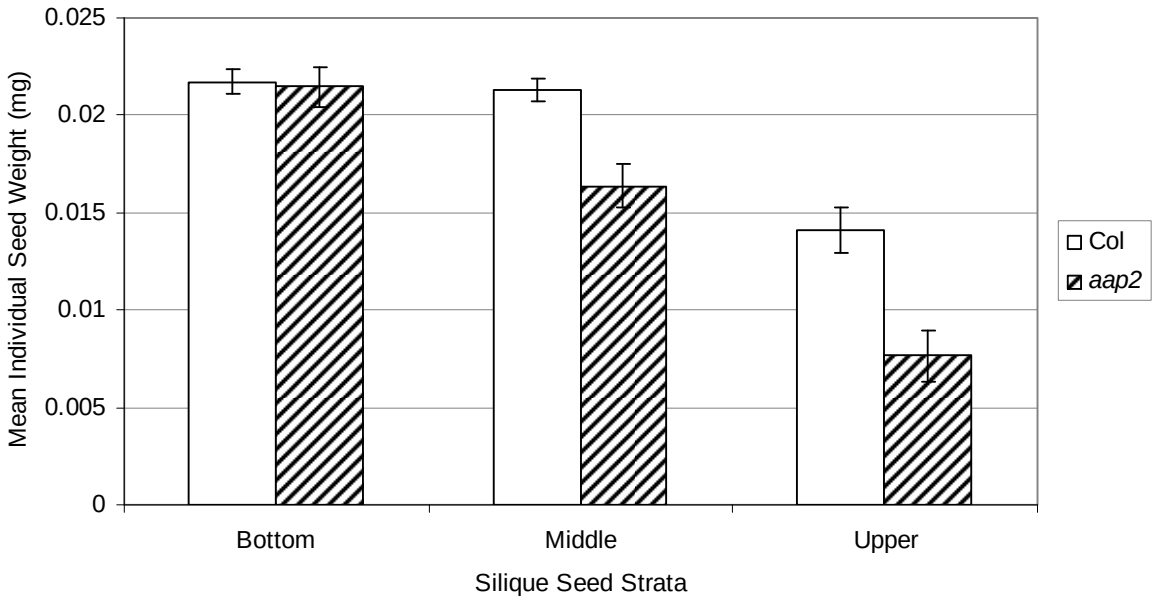

Figure 2.8 – Graph showing the mean dry weight of individual seeds from siliques sampled from the varying strata of plant development in *aap2* (n=26) and control plants (n=30). Sample size numbers refer to the number of siliques sampled. Error bars represent ± s.e. Data was analysed using a GLM ANOVA. The lower, middle and upper silique strata within the plant were identified and samples taken from these areas in order to allow for any developmental effects on silique seed weight to be accounted for.

2.5.4 Chlorophyll Analysis

2.5.4.1 Chlorophyll concentration analysis of *aap1* plants

Chlorophyll concentrations for chlorophyll a have been acquired for *aap1* (n=10) and their wildtype control plants (n=20). Data was analysed using a two-sample t-test. The chlorophyll a concentrations of *aap1* and wildtype plants were found not to be significantly different (p = 0.840). Refer to figure 2.9.

Chlorophyll concentrations for chlorophyll b have been acquired for *aap1 (n=10)* and their wildtype control plants (n=20). Data was analysed using a two-sample t-test, the chlorophyll b concentrations of *aap1* and wildtype plants were found to be significantly different (p = 0.023). Refer to figure 2.9.

Chlorophyll concentrations for chlorophyll a+b have been acquired for *aap1* (n=10) and their wildtype control plants (n=20). Data was analysed using a two-sample t-test. The chlorophyll a+b concentrations of *aap1* and wildtype plants were found not to be significantly different (p = 0.435). Refer to figure 2.9.

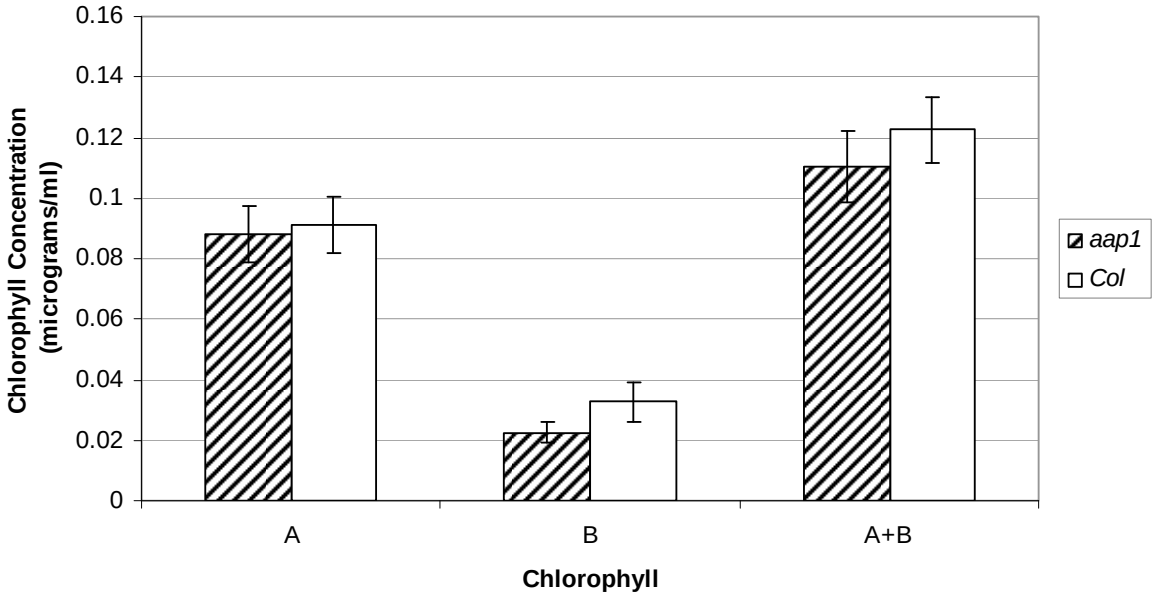

Figure 2.9 – Graph showing the mean chlorophyll concentration of chlorophyll a, b and a+b in *aap1 mutants* (n=10) compared to wildtype plants (n=20). Error bars represent the ± s.e. Data was analysed using a two sample t-test.

2.5.4.2 Chlorophyll concentration analysis of *aap2* plants

Chlorophyll concentrations for chlorophyll a have been acquired for *aap2* (n=10) and their wildtype control plants (n=20). Data was analysed using a two-sample t-test. The chlorophyll a concentrations of *aap2* and wildtype plants were found not to be significantly different (p = 0.508). Refer to figure 2.10.

Chlorophyll concentrations for chlorophyll b have been acquired for *aap2 (n=10)* and their wildtype control plants (n=20). Data was analysed using a two-sample t-test. The chlorophyll b concentrations of *aap2* and wildtype plants were found to be significantly different (p = 0.014). Refer to figure 2.10.

Chlorophyll concentrations for chlorophyll a+b have been acquired for *aap2* (n=10) and their wildtype control plants (n=20). Data was analysed using a two-sample t-test. The chlorophyll a+b concentrations of *aap2* and wildtype plants were found not to be significantly different (p = 0.074). Refer to figure 2.10.

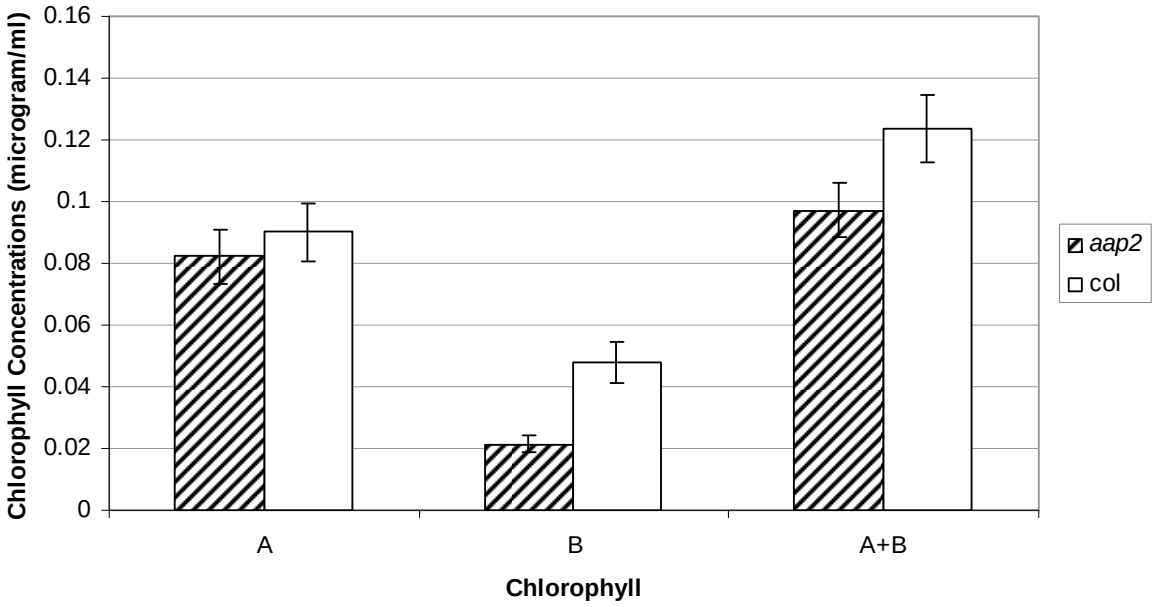

Figure 2.10 – Graph showing the mean chlorophyll concentration of chlorophyll a, b and a+b in *aap2* mutants (n=10) compared to wildtype plants (n=20). Error bars represent the ± s.e. Data was analysed using a two sample t-test.

2.5.5 Plant Growth Under Varying Nitrogen Conditions

2.5.5.1 Plant growth of *aap1, aap2* and wildtype plants under nitrogen supplemented and non-supplemented watering regimes

In order to assess the affects of supplementary nitrogen in *aap1* and *aap2* plants, plants of both genotype were grown under standard nutrient regimes and also supplemented nutrient regimes. Plants grown to day 50 on a standard watering regime and then measured according to methods in section 2.4.2 were observed to exhibit a significant effect of genotype. The *aap1* plants showed significantly reduced numbers of siliques ($p < 0.000$), reduced rosette leaf numbers ($p < 0.000$) and reduced plant height ($p = 0.013$) when compared to control wildtype plants (see table 2.4). Other parameters measured were not significantly affected in *aap1* plants.

At day 50 several characteristics were also significantly altered in *aap2* plants compared to wildtype plants. A significant reduction in rosette leaf number ($p < 0.000$), rosette leaf width ($p = 0.003$), plant height ($p < 0.000$) and silique number ($p < 0.000$) was observed in *aap2* plants when compared to wildtype, control plants. Statistical analysis using a General Linear Model (GLM) ANOVA indicated the *aap1* plants that were watered with a nitrogen supplemented solution did not show significantly altered growth in any of the plant characteristics measured when compared to plants of the same genotype that were watered with a standard water regime ($p > 0.05$)(see table 2.11). Similarly the *aap2* plants that were watered with a nitrogen supplemented solution did not show significantly altered growth in any of the plant characteristics measured when compared to plants of

the same genotype that were watered with a standard water regime ($p > 0.05$) (see table 2.11). Wildtype plants that were watered with a nitrogen supplemented solution did show significantly increased growth in some of the characteristics measured when compared to wildtype plants on the standard watering regime. Rosette leaf number ($p < 0.000$) and rosette leaf width ($p < 0.000$) were significantly increased in wildtype plants supplemented with nitrogen (see table 2.11). Above root plant mass ($p = 0.105$), plant height ($p = 0.058$) and silique number ($p = 0.818$) were not significantly affected by the nitrogen supplementation. Interestingly, when nitrogen supplemented *aap1* and *aap2* plants were compared with nitrogen supplemented wildtype (Col0) plants statistically significant differences were observed for all characteristics measured. The data analysed using one-way ANOVA tests showed plant height (*aap1* $p < 0.000$, *aap2* $p < 0.000$), rosette leaf number (*aap1* $p < 0.000$, *aap2* $p < 0.000$), rosette leaf width (*aap1* $p < 0.000$,*aap2* $p < 0.000$), above soil plant mass (*aap1* $p = 0.021$, *aap2* $p = 0.005$) and total number of siliques (*aap1* $p < 0.000$ and *aap2* $p < 0.000$) were all significantly lower in nitrogen supplemented *aap1* and *aap2* plants when compared to nitrogen supplemented wildtype plants.

Table 2.11 illustrates the mean values for plant characteristics measured at day 50 after sowing for *aap1, aap2* and wildtype plants under standard and nitrogen supplemented watering regimes.

Plant Characteristics (at day 50 after sowing)	*aap1* **Water regime**	*aap1* **Nitrogen Regime**	*aap2* **Water Regime**	*aap2* **Nitrogen Regime**	**Col Water Regime**	**Col Nitrogen Regime**
Plant Height (cm)	**34.2 (±0.804) n=39**	**34.0 ** (±0.835) p < 0.000 n=39**	**32.2 (±0.828) n=40**	**31.1 ** (±0.776) p < 0.000 n=40**	**37.2 (±0.900) n=35**	**39.8 (±0.986) p > 0.05 n=35**
Rosette Leaf Number	**14.3 (±0.381) n=39**	**14.0 ** (±0.361) p < 0.000 n=39**	**13.7 (±0.245) n=40**	**13.5 ** (±0.343) p < 0.000 n=40**	**17.6 (±0.494) n=35**	**21.0* (±0.632) p < 0.000 n=35**
Rosette Leaf Width (cm)	**8.5 (±0.365) n=39**	**8.37 ** (±0.359) p < 0.000 n=39**	**7.76 (±0.308) n=40**	**7.65 ** (±0.329) p < 0.000 n=40**	**9.2 (±0.264) n=35**	**11.78* (±0.335) p < 0.000 n=35**
Above Soil Plant Mass (g)	**0.49 (±0.040) n=39**	**0.51** (±0.041) p = 0.021 n=39**	**0.49 (±0.033) n=40**	**0.50 ** (±0.034) p = 0.005 n=40**	**0.55 (±0.038) n=35**	**0.63 (±0.029) p > 0.05 n=35**
Total Number of Siliques	**72.8 (±5.77) n=34**	**72.9 ** (±5.71) p > 0.05 n=34**	**71.5 (±5.30) n=34**	**72.2 ** (±5.34) p > 0.05 n=34**	**102.5 (±4.87) n=34**	**104.0 (±4.65) p > 0.05 n=34**

Table 2.11 – Table showing the mean plant parameters measured in *aap1* (n=39), *aap2* (n=40) and control plants (n=35) following standard and nitrogen supplemented watering regimes. Values in brackets refer to ± s.e. Data analysed using General Linear Model (GLM) ANOVA and one-way ANOVA. * denotes a statistically significant result when comparing standard and nitrogen supplemented treatments of the same genotype. ** denotes a statistically significant result when comparing nitrogen supplemented plants of different genotypes.

2.5.6 Plant Growth through Aphid Infestation

2.5.6.1 Plant growth of *aap1, aap2* and wildtype plants through aphid infestation with *M. persicae*

Aphid infestations are known to be a stress factor capable of inducing varying plant defences (Couldridge, 2007). This aim of this experiment was to observe how stress induced by aphid infestation affected plant growth in *aap1* and *aap2* mutants with potentially disrupted amino acid transport. Data in section 2.5.1 indicated plants grown to day 50 free of aphid infestations and then measured according to methods in section 2.4.2 exhibited a significant effect of plant genotype. The *aap1* plants showed significantly reduced numbers of siliques ($p < 0.000$), reduced rosette leaf numbers ($p < 0.000$) and reduced plant height ($p = 0.013$) when compared to control wildtype plants (table 2.4). Other parameters measured were not significantly affected in *aap1* plants.

At day 50 several characteristics were also significantly altered in *aap2* plants compared to wildtype plants. A significant reduction in rosette leaf number ($p < 0.000$), rosette leaf width ($p = 0.003$), plant height ($p < 0.000$) and silique number ($p < 0.000$) was observed in *aap2* plants when compared to wildtype, control plants. Statistical analysis using a General Linear Model (GLM) ANOVA indicated that the *aap1*, *aap2* and wildtype plants that were infested with *M. persicae* did not show any significantly adverse aphid induced effects (for example, excessive leaf tissue damage or growth stunting) on the plant characteristics measured when compared to the un-infested *app1*, *aap2* and wildtype plants of similar age (see

table 2.12). All plant characteristics measured in *aap1*, *aap2* and control plants were unaffected by the presence of the aphid infestations ($p > 0.05$).

Plant Characteristics (mean values at day 50 after sowing)	*aap1* **Without aphid infestation**	*aap1* **With aphid infestation**	*aap2* **Without aphid infestation**	*aap2* **With aphid infestation**	**Col) Without aphid infestation**	**Col0 With aphid infestation**
Plant Height (cm)	**34.2 (±0.804) n=39**	**33.9 (±0.799) n=40 p > 0.05**	**32.2 (±0.828) n=40**	**33.1 (±0.756) n=40 p > 0.05**	**37.2 (±0.90) n=35**	**38.4 (±0.687) n=40 p > 0.05**
Rosette Leaf Number	**14.3 (±0.381) n=39**	**15.1 (±0.340) n=40 p > 0.05**	**13.7 (±0.245) n=40**	**14.2 (±0.299) n=40 p > 0.05**	**17.6 (±0.494) n=35**	**18.3 (±0.353) n=40 p > 0.05**
Rosette Leaf Width (cm)	**8.5 (±0.365) n=39**	**8.9 (±0.253) n=40 p > 0.05**	**7.76 (±0.308) n=40**	**7.4 (±0.301) n=40 p > 0.05**	**9.2 (±0.264) n=35**	**8.9 (±0.275) n=40 p > 0.05**
Above Soil Plant Mass (g)	**0.49 (±0.040) n=39**	**0.46 (±0.034) n=40 p > 0.05**	**0.49 (±0.033) n=40**	**0.48 (±0.031) n=40 p > 0.05**	**0.55 (±0.038) n=35**	**0.53 (±0.032) n=40 p > 0.05**
Total Number of Siliques	**72.8 (±5.77) n=34**	**73.1 (±5.52) n=35 p > 0.05**	**71.5 (±5.30) n=34**	**71.9 (±4.39) n=35 p > 0.05**	**102.5 (±4.87) n=34**	**100.2 (±4.98) n=35 p > 0.05**

Table 2.12 – Table showing the mean plant parameters measured in *aap1* (n=39), *aap2* (n=40) and control plants (n=35) following aphid infestation of *M. persicae*. Values in brackets refer to ± s.e. Data analysed with a General Linear Model (GLM) ANOVA indicated no statistically significant differences ($p > 0.05$)

2.5.6.2 Plant growth of *aap1, aap2* and wildtype plants through aphid infestation with *B. brassicae*

As mentioned previously in section 2.4.6.2 the *aap1* plants grown to day 50 free of aphid infestations and then measured according to methods in section 2.3.2 indicated there was a significant effect of genotype for several of the plant characteristics measured (see table 2.4). Other parameters measured were not significantly affected in *aap1* plants. Similarly, several characteristics were also significantly altered in *aap2* plants compared to wildtype plants (see table 2.4).

When analysed with a General Linear Model (GLM) ANOVA the data from *aap1*, *aap2* and wildtype control plants that were purposefully infected with infestations of *B. brassicae* did not show any aphid induced significant effects on the plant characteristics measured when compared to the un-infested *app1*, *aap2* and wildtype plants of similar age. All plant characteristics measured in *aap1*, *aap2* and control plants were found to be significantly unaffected by the presence of the aphid infestations ($p > 0.05$). See table 2.13.

Plant Characteristics (mean values at day 50 after sowing)	*aap1* **Without aphid infestation**	*aap1* **With aphid infestation**	*aap2* **Without aphid infestation**	*aap2* **With aphid infestation**	**Col0 Without aphid infestation**	**Col0 With aphid infestation**
Plant Height (cm)	**34.2 (±0.804) n=39**	**35.1 (±0.768) n=40 p > 0.05**	**32.2 (±0.828) n=40**	**32.9 (±0.741) n=40 p > 0.05**	**37.2 (±0.900) n=35**	**36.9 (±0.833) n=40 p > 0.05**
Rosette Leaf Number	**14.3 (±0.381) n=39**	**13.8 (±0.298) n=40 p > 0.05**	**13.7 (±0.245) n=40**	**13.9 (±0.308) n=40 p > 0.05**	**17.6 (±0.494) n=35**	**18.0 (±0.364) n=40 p > 0.05**
Rosette Leaf Width (cm)	**8.5 (±0.365) n=39**	**8.2 (±0.193) n=40 p > 0.05**	**7.76 (±0.308) n=40**	**7.1 (±0.265) n=40 p > 0.05**	**9.2 (±0.264) n=35**	**9.5 (±0.266) n=40 p > 0.05**
Above Soil Plant Mass (g)	**0.49 (±0.040) n=39**	**0.45 (±0.030) n=40 p > 0.05**	**0.49 (±0.033) n=40**	**0.51 (±0.026) n=40 p > 0.05**	**0.55 (±0.038) n=35**	**0.57 (±0.028) n=40 p > 0.05**
Total Number of Siliques	**72.8 (±5.77) n=34**	**71.5 (±4.92) n=35 p > 0.05**	**71.5 (±5.30) n=34**	**72.4 (±5.18) n=35 p > 0.05**	**102.5 (±4.87) n=34**	**104.4 (±6.02) n=35 p > 0.05**

Table 2.13 – Table showing the mean plant parameters measured in *aap1* (n=39), *aap2* (n=40) and control plants (n=35) following aphid infestation of *B. brassicae*. Values in brackets refer to ± s.e. Data analysed with a General Linear Model (GLM) ANOVA indicated no statistically significant differences ($p > 0.05$)

2.6 Discussion

Experiments were carried out to determine whether the loss of function mutations in AAP1 and AAP2 amino acid transporters affected the growth, morphology, and/or reproductive fitness of the plants. The results of these experiments are reviewed in this discussion.

2.6.1 Phenotype Characterisation

Results of a phenotypic screening prior to day 50 indicated no observed significant differences between *aap1* plants and the control plants in all of the growth scoring characteristics measured ($p > 0.05$) (see section 2.4.2.1). Similarly, prior to day 50 there were no observed differences (for all characteristics measured) between *aap2* plants and their control wildtype comparison plants ($p > 0.05$).

At day 50 the plants measured exhibited several characteristics that were significantly altered in *aap1* plants compared to control plants. These results included a significant reduction in rosette leaf number ($p < 0.000$), a reduction in plant height ($p= 0.013$) and a lowered silique number ($p < 0.000$) observed in *aap1* plants compared to control plants. All other parameters measured including rosette leaf width and above root plant mass were not significantly affected by the disruption to AAP1.

At day 50 several characteristics were also significantly altered in *aap2* plants compared to wildtype plants. A significant reduction in rosette leaf number

($p < 0.000$), rosette leaf width ($p = 0.003$), plant height ($p < 0.000$) and silique number ($p < 0.000$) was observed in *aap2* plants when compared to wildtype, control plants. Above root plant mass was not significantly affected by the disruption to AAP2.

The disruption to the AAP1 and AAP2 transporters and thus the disruption of the normal function of amino acid transportation may have been sufficient to cause a difference in the growth pattern of the *aap1 and aap2* plants. Interestingly, AAP1 has been previously identified to be a plasma membrane transporter functioning within the root epidermal cells (including throughout the root tip hair cells of the main and lateral roots) in order to uptake amino acids and translocate them to the actively growing shoots. AAP1 inhibition work in potato plants demonstrated a reduction in the levels of free amino acids within the potato tubers (Koch et al., 2003) whereas over expression experiments performed in leguminous plants showed increases in the storage proteins within seeds. Work involving the successful growth of At*aap1* plants on toxic levels of amino acids indicates these *aap1* mutants have a reduced ability to uptake a broad spectrum of amino acids (Lee et al., 2003). The localisation of AAP1 throughout the root tissue suggests that AAP1 facilitates amino acid uptake into the root cells and along the amino acid transport pathway. This amino acid transport directs the nitrogen into the vasculature for the long distance transport to the shoots (Lee et al., 2007). Upon considering this evidence the results suggest that the disruption to AAP1 may have been sufficient to reduce this initial uptake of amino acids into the roots of the mutant plants, impairing the transport of nitrogen into the vasculature and then onwards to the developing regions, thus accounting for the reduction in rosette

leaf number and reduced plant height observed in *aap1* plants. The observed delay in response (after day 50) may be attributed to the changing resource allocation in aging plants.

Similarly AAP2 has previously been shown to be expressed within the vascular strands of the funiculi within siliques (Hirner et al., 1998) and is it is also thought that AAP2 is believed to be responsible for loading amino acids into the phloem, supplying the roots tips with nitrogen needed for growth (Hirner et al., 1998; Okumoto et al., 2004). The role of AAP2 in phloem loading is clearly important for the normal development and growth of the plant, potentially explaining why *aap2* mutants in this study are observed to show reduced rosette leaf numbers, reduction in rosette leaf width, and a reduction in plant height.

2.6.2 Silique Analysis – Whole Silique and Seed Analysis

As previously mentioned, *aap1* and *aap2* plants exhibited a significantly altered phenotype for several characteristics after day 50. Further analysis of the mutant genotypes indicated plant fitness in terms of the number of siliques produced was significantly decreased in both *aap1* and *aap2* plants ($p < 0.005$) compared to control plants.

The mean dry mass of seeds sampled from individual siliques was found to be significantly less in *aap1* ($p = 0.001$) and *aap2* ($p < 0.000$) plants compared to control plants. When a comparison was made between the two mutant genotypes

the dry mass of seeds from individual siliques of *aap2* plants did not differ significantly from the dry mass of seeds sampled from *aap1* mutants ($p = 0.102$).

After further analysis, the mean individual seed weights of the *aap1* plants were not found to be significantly lower ($p = 0.066$). The mean individual seed weights of the *aap2* plants were indeed significantly lower than those of wildtype plants ($p < 0.001$).

These results are perhaps best understood when considering the role of amino acid transporters in the nitrogen loading from source regions of the plant into the seed sink regions of the plant. Seed development and the accumulation of stored nitrogen are heavily dependant on the success of nitrogen import into the seed embryo. This nitrogen is assimilated within the leaves or roots of the plants and translocated to the developing seed in the form of amino acids. AAP1 has previously been identified to be a plasma membrane transporter functioning to mediate this amino acid import into developing seeds. AAP2 has also been shown to be expressed within the vascular strands of the funiculi within siliques (Hirner et al., 1998) and has been demonstrated to be important in the process of loading amino acids into the phloem. Previous RNA work with AAP1 and AAP2 have shown that both genes appear to be co-regulated developmentally during the process of silique maturation (Fischer et al., 1998; Fischer et al., 2002) This may explain why both *aap1* and *aap2* have exhibited significantly reduced silique numbers, with both *aap1 and aap2* exhibiting reduced total seed mass and *aap2* showing significantly reduced seed weight. These changes all suggest that both AAP1 and AAP2 are important in affecting seed development (Sanders et al., 2009). If

nitrogen loading and translocation around the plant (ultimately to the seed sink region) is affected by the lack of AAP1 or AAP2 proteins, plant fitness in the way of general plant growth, silique number and the number and size of viable seeds produced has shown to be significantly reduced. The lowered mean silique numbers observed in *aap1* plants could be attributed as before to the disruption to amino acid uptake into the roots of the mutant plants, the disruption of the *aap1* plant's ability to uptake amino acids impairs the plants ability to acquire the N needed for normal growth and development. AAP1 role in mediating amino acid loading into the seed may also have an effect. The sink number, in this case the developing siliques, of a plant can be affected greatly by the nitrogen supply from the source regions of the plant (Cakmak and Engels, 1999). The fact that *AAP1* and *AAP2* are co-regulated during the period of silique maturation also explains the lack of phenotype prior to the plant's reproductive maturity, clearly the disruption to the co-regulation system in both *aap1* and *aap2* plants may have disrupted the process of silique development and seed filling.

Disruption to nitrogen transport processes within the *aap1* and *aap2* plants and the potential changes to nitrogen levels within seeds may also potentially create a seed 'sink feedback' whereupon the source (leaf) metabolism or transport is regulated accordingly. The observed reduction in silique number is also observed in *aap2* plants, suggesting their role in root phloem loading is clearly important for the normal development and growth of the plant, however *aap2* plants also show a reduction in the mean individual seeds weights when compared to control plants. This significant reduction to mean individual seed weights observed in *aap2* plants could be explained by the fact that AAP2 is expressed in the vascular strands of

the funiculi within the siliques. This suggests the *aap2* loss of function mutants exhibit reduced seed size due to the disruption to amino acid loading into the vascular strands of the funiculi within the siliques, reducing the level of resources allocated to the seed.

It may also be possible that the absence of AAP2 affected individual seed weight due to other alterations in plant growth. The fact that the *aap2* plants also exhibited reduced rosette leaf number and rosette leaf width may create a secondary affect within the plant. The reduced rosette leaves of the *aap2* leaves may have limited the level of stored resources and the level of available light captured for photosynthesis. This limited level of photosynthesis could potentially lower the level of resources available for allocation to the seed, accounting for the lowered seed weight. Evidence in the literature has shown that the rosette leaves of *A. thaliana* play an important role in the continued assimilation of resources during seed maturation. This was supported by the low seed yield in plants that had their rosette leaves removed or covered (Schulze et al., 1994). This may also be true for *aap1* plants.

2.6.3 Leaf Chlorophyll Analysis

Concentrations of chlorophyll measured in *aap1* were determined not to be altered for chlorophyll a ($p = 0.840$) and chlorophyll a+b ($p = 0.453$) in comparison to wildtype plants. The concentration of chlorophyll b was however significantly reduced in *aap1* plants in comparison to wildtype plants ($p = 0.023$). Similarly the concentrations of chlorophyll measured in *aap2* were also determined not to be

altered for chlorophyll a ($p=0.508$) and chlorophyll a+b ($p = 0.074$) when compared to wildtype plants. The concentration of chlorophyll b was however significantly reduced in *aap2* when compared to wildtype plants ($p = 0.014$).

This data suggests that the disruption to the AAP1 and AAP2 transporters did not have a significant effect on the chlorophyll concentrations of chlorophyll a and a+b, however a significant effect on the concentration of chlorophyll b was observed. Combined with the reduced rosette leaf width observed in *aap1* and *aap2* plants this data suggests that the level of photosynthesis occurring within these leaves may be lower than that of the wildtype plants. With the reduced leaf surface area of the mutant plants physically limiting the amount of available light, it might be expected that the levels of photosynthetic pigments would be increased in order to compensate for the physical reduction in leaf size. A form of compensation in terms of the chlorophyll concentrations does not appear to have happened in *aap1* or *aap2* plants. There were no significant changes in the concentration of chlorophyll a and a+b ($p > 0.05$), and for chlorophyll b a significant decrease in concentration was observed ($p < 0.05$). These results suggest that the level of photosynthesis occurring in the mutant plants may be reduced, further supporting the previously discussed plant growth and seed size data.

2.6.4 Analysis of Plant Growth with Varying Nitrogen Conditions

When assessing plant characteristics under nitrogen supplementation the *aap1* plants that were watered with a nitrogen supplemented solution did not show

significantly altered growth in any of the plant characteristics measured when compared to plants of the same genotype that were watered with a standard nutrient regime (water) ($p > 0.05$). Similarly the *aap2* plants that were watered with a nitrogen supplemented solution did not show significantly altered growth in any of the plant characteristics measured when compared to plants of the same genotype that were watered with a standard nutrient regime (water) ($p > 0.05$). Wildtype plants that were watered with a nitrogen supplemented solution showed significantly increased growth in some of the characteristics measured when compared to wildtype plants on the standard watering regime. Rosette leaf number ($p < 0.000$) and rosette leaf width ($p < 0.000$) were significantly increased in wildtype plants supplemented with nitrogen. Above root plant mass ($p = 0.105$), plant height ($p = 0.058$) and silique number ($p = 0.818$) were not significantly affected by the nitrogen supplementation. Interestingly, when nitrogen supplemented *aap1* and *aap2* plants were compared with nitrogen supplemented wildtype (Col0) plants all characteristics measured were observed to be significantly lower in the nitrogen supplemented *aap1* and *aap2* plants compared to the nitrogen supplemented wildtype plants ($p < 0.05$).

Wildtype plants watered with a nitrogen supplemented solution exhibited increased plant growth in terms of rosette leaf number and rosette leaf width. This increase in plant growth can be explained by the increase in available nitrogen within the soil which was available for absorption and allocation to actively growing regions of the plant. In comparison the *aap1* and *aap2* plants did not exhibit any increased growth when watered with a nitrogen supplemented solution. Due to the nature of the nitrogen application this result is explained by

the role of AAP1 and AAP2 in the uptake of amino acids within the roots. AAP1's role as a plasma membrane transporter within root epidermal cells facilitate amino acid uptake from the soil,

AAP1 has been previously identified to be a plasma membrane transporter functioning within the root epidermal cells (including throughout the root tip hair cells of the main and lateral roots) in order to uptake amino acids and translocate them to the actively growing shoots (Hirner et al., 1998; Okumoto et al., 2004). AAP2 also plays an important role in the loading of amino acids into the root phloem, supplying actively growing regions of the plant. The *aap1* and *aap2* plants lacking these transporters will therefore have a reduced ability to uptake a broad spectrum of amino acids, negating the effects of the nitrogen supplementation. Previous studies involving the successful growth of At*aap1* plants on toxic levels of amino acids indicate that *aap1* mutants have a reduced ability to uptake a broad spectrum of amino acids (Lee et al., 2003), supporting observations taken from the nitrogen supplementation analysis.

2.6.5 Analysis of Plant Performance Under Aphid Infestation

The effects of external stress, in the form of aphid infestations, were examined in order to determine whether *aap1* and *aap2* would be able to maintain similar levels of growth and fitness without their functioning amino acid transporters. When assessing plant characteristics following aphid infestation the *aap1*, *aap2* and wildtype control plants that were purposefully infected with aphid infestations did

not show any aphid induced significant effects on the plant characteristics measured ($p > 0.05$).

The result meant that the infested *aap1* and *aap2* plants did not suffer significantly more than the wildtype plants also being similarly infested with aphids. This suggests that although the *aap1* and *aap2* plants exhibit altered growth in terms of reduced rosette leaf width and number, reduced plant height and reduced silique numbers, they are still equally equipped to respond to the added stress of an aphid infestation. The majority of plants are able to adapt their physiology in order to cope with various environmental stresses. These adaptations to plant physiology are usually achieved without the need for significant morphological change (Bouché and Bouchez, 2001). Plants in natural, undomesticated situations are subject to a large number of stresses, one of which may be infestation by insects. For an organism which is physically unable to move away from a source of stress, it is crucial for the overall fitness of the organism to be dynamic in their response to external stress, being well equipped and able to adjust physiological processes when necessary (Kuelheim et al., 2002).

2.7 Conclusions

No differences in plant phenotype were found prior to day fifty in either *aap1* or *aap2* plants, however after this point the absence of the AAP1 and AAP2 transporters did cause several significant phenotypic changes, including reductions in rosette leaf growth and the silique and seed yield of the plant.

Until recently previous studies have shown very few major phenotypic changes associated with a mutation in an amino acid transporter (Koch et al., 2003, Okumoto et al., 2004). Due to the numerous amino acid transporter families present within plants (Fischer et al., 1995; Frommer et al., 1993; Hsu et al., 1993; Rentsch et al., 1996) the lack of significant phenotypic changes might be expected. A multitude of these transporters all functioning within a plant to regulate phloem amino acid content (Lam et al., 1995) may compensate for the loss of function of one particular transporter. This functional redundancy explanation may be why plants exhibit no major phentoypic changes under certain circumstances. This may also help to explain why phenotypic changes were observed in *aap1* and *aap2* plants over 50 days old, but not in those younger than 50 days old. As plants age and reach their reproductive maturity, more resources throughout the plant are allocated to seed filling. As previously reviewed, RNA work with *AAP1* and *AAP2* have shown that both genes appear to be co-regulated developmentally during the process of silique maturation (Fischer et al., 1998; Fischer et al., 2002), disruption to this co-regulation in *aap1* and *aap2* plants may explain the seed phenotypes described previously. Recent work is consistent with the findings of this study, supporting the suggestion that AAP1 and AAP2 are important in the process of embryo amino acid loading (Sanders et al., 2009).

In this investigation experiments were not conducted to determine the total carbon, nitrogen and amino acid content so it is not known if the composition of the *aap1* and *aap2* seeds differed from wildtype seeds. If these measurements were made it would allow for a more accurate assessment of the level at which

seed filling had been affected in *aap1* and *aap2* plants. Although it is generally difficult to assess the level of a plant's fitness, the seed composition, in terms of seed nutrient status, would be a helpful determining factor. It is possible that the seed composition does vary between plants types and so it would be advisable to proceed with this method in a further investigation. Findings from this study at present suggest that the fitness of *aap1* and *aap2* was affected by the mutations in *AAP1* and *AAP2*.

Chapter 3

How is the Feeding Behaviour and Metabolism of Two Aphid Species Affected by AAP1 and AAP2 Transporter Mutations?

3.1 Abstract

Experiments were carried out to study the relationship between changes to host plant amino acid transport and the consequential effect on aphid feeding behaviour and metabolic function in two different aphid species.

The aphid probing and feeding behaviour of *M. persicae* and *B. brassicae* on *app1* and *aap2* mutant plants compared to wildtype plants was assessed. Aphid probing and feeding behaviour, determined by EPG analysis, was found to be significantly different between mutant and wildtype plants. The time taken for both *M. persicae and B. brassicae* to reach sustained ingestion (E2) was significantly greater for aphids feeding on *aap1* plants compared to those feeding on wildtype plants.

During the first and second hours of the experiments the time spent in E2 sustained feeding was significantly greater for aphids feeding on wildtype plants (for *M. persicae* 472 (SE ±128) and 3326 (SE ±154) seconds and for *B. brassicae* 1315 (SE ±93) and 2661 (SE ±149) seconds respectively) compared to aphids feeding on *aap1* plants (for *M. persicae* 175 (SE ±120) and 796 (SE ±293) seconds and for *B.*

brassicae 327 (SE ±112) and 1303 (SE ±189) seconds respectively). Interestingly, the time spent in E2 sustained sieve element feeding during hours one and two was significantly greater for aphids feeding on wildtype plants (for *M. persicae* 510 (SE ±116) and 2987 (SE ±42) seconds and for *B. brassicae* 639 (SE ±91) and 1784 (SE ±69) seconds respectively) compared to aphids feeding on *aap2* plants (for *M. persicae* 235 (SE ±122) and 912 (SE ±154) seconds and for *B. brassicae* 195 (SE ±119) and 689 (SE ±35) seconds respectively). During hour two of the experiments the time spent in pathway was also found to be significantly greater for both species of aphid feeding on *aap1* plants compared to that of aphids feeding on the wildtype plants ($p < 0.05$). This relationship is reciprocal to the result for mean time spent in E2. Other parameters being measured, including time spent in E1 and time spent out (not penetrating) showed no significant differences ($p > 0.05$) between either aphid species feeding on the two plant genotypes. The time taken for both *M. persicae and B. brassicae* to reach sustained ingestion (E2) was significantly greater on *aap1* and *aap2* plants compared to those feeding on control ($p < 0.05$).

The aphid feeding rate of *M. persicae* and *B. brassicae* on *aap1* and *aap2* mutants compared to wildtype plants was assessed. Honeydew droplet frequency was found to be significantly reduced for both aphid species when feeding on *aap1* and *aap2* mutants compared to wildtype control plants ($p < 0.05$). Honeydew droplet volume was not significantly affected in *M. persicae* when feeding on *aap1* and *aap2* mutants when compared to wildtype plants ($p = 0.156$), similarly droplet volume was not significantly affected in *B. brassicae* feeding on *aap2* mutants when compared to wildtype plants ($p = 0.357$). Honeydew droplet volume was however

significantly lower in *B. brassicae* feeding on *aap1* when compared to wildtype plants ($p = 0.026$). Using the honeydew drop frequencies and honeydew drop volumes the aphid feeding rates were determined for both aphid species on *aap1, aap2* and control plants. For both aphid species the feeding rates were significantly lower when feeding on *aap1* and *aap2* plants when compared to the feeding rates on control plants ($p < 0.000$).

3.2 Introduction

3.2.1 Aphid Plant Interactions – Feeding From the Host Plant

Probing of the host plant by aphids follows tarsal contact (Ibbotson and Kennedy, 1959; Powell et al., 2006). Initial penetrations remain within the epidermis lasting for relatively brief periods (Sauge et al., 1998). Upon arrival on a plant and following antennation behaviour, an alate will often probe perhaps only once or twice before flying away (Nault and Stryer, 1972; Powell, 1991; Caillaud and Via, 2000) or moving to another feeding site, for instance the abaxial leaf surface (Wensler, 1962; Calabrese and Edwards, 1976). Although initial probing can determine acceptance or rejection, surprisingly both the stylets and labium are thought to lack any external 'contact chemoreceptors' (Wensler, 1974; Wensler, 1977; Tjallingii, 1978).

Penetration into the stylet 'pathway' within the mesophyll and parenchyma tissues (Caillaud and Via, 2000) usually occurs in longer more sustained stylet penetrations (approximately 30 s to 1 min). Upon stylet penetration the aphid recognises specific cues within the 'pathway' (epidermal, mesophyll, and phloem tissues). This process of chemoreception is believed to be the most important stage in determining whether or not the aphid accepts or rejects the plant (Powell et al., 2006) although as yet not much is known about these cues. Evidence within the literature also suggests that deterrent agents may act within the pathway to deter the aphid from accepting the plant and then feeding from it (Sauge et al., 1998).

Once the aphid has successfully penetrated the plant and moved into pathway phase, the tissue between the epidermis and the sieve element, the aphid will periodically puncture a cell to imbibe and using its epipharyngeal organ effectively taste the cell content, testing for suitability and the presence of any harmful allelochemicals (Schoonhoven et al., 1976). If the plant composition is deemed suitable the aphid will continue probing, eventually feeding and reproducing, however if after the initial puncture the aphid does not deem the plant suitable in terms of nutritional quality, or if harmful allelochemicals are present the plant will be rejected. At the time of penetration various plant defences may be initiated (Walling, 2000; Moran and Thompson, 2001), possibly inducing the production of chemical signals involved in the plant's defence pathways. These chemical signals may act as cues although they are generally considered to be less important in host selection due to the fact that the transcription of the pathway genes would occur over many hours, whereas selection usually occurs over a much more rapid timescale. Again these chemical compounds would be detected sensitively by the aphids gustatory organ present in the epipharynx thus allowing the aphid to avoid any significant intoxication (Mittler and Dadd, 1964; Wensler, 1969; Prado and Tjallingii, 1994).

As discussed in chapter one (see section 1.6.4) the host plant acceptance by a feeding aphid is a complex process. Pathway tissue cues or deterrents, pathway tissue composition and the palatability of the sieve element sap all contribute to the overall decision of acceptance or rejection made by the aphid. The use of artificial diet work has been particularly helpful in elucidating the powerful phagostimulant cues present in the sieve element, namely sucrose and certain

amino acids such as methionine (Mittler and Dadd, 1962; Mittler, and Dadd, 1963; Mittler, 1967; Mittler, 1970).

3.2.2 Comparison of Aphid Species

Different aphid species have different plant host ranges, some aphids are known as 'generalist feeders' or polyphagus aphids, those species of aphids that are able to feed on a vast range of plant species. There are also species of aphids known as 'specialist feeders', aphids that have a limited plant host range, they are more restricted on the range of plants that they can successfully feed on. These species are often only able to survive on a particular family of plants due to the presence of a particular chemical cue. Previous studies have closely investigated various aspects of aphid feeding behaviour by using particular species of aphid as test aphids on specific test host plants.

This project utilises two aphid species, *Myzus persicae* and *Brevicoryne brassicae*, to investigate how two amino acid transporters affect the feeding behaviour of two aphid species with qualitatively different life history strategies. *M. persicae*, also known as the green peach aphid, is a world wide, economically important pest species which is capable of feeding on more than 40 plant families (Ramsey et al., 2007). In comparison *B. brassicae*, also known as the mealy cabbage aphid, is an aphid species that is restricted to feeding only on members of the Cruciferae family. This host plant range of *B. brassicae* is comparatively more limited and specialized than that of the polyphagous *M. persicae*. As both these aphid species are capable of feeding on *A. thaliana* they are capable of being used as model

aphid species throughout the study, providing a novel opportunity to investigate the questions raised in this study from a dual behavioural perspective.

3.2.3 Using Electrical Penetration Graphs (EPG) to study aphid probing and feeding behaviour of two aphid species on both *aap1* and *aap2* plants

A useful technique used to study aphid feeding is that of the 'electrical penetration graph' (EPG) analysis (McLean and Kinsey, 1964; Tjallingii, 1978). This technique was developed in 1964 by McLean and Kinsey in order to investigate aphid stylet activities throughout the process of plant probing. In this technique an aphid is wired into a circuit with the insect connected to the input of an amplifier. The test plant that the aphid is allowed to feed on is then entered into the circuit by applying a small DC current into the soil via an electrode. The aphid's stylets act as microelectrodes and upon the aphid piercing the plant cuticle the circuit is closed allowing current to flow through the fluid filled maxillary food and salivary canals and thus generating an electrical signal at the amplifier input (Tjallingii, 1987). Every time the aphid penetrates a cell membrane during the course of its feeding the recording software records the negative transmembrane potential as an abrupt drop in the recording signal (Tjallingii, 1985).

A series of characteristic waveforms are produced depending on the cell compartments being penetrated (see table 3.1 and figure 3.2). A diagrammatic representation of this method is shown in figure 3.3. The characteristic waveforms produced during an EPG have taken much effort to interpret, primarily aphids were analysed while feeding on artificial diet sachets (McLean and Kinsey 1964,

Tjallingi, 1978; Tjallingi, 1985) before more recent attempts involved video observation of the feeding aphid with which to correlate the simultaneously recorded waveforms (Hardie et al., 1992). Ultimately the most revealing method of successfully interpreting the EPG waveforms has been that of using histological sectioning of plant tissue with the probing aphid's stylet in situ during specific waveforms throughout the probing and feeding process (Tjallingi, 1978, Tjallingi, and Hogen Esch, 1993 and Tjallingi and Gabrys, 1999).

Pattern	Behaviour Relation	Description
np	Non-probing behaviour. There is no stylet penetration of the leaf by the aphid.	No electrical stylet contact.
pathway	Collective term for stylet penetration, salivary sheath formation and pathway of stylets through the plant tissue.	Irregular frequency with numerous pd.
pd	Potential drop indicating intracellular puncture.	Square negative wave. Value corresponds with plant cell membrane potential.
E1	Salivation (watery) into sieve element without ingestion.	Tall up-spikes. Usually brief, 10-15 seconds.
E2	Salivation and sieve element ingestion.	Asymmetric wav with rhythmic downwards spike. Always proceeded by a pd.
G	Xylem ingestion.	Symmetrical square wave. Part of intercellular pathway therefore not proceeded by a pd.

Table 3.1 – Table of Electrical Penetration Graph (EPG) waveform characteristics observed within this study and their descriptions (Tjallingii, 1988; 1990).

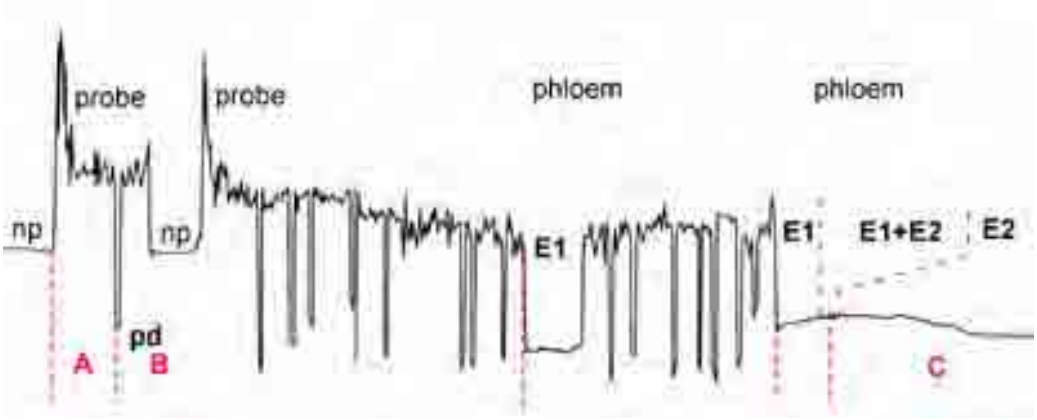

Figure 3.2 – An example of the type of trace observed using the EPG technique.

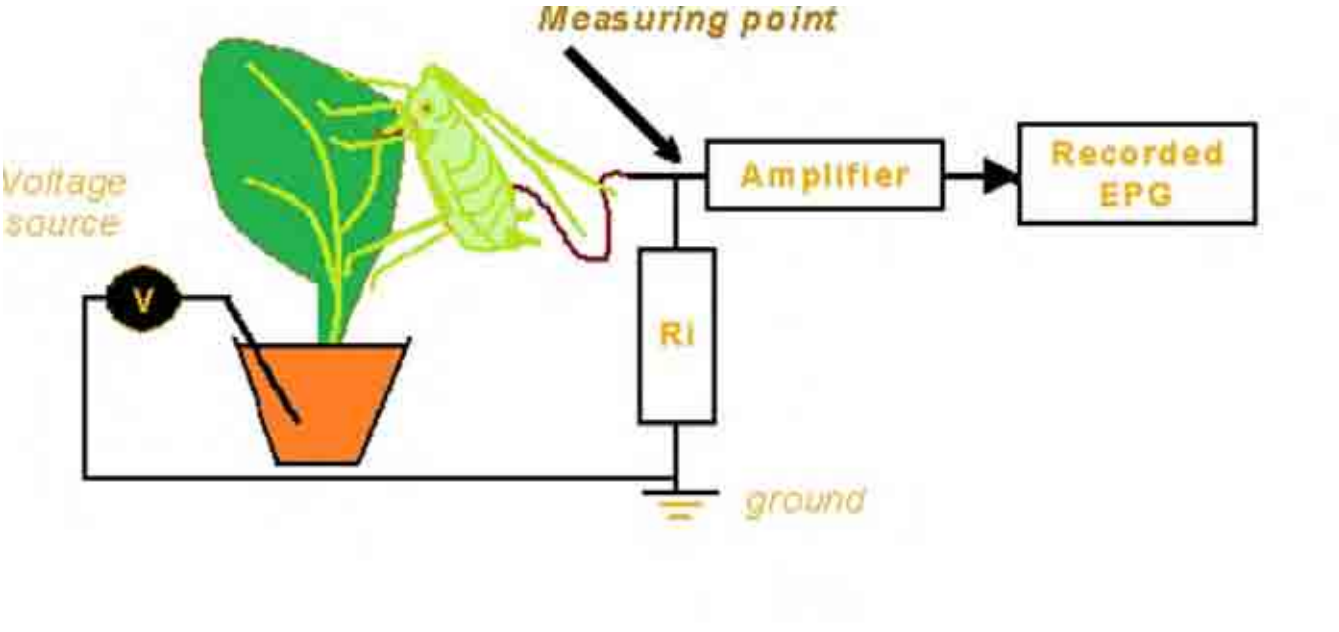

Figure 3.3 – A simplified diagrammatic illustration of the Electrical Penetration Graph (EPG) method (Tjallingi, www.epgsystems.eu).

3.2.4 Using honeydew production analysis to study aphid feeding rate of *M. persicae* and *B. brassicae* on *aap1* and *aap2* mutants

The previously described EPG technique is an extremely valuable technique which is used as a means of studying aphid probing and feeding behaviours. It can provide detailed information as to the initiation of ingestion and the duration of feeding however this technique cannot provide a rate of ingestion. The flow rate of ingested sap is ultimately controlled by the aphid's cibarial pump (Dixon, 1998). Environmental variables and diet composition are contributing factors in determining the aphid's rate of sap ingestion however the aphid is able to utilise the cibarial pump mechanism in order to vary the rate of ingestion thus compensating for fluctuations in diet quality (Mittler and Meikle 1991, and Schoonhoven et al., 1998). For example, aphids are able to selectively regulate the rate of ingestion according to the presence of specific amino acids (Weibull, 1987). Should the nutritional quality of the diet fall below a 'limiting' nitrogen threshold and become poor, the aphid is able to regulate the situation by increasing the quantity of sap ingested. The increased throughput of sap allows the aphid to process an increased level of nitrogen. Prosser et al., (1992) demonstrated this form of compensatory feeding using aphids fed on artificial diets of varying nitrogen qualities (45–270 mM amino acids). This study showed that *Acyrthosiphon pisum* increased dietary uptake in response to decreasing levels of amino acid concentration.

This is a highly versatile mechanism which allows for optimal feeding and in some situations the aphid is able to reduce its rate of ingestion in order to avoid the negative osmotic effects of excess sucrose ingestion. Should phloem sucrose levels reach very high levels aphids are known to reduce ingestion in an attempt to avoid osmotic stress (Hale et al., 2003).

Clearly the feeding rate of an aphid is an important factor to be studied in aphid plant interactions. In 1964 Banks and McCaulay suggested that the feeding rate of an aphid could be determined by measuring the volume of honeydew produced over a defined period of time. Honeydew is the sugary excretion regularly produced in uniform sized droplets every 15-45 minutes by a feeding aphid (Banks and McCaulay, 1964; Mittler, 1970; Mittler and Sylvester, 1961).

The rate of honeydew production is dependant on the aphid plant combination and ultimately the interaction between host quality and the aphid's ability to utilise the diet (Douglas, 2003). The universal method for measuring the honeydew production of an aphid is the 'honeydew clock' technique. This method consists of an aphid feeding on a horizontally positioned plant above a 360 degree rotating disc. The aphid which is positioned to feed on a host plant excretes honeydew which falls onto the rotating disc below. The disc platform is attached to the hour hand spiggot of the clock and consequently rotates at 360 degrees within 12 hours. In order to gain a visible trace of the honeydew droplets a filter paper disc is treated with a pH indicator and placed on the rotating disc platform. These droplets are counted to provide a honeydew frequency measurement.

Similarly a volume measurement for the honeydew droplets can be calculated by collecting the honeydew in a silanised glass dish containing Dow Corning Silicone Oil and measuring the droplet of suspended honeydew in the oil converting the diameter measurement into the volume of a sphere (Hale et al., 2003; Mittler, 1970; Mittler and Meikle, 1991), measuring the area of the droplets (Lei and Xu, 1993) or by measuring the distance which the droplet travels up a capillary tube (Rhodes et al., 1996).

In order to rule out the affect of aphid size or developmental stage on the size of honeydew droplets or the frequency of honeydew production, aphids of the same age and developmental stage should be used (Febvay et al., 1999; Prado and Tjallingi, 1999).

3.3 Chapter Aims

In the previous chapter various techniques were used to screen the effects of the loss of function mutations in the AAP1 and AAP2 transporters in *A. thaliana*. The results from this chapter indicated that the disruption to AAP1 and AAP2 transporters may have affected amino acid transport within the mutant plants significantly enough to reduce aspects of plant growth and potentially reduce whole plant fitness. A study by Hunt (2005) supports chapter one's findings, demonstrating a physiological role for the closely related amino acid transporter AAP6 in regulating sieve element sap composition. Measurements for sieve element total amino acid concentration were significantly lower in the *aap6* plants compared to the wildtype plants. These results suggest that plant-aphid interactions in terms of aphid feeding behaviour and metabolic activity may also be affected by the altered amino acid transport present in *aap1* and *aap2* plants.

The first aim of this chapter is to investigate the initial behaviours exhibited by *M. persicae* and *B. brassicae* immediately upon their introduction to *aap1* and *aap2* mutant plants compared to the wildtype control plants. These initial stage behaviours were interpreted to provide an early measure of host plant preference. The second aim of the chapter was to investigate probing and feeding behaviours of *M. persicae* and *B. brassicae* on *aap1* and *aap2* mutants by utilising the Electrical Penetration Graph (EPG) approach.

The final aim of the chapter was to determine another measure of aphid feeding behaviour by analysing the honeydew production rate and honeydew droplet

volume of the two aphid species when feeding on *aap1* and *aap2* mutant plants. This analysis of the frequency and volume of honeydew drops produced allowed for a further aim of deriving the aphid's feeding rate for both aphid species on both *aap1* and *aap2* mutants.

3.4 Materials and Methods

3.4.1 Aphid Culture

Myzus persicae and *Brevicoryne brassicae* were taken from a long term culture raised within the University of Birmingham insect room. These separate clonal cultures were initially started from a single aphid. These aphids were maintained on *A. thaliana* (Columbia – 0) plants grown in regularly watered compost in a controlled environment growth room maintained at 20-22ºC with an 18-6 light:dark regime. Only adults were used throughout the study. All adults used were synchronised in terms of age, being raised from nymphs born and selected from a 24 hour window.

Figure 3.4 – This is an image of an aphid culture of *B. brassicae* on wildtype *A. thaliana.*

3.4.2 Initial Host Preference Analysis

3.4.2.1 Plant Material

Homozygous *A.thaliana* seed with an insertional mutation in the AAP1 and AAP2 transporter genes were obtained along with Col0 seed which was used as wildtype 'control' plants. Both Col0 (wildtype plants), *aap1* and *aap2* plants were cultivated according to the methods featured in section 2.4.1. These plants were selected for use in the initial host preference analysis when at their optimum stage of growth (30-35 days old from sowing).

3.4.2.2 Aphid Culture

For this experiment only, adult alate *M. persicae* and *B. brassicae* were used in the initial host preference analysis (see section 3.4.1 for details).

3.4.2.3 Initial Host Preference Analysis

Aphids that were cultured according to the above method were synchronised so that only adult alate aphids of the same age were used. This synchronisation ensured the adults were the same age (within a 24 hour margin). Alates were chosen in place of apterous aphids in order to more closely simulate the alate colonisation of a new host plant observed in the wild. Synchronised adult aphids were removed from their Col0 culture plants and half were marked on their dorsum with silver conductive paste using a fine paintbrush and the other half were left unmarked. This was the marking element of the 'mark-release-

recapture' method, an ecology based method of marking specimens, releasing them into a specified environment for a period of time and then recapturing the specimens again to determine their movement or favoured position within the environment. According to their marking status the aphids were immediately introduced via fine paintbrush onto the mid stem region of their 'starting' host plant. For each experiment all of the marked aphids were positioned on the same plant genotype and the unmarked aphids were positioned on the opposing genotype.

Once positioned on their starting plant genotype, in order to allow for the aphids to freely choose their preferred host plant, the plants were arranged in a closely packed alternating arrangement with alternating mutant and control plants. Alternating the plant genotypes in a tightly packed secure space allowed the aphid's unrestricted movement between plants, ultimately allowing free choice of the aphid over which is the preferred host plant. After 24 hours of unrestricted movement the aphids, both unmarked and marked aphids were identified amongst the plants and their position on which plant genotype was recorded. The aphids were given free choice to roam over and ultimately settle on either; 1) *aap1* or wildtype plants, 2) *aap2* or wildtype plants or 3) *aap1* or *aap2* plants. For each experimental run 10 aphids were released, 5 marked aphids and 5 unmarked aphids. Marked and unmarked aphids would have their starting plant position alternated, for example, for the first experimental run the marked aphids would be positioned on the *aap1* plants then in the next experimental run, the marked aphids would be positioned on the Col0 plants at the start of the experiment. See figure 3.5 for a representation of experimental set up.

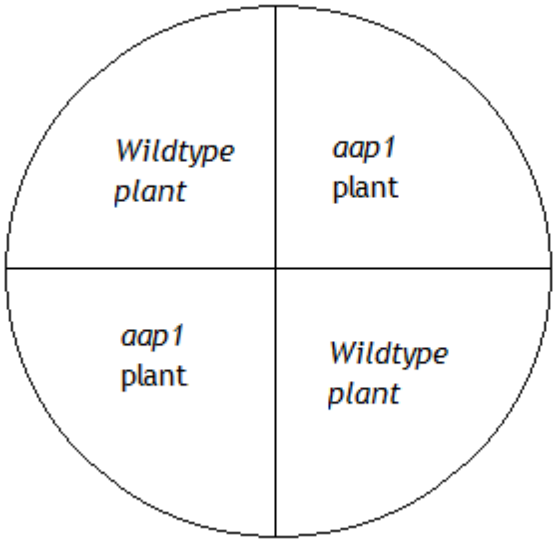

Figure 3.5 – Diagrammatic representation of the 'Initial host preference analysis' set up.

3.4.2.4 Statistical Analysis

Data collected was first tested for normality using an Anderson-Darling test and analysed using a Chi-Square analysis to test for homogeneity or randomness. In order to ascertain whether the aphids had made a true choice in their selection of host plant two hypotheses were set up in order for the eventual result to provide a clear conclusion. See appendix 2.1 for details of this statistical analysis.

The statistical hypotheses are:

H_0 : Aphids show no choice for their host and so disperse randomly across both plant genotypes

H_1 : Aphids show an active choice for their favoured host plant and therefore their distribution over both plant genotypes is not random

3.4.3 EPG Analysis

3.4.3.1 Plant Material

Both Col0 (wildtype plants), *aap1* and *aap2* plants were cultivated according to the methods featured in section 2.4.1. These plants were selected for use in the EPG analysis when at their optimum stage of growth (30-35 days old from sowing).

3.4.3.2 Aphid Culture

Adult apterous *M. persicae* and *B. brassicae* were used in the Electrical Penetration Graph (EPG) analysis (see section 3.4.1 for details).

3.4.3.3 Wiring of Aphids for EPG

Previous studies have indicated that the wiring up of the experimental aphid as part of the EPG technique has an effect on the aphids behaviour (Annan et al., 1997, Lei et al., 1997, Hunt, 2005). Studies have shown the wire attachment does limit an aphid's movement, with 'tethered aphids' taking up to four times as long to settle and feed from a host plant. This effect is believed to be due to the wire creating torsion which would act to reduce the aphids overall manoeuvrability (Tjallingii, 1986; van Helden and Tjallingii, 2000). Longer term studies of tethered aphids have shown reduced levels of longevity and reproductive output, potentially due to a reduction in feeding (Tjallingii, 1986). However, studies have not suggested that wiring an aphid for EPG has any effect on the probing and

penetration behaviour of a feeding aphid (Tjallingii, 1986; Annan et al., 1997). Any 'tethering effects' can be negated due to the comparative nature of this experiment. All combinations of aphid species and plant genotypes studied will be treated similarly and therefore the effects of the wiring can be disregarded.

Apterous adult aphids were taken out of the previously described cultures and were left within a sealed culture dish for one hour prior to any experimentation. This one hour period acted as a starvation period. After the starvation period the aphids were manipulated individually with a fine paintbrush so that they remained motionless within the culture dish with their dorsum exposed. A 2cm length of 25 micrometre diameter gold wire (Goodfellow) was dipped into silver conductive paint (RS Components) until a droplet accumulated at the tip of the wire. The accumulated droplet of silver conductive paint at the tip of the wire was brought into contact with the aphid's dorsum and held securely until the paint had dried and a secure bond had formed. At the opposing end of the wire, a connector (a metal pin) was secured to the wire using the same silver conductive paint. Upon the aphids being wired the apparatus within the Faraday cage was prepared. The Faraday cage made from mesh worked by blocking out any external static electric fields which may have created electrical interference and disruption to the EPG recording.

Eight EPG channels were connected to an amplifier via their individual wire. The chosen number of plants (a maximum of eight) were placed in front of the eight channels with an electrode inserted into the soil close to the base of each plant. At this stage the EPG data recording software Stylet 3.7 ® was initiated. The

aphids and their attached wires were then plugged into one of eight EPG channels via the connector. Once all the aphids were wired up they were lowered individually onto their respective plants stem, at a height of approximately 3.5cm above the rosette. In each experiment 8 aphids were recorded for each experiment, four on *aap1* or *aap2* plants and four on control wildtype plants. Monitoring then commenced. Aphids were observed for the initial hour to ensure that they had successfully settled.

If any aphids fell from their plants within the initial hour they were replaced, aphids that fell off after this time were excluded from the analysis. All experiments were started in the afternoon, running for 8 hours under constant illumination at 18-20ºC.

3.4.3.4 Special treatment required for the wiring of *B. brassicae*

In order for *B. brassicae* to be wired effectively with the gold wire and silver conductive paste the waxy covered dorsum of the adult *B. brassicae* were first brushed with a fine paintbrush and a delicate stroke. This allowed for the fine coating of wax to be removed prior to wiring so that a good contact between the aphid dorsum to wire interface could be made.

The waxy coating which would prevent a good electrical connection forming is illustrated in figure 3.6.

Figure 3.6 – This is a photograph taken with U-lead Photoexplorer® software under microscope of a developing nymph which will eventually become an alate adult. This image highlights the waxy covering typical of *B. brassicae*.

3.4.3.5 Statistical Analysis

Data collected from the EPG analysis was analysed using Stylet 3.7 ® software. Individual EPGs were analysed using a guide set of previously identified waveforms. The parameters that were measured in each trace included; mean time to first cell penetration, occurrence and mean duration of cell penetrations, occurrence of non-probing events, pathway activities, occurrence of cell penetrations, occurrence and duration of xylem feeding, occurrence and duration of sieve

element salivation (E1), mean time to reach first period of sieve element ingestion (E2) and occurrence and duration of sieve element ingestion (E2).

In order to determine statistical significance a non-parametric test was used to analyse the EPG data. A non-parametric test was chosen since the sets of data were not normally distributed (as determined by the Anderson-Darling normality test). The Mann-Whitney U-test was used in order to compare the many EPG parameters of aphids feeding on *aap1* and *aap2* plants with those feeding on the wildtype, control plants. See appendix 2.2 for details of this statistical analysis.

3.4.4 Honeydew Droplet Frequency Analysis

3.4.4.1 Plant Material

The *aap1*, *aap2* and Col0 plants were cultivated according to the methods featured in section 2.4.1. These plants were selected for use in the honeydew frequency analysis when at their optimum stage of growth (30-35 days old from sowing).

3.4.4.2 Aphid Culture

Adult apterous *M. persicae* and *B. brassicae* of the same age and therefore developmental stage were used in the honeydew frequency analysis (see section 3.4.1 for details).

3.4.4.3 Honeydew Frequency Collection

The frequency and volume of honeydew drops excreted by starved, apterous adult *M. persicae* and *B. brassicae* feeding on *aap1*, *aap2* and control plants were determined using a modified 'honeydew clock'. In brief, aphids were placed individually for drop frequency measurements on the mid stem regions of the plants. After a 1h settling period, honeydew drops excreted by the aphids were collected.

In order to determine the frequency of honeydew drops, each plant was suspended horizontally above the open half of a petri dish (14 cm diameter) connected to the hour-hand spigot of a kitchen clock. The clocks mechanism allowed the petri dish to rotate 360^{o} in a 12 h period. See figure 3.7. Contained within the petri dish was a circular piece of filter paper (125 mm diameter; Fischer Scientific, UK) pre-treated with 0.1% bromophenol blue (Sigma-Aldrich Company Ltd., UK) and 0.01 M HCl (Sigma-Aldrich Company Ltd.) See figure 3.8.

The feeding aphid was positioned onto the suspended plant held directly above the filter paper. Any honeydew drops excreted by the aphid fell directly onto the filter paper, where they were clearly visualised by a colour change from yellow to blue. Following the 10 h experimental period the numbers of drops in each segment of the filter paper were counted. Any filter papers not showing a regular pattern of drops were excluded from analysis. See figure 3.8 and 3.9.

Figures 3.7 - This image illustrates the basis for the honeydew clock method of collecting honeydew drops over a period of time. The hands of the clock have been removed and a pipette tip with the tip cut off was securely fastened to the hour hand spigot of the clock. To the pipette tip a small piece of blue tack was used to fasten a large plastic petri dish horizontally over the clock face.

Figure 3.8 – This figure illustrates a typical honeydew trace produced by a feeding aphid suspended over the honeydew clock set up. The characteristic blue dots are the result of honeydew droplets interacting with the treated filter paper disc. The filter paper was pre-treated with 0.1% bromophenol blue (Sigma-Aldrich Company Ltd., UK) and 0.01 M HCl (Sigma-Aldrich Company Ltd) allowing honeydew droplets to be visualised by a colour change from yellow to blue.

Figure 3.9 – This figure illustrates the entirety of the honeydew clock set up. The plant and its feeding aphid are positioned horizontally over the revolving filter paper.

3.4.4.4 Statistical Analysis

As the data was determined to be normal using an Anderson-Darling test, General Linear Model (GLM) ANOVAs were used to analyse the mean honeydew droplet frequency of *M. persicae* and *B. brassicae* feeding on *aap1, aap2* and wildtype (Col0) plants. See appendix 2.3 for details of this statistical analysis.

3.4.5 Honeydew Droplet Volume Analysis

3.4.5.1 Plant Material

The *aap1*, *aap2* and Col0 plants were cultivated according to the methods featured in section 2.4.1. These plants were selected for use in the honeydew droplet volume analysis when at their optimum stage of growth (30-35 days old from sowing).

3.4.5.2 Aphid Culture

Adult apterous *M. persicae* and *B. brassicae* of the same age and therefore developmental stage were used in the honeydew droplet volume analysis (see section 3.4.1 for details).

3.4.5.3 Mean Honeydew Droplet Volume Analysis

Approximately 70 adult aphids were taken from each sub-culture of synchronised aphids and caged within purposefully made 'settling cages' on the mid stem region

of the *aap1, aap2* and control Col0 plants. The settling cages were fabricated from eppendorf tubes cut to create an open ended collar structure. The open ended collar allowed for easy placement around the plant, positioned around the aphid on the mid stem region and closed using cotton wool bungs. The aphids were caged overnight in this way to allow the aphid to settle and begin feeding within their restricted area of the plant. The following morning the cages were removed from the mid stem region of each plant and the plant secured in a horizontal position above a silanized Petri dish containing silicone oil (200/50 cS Dow Corning, USA). This dish was positioned accordingly below the plant in order to collect falling honeydew droplets being excreted from the feeding aphid. The use of the silanised dish and the silicone oil allowed for perfectly spherical droplets to be collected, maintaining the integrity of the droplets which were used for volume calculations.

After 3 hours of aphid feeding the honeydew droplets were removed from the dish using a back filled microcapillary tube (back filled with Dow Corning silicone oil) and expelled from this microcapillary tube into another silanised dish of silicone oil under microscope. The diameters of the droplets were measured and from these values the volume of the honeydew droplets could be determined using the equation for the volume of a sphere, $((4/3)\ \pi r^3)$.

3.4.5.4 Statistical Analysis

The data was found to be normal using an Anderson-Darling test of normality. The data was then analysed using a one way ANOVA. See appendix 2.4 for details of this statistical analysis.

3.4.6 Aphid Feeding Rate Estimation

3.4.6.1 Plant Material

The *aap1*, *aap2* and Col0 plants were cultivated according to the methods featured in section 2.4.1. These plants were selected for use in the honeydew frequency analysis and honeydew droplet volume analysis when at their optimum stage of growth (30-35 days old from sowing).

3.4.6.2 Aphid Culture

Adult apterous *M. persicae* and *B. brassicae* of the same age and therefore developmental stage were used in the honeydew frequency analysis and honeydew droplet volume analysis (see section 3.4.1 for details).

3.4.6.3 Estimation of Aphid Feeding Rate

Previous work has shown that aphids excrete honeydew droplets of a uniform size therefore allowing for an estimation of aphid feeding rate to be derived from the measured mean honeydew droplet volumes (nl) and the hourly rate of honeydew production (drops/h).

Aphid feeding rate (nl/h) = honeydew drops/h x honeydew drop volume (nl)

3.4.6.4 Statistical Analysis

After determining the data was normal using an Anderson-Darling test, Students t-tests were used to analyse i) the number of honeydew drops produced over the 10 hour period, ii) the mean honeydew droplet volume and iii) the aphid feeding rate. See appendix 2.5 for details of this statistical analysis.

3.5 Results

3.5.1 Initial Host Preference Analysis of *M. persicae* and *B. brassicae*

Initial host preference, measured using a choice testing approach, was found not to be significantly affected in either *M. persicae* or *B. brassicae* when feeding on *aap1* and *aap2* compared to wildtype plants ($p > 0.05$). After 24 hours of free movement the unmarked and marked aphids of both species were found to be randomly distributed over *aap1, aap2* and wildtype plants. Refer to table 3.10.

Aphid Species	Mean Number of Aphids Present on Respective Plant Genotype after 24hr Choice Test Period		
	aap1 (n=30)	*aap2* (n=30)	Col0 (n=30)
M. persicae	7(±1.71)	6(±1.23)	6(±2.12)
B. brassicae	6(±1.18)	5(±2.67)	4(±1.85)

Table 3.10 – A table of the mean numbers of aphids found on each plant genotype following free roaming host choice tests. Mean values are determined from the results of a total of thirty 24 hr long host choice tests (n=30). Values in brackets represent ± s.d. No significant effects were found following a chi-squared analysis ($p > 0.05$).

3.5.2 EPG Analysis

During the first and second hours of the experiments the time spent in E2 sustained feeding was significantly greater for aphids feeding on wildtype plants (for *M. persicae* 472 (±128) and 3326 (±154) seconds and for *B. brassicae* 1315 (±*93)* and 2661 (±149) seconds respectively) compared to aphids feeding on *aap1* plants (for *M. persicae* 175 (±120) and 796 (±293) seconds (see figure 3.15) and for *B. brassicae* 327 (±112) and 1303 (±189) seconds respectively) ($p < 0.05$) (see figures 3.17).

Interestingly, the time spent in E2 sustained sieve element feeding during hours one and two was significantly greater for aphids feeding on wildtype plants (for *M. persicae* 510 (±116) and 2987 (±42) seconds and for *B. brassicae* 639 (±91) and 1784 (±69) seconds respectively) compared to aphids feeding on *aap2* plants (for *M. persicae* 235 (±122) and 912 (±154) seconds (see figure 3.16) and for *B. brassicae* 195 (±119) and 689 (±35) seconds respectively) ($p < 0.05$) (see figures 3.18).

During hour two of the experiments the time spent in pathway was also found to be significantly greater for both species of aphid feeding on *aap1* and *aap2* plants compared to those feeding on the wildtype plants ($p < 0.05$). This relationship is reciprocal to the result for mean time spent in E2. See figures 3.11 – 3.14. Other parameters being measured, including time spent in E1 and time spent out showed no significant differences ($p > 0.05$) between either aphid species feeding on the two plant genotypes. See figures 3.11 – 3.14.

The time taken for both *M persicae and B brassicae* to reach sustained ingestion (E2) was significantly greater for aphids feeding on *aap1* and *aap2* plants compared to those feeding on wildtype plants ($p < 0.05$).

Figure 3.11 illustrates the main EPG parameters measured in the EPG analysis. The mean total duration (sec) of each probing activity over the course of the 8 hour period has been determined for plant aphid species on each plant genotype. The full data for this analysis, including means ± s.e, can be found in appendix 5.1.

Figures 12-14 illustrate the first two hours of EPG analysis and the mean duration of time (s) *M. persicae* and *B. brassicae* spent in the EPG parameters measured when feeding on *aap1, aap2* and wildtype plants.

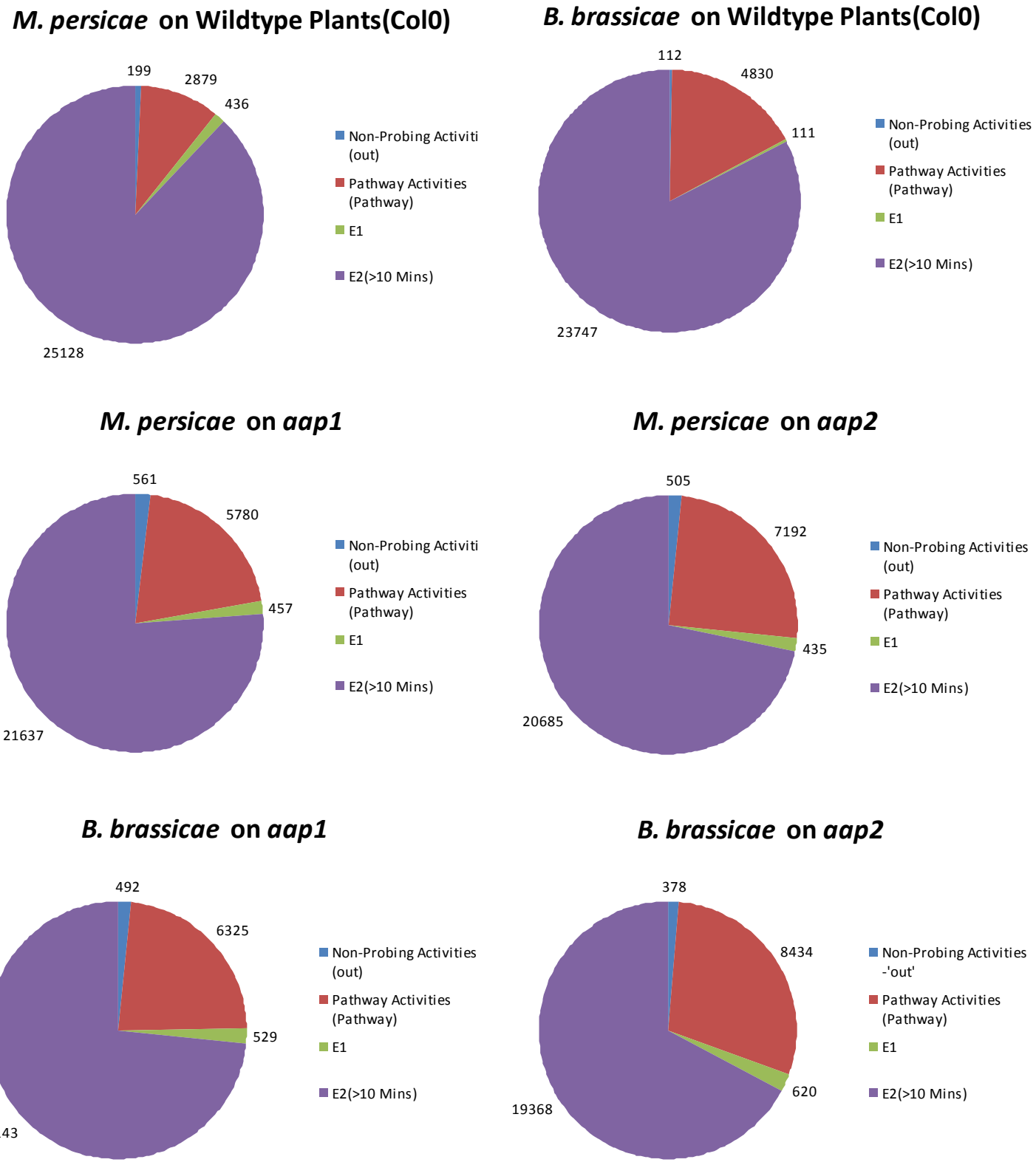

Figure 3.11 – Pie charts representing the total mean duration (sec) of EPG parameters measured for *M. persicae* and *B. brassicae* feeding on *aap1, aap2* and wildtype plants (Col0) over an 8 hour experimental period. Values shown are mean values (mean values ± s.e. are provided in table appendix 5.1). *'s indicate statistically significant differences ($p < 0.05$) after analysis with a Mann-Whitney U test. Sample sizes are indicated as follows, wildtype (Col0) n=13, *aap1* n=14 and *aap2* n=15.

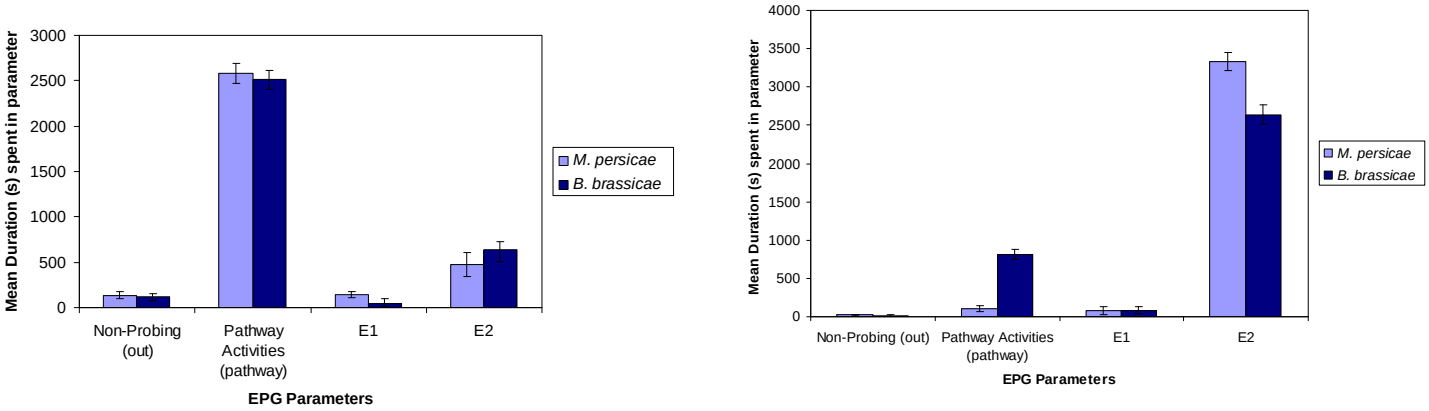

Figure 3.12 – Mean Duration of time spent (s) by *M. persicae* (n=13) and *B. brassicae (n=13)* in EPG parameters during hour one (left graph) and hour two (right graph) of feeding on wildtype plants, Col0. Error bars represent ± s.e. * represents a statistically significant difference of mean time spent in 'pathway' between aphid species (analysis using a Mann-Whitney U-test, $p < 0.05$).

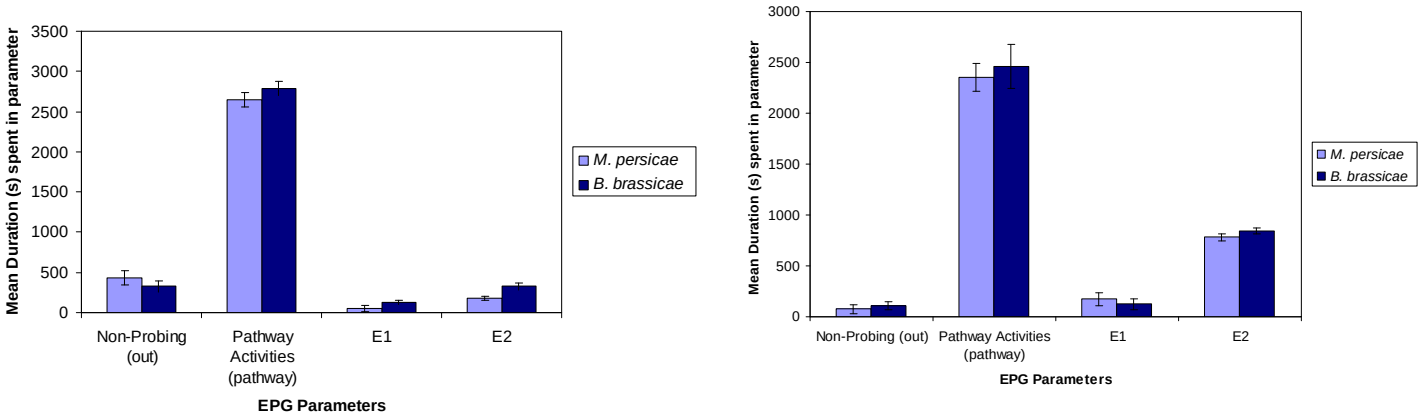

Figure 3.13 – Mean Duration of time spent (s) by *M. persicae* (n=14) and *B. brassicae* (n=14) in EPG parameters during hour one (left graph) and hour two (right graph) when feeding on *aap1* plants. Error bars represent ± s.e. Analysis using a Mann-Whitney U-test indicated no significant effect of aphid species ($p > 0.05$).

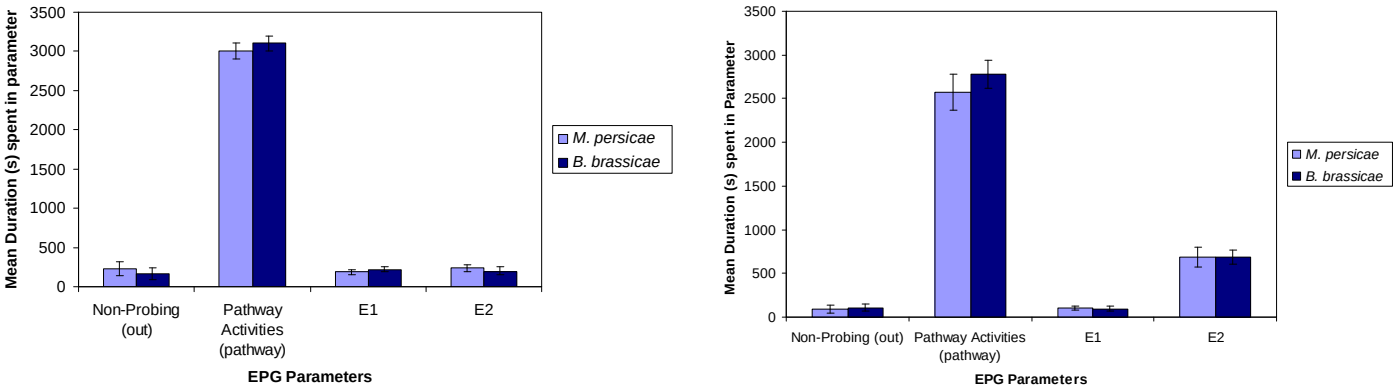

Figure 3.14 – Mean Duration of time spent (s) by *M. persicae* (n=15) and *B. brassicae (n=15)* in EPG parameters during hour one (left graph) and two (right graph) when feeding on *aap2* plants. Error bars represent ± s.e. Analysis using a Mann Whitney U-test indicated no significant effect of aphid species ($p > 0.05$).

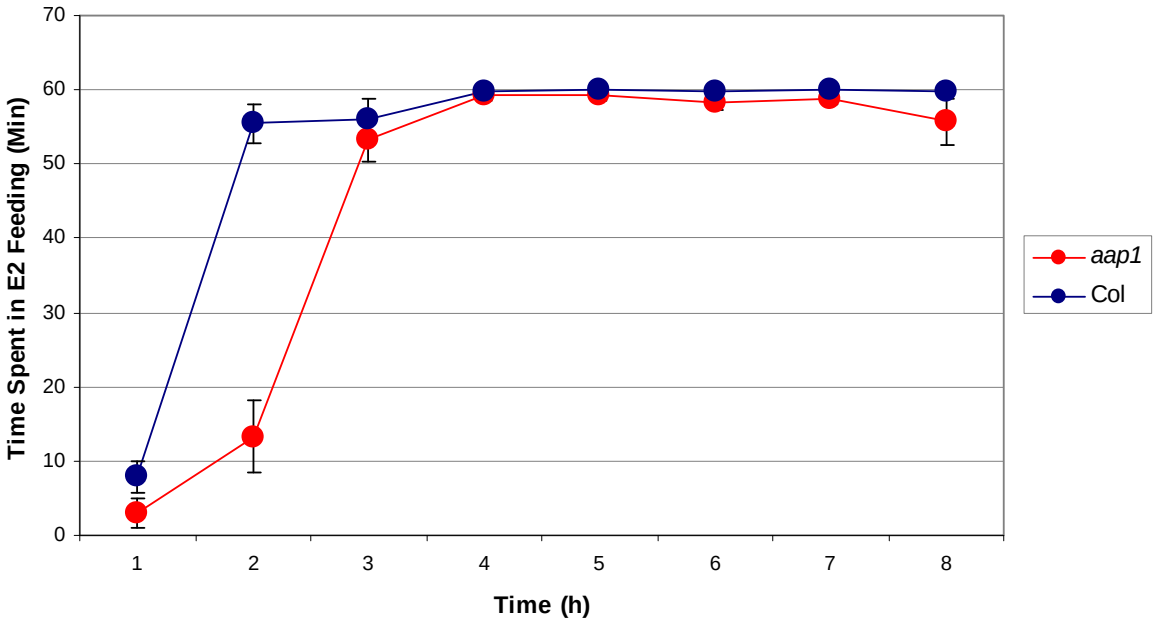

Figure 3.15 – A graph representing the mean time *M. persicae* spent in E2 during the 8 hour experimental period when feeding on *aap1*(n=14) and control plants (n=13). Error bars represent the ± s.e. The Mann Whitney U test indicated a significant effect of genotype ($p < 0.05$).

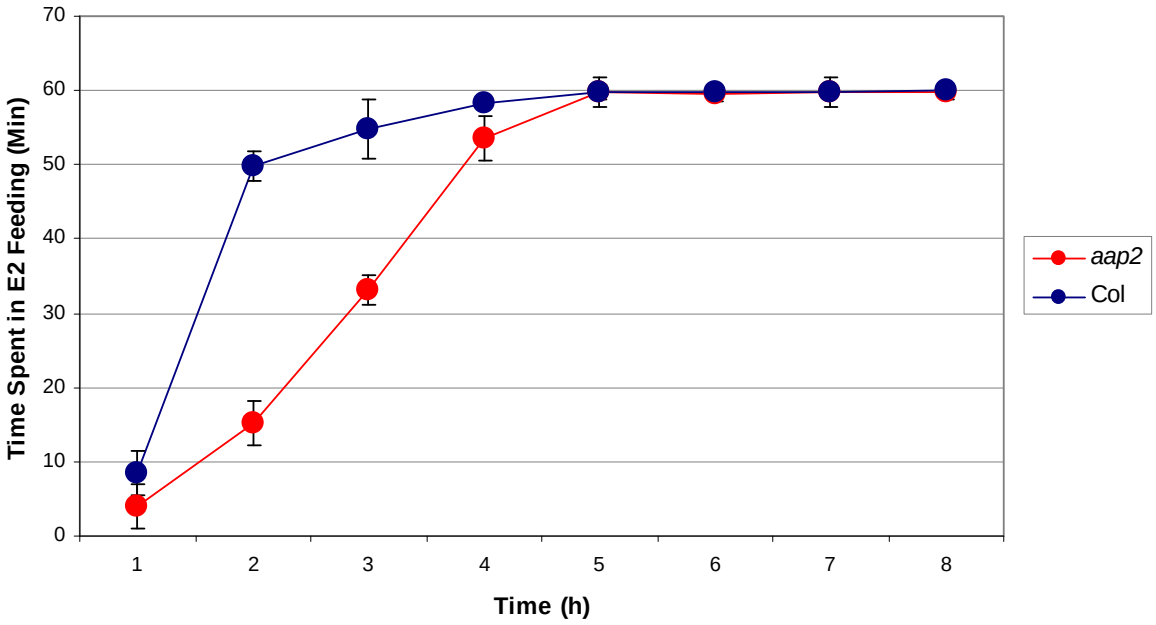

Figure 3.16 – A graph representing the mean time *M. persicae* spent in E2 during the 8 hour experimental period when feeding on *aap2*(n=15) and control plants (n=13). Error bars represent the ± s.e. The Mann Whitney U test indicated significant effect of genotype ($p < 0.05$).

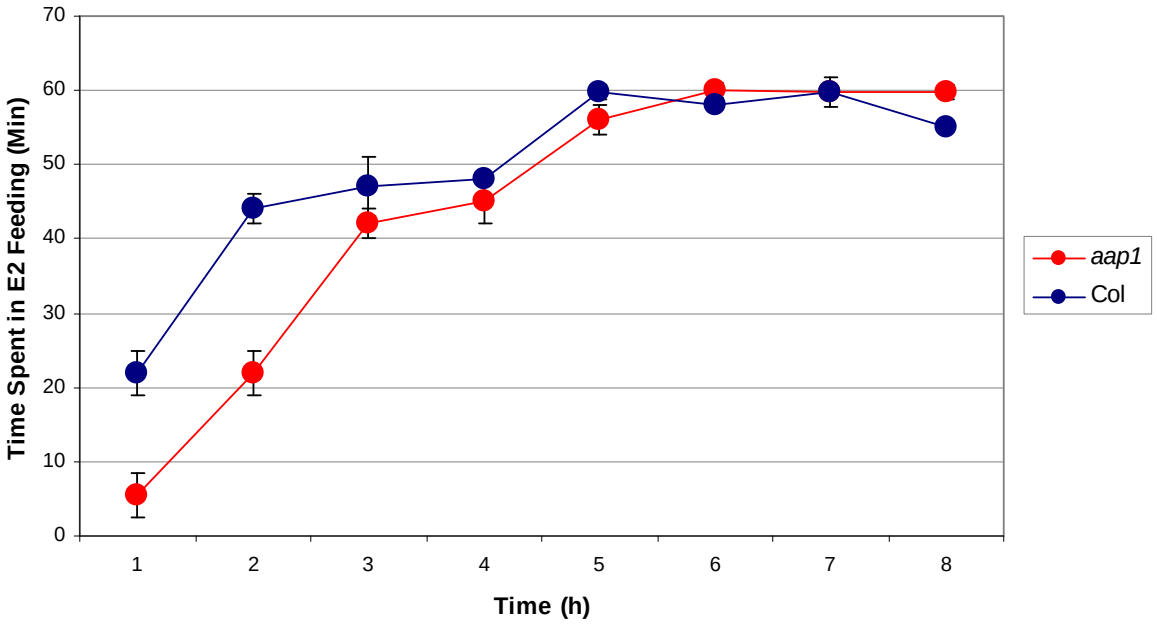

Figure 3.17 – A graph representing the mean time *B. brassicae* spent in E2 during the 8 hour experimental period when feeding on *aap1*(n=14) and control plants (n=13). Error bars represent the ± s.e. The Mann Whitney U test indicated a significant effect of genotype ($p < 0.05$).

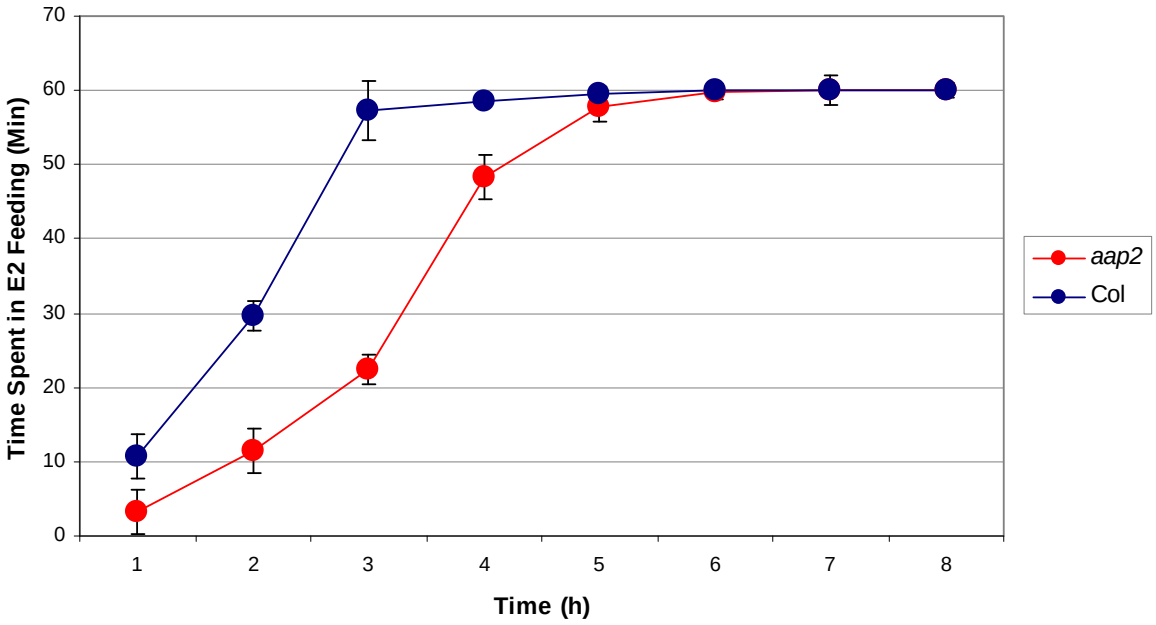

Figure 3.18 – A graph representing the mean time *B. brassicae* spent in E2 during the 8 hour experimental period when feeding on *aap2*(n=15) and control plants (n=13). Error bars represent the ± s.e. The Mann Whitney U test indicated a significant effect of genotype ($p < 0.05$).

3.5.3 Honeydew Frequency Analysis

3.5.3.1 Honeydew Production Analysis of *M. persicae* on *aap1* and wildtype plants

Aphids feeding on wildtype plants produced a mean number of 22.6 (±2.7) honeydew droplets over a 10 hour feeding period. The aphids feeding on *aap1* plants produced a significantly lower mean number of 18 (±1.9) droplets over a 10 hour feeding period ($p < 0.000$). See figure 3.19. A significant effect of time was also observed ($p < 0.000$).

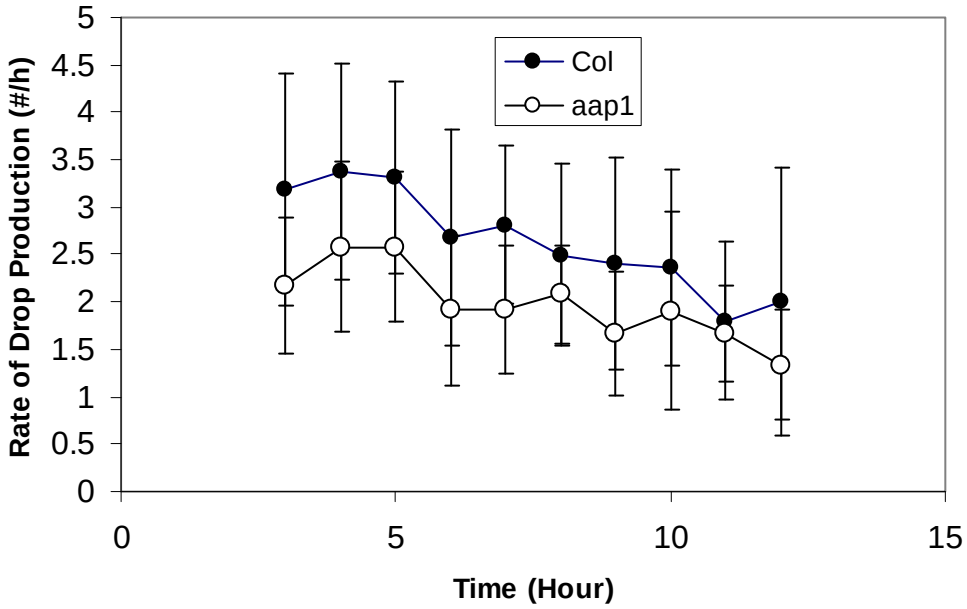

Figure 3.19 - Rate of honeydew production by *M. persicae* feeding on *aap1 mutants* and wildtype plants over a 10 hr period. For wildtype plants each point is the mean of 12 determinations of droplet production rate ± s.e, for *aap1* plants each point is the mean of 16 determinations of droplet production rate ± s.e. A General Linear Model (GLM) ANOVA indicated a significant effect of genotype ($p < 0.000$).

3.5.3.2 Honeydew Production Analysis of *B. brassicae* on *aap1* and wildtype plants

Aphids feeding on wildtype plants produced a mean number of 22.7 (±2.5) honeydew droplets over a 10 hour feeding period. The aphids feeding on *aap1* plants produced a significantly lower mean number of 16 (±1.5) droplets over a 10 hour feeding period ($p < 0.000$). See figure 3.20. A significant effect of time was also observed ($p < 0.000$).

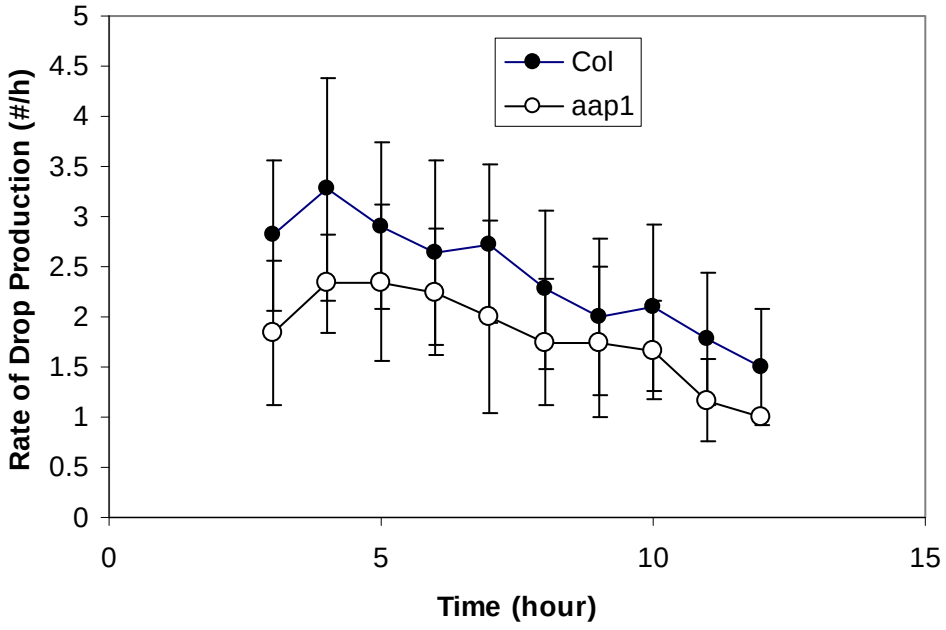

Figure 3.20 - Rate of honeydew production by *B. brassicae* feeding on *aap1 mutants* and wildtype plants over a 10 hr period. For wildtype plants each point is the mean of 11 determinations of droplet production rate ± s.e , for *aap1* plants each point is the mean of 12 determinations of droplet production rate ±s.e. A General Linear Model (GLM) ANOVA indicated a significant effect of genotype ($p < 0.000$).

3.5.3.3 Honeydew Production Analysis of *M. persicae* on *aap2* and wildtype plants

Aphids feeding on wildtype plants produced a mean number of 24.5 (±2.1) honeydew droplets over a 10 hour feeding period. The aphids feeding on *aap2* plants produced a significantly lower mean number of 17.8 (±1.7) droplets over a 10 hour feeding period ($p < 0.000$). See figure 3.21. A significant effect of time was also observed ($p < 0.000$).

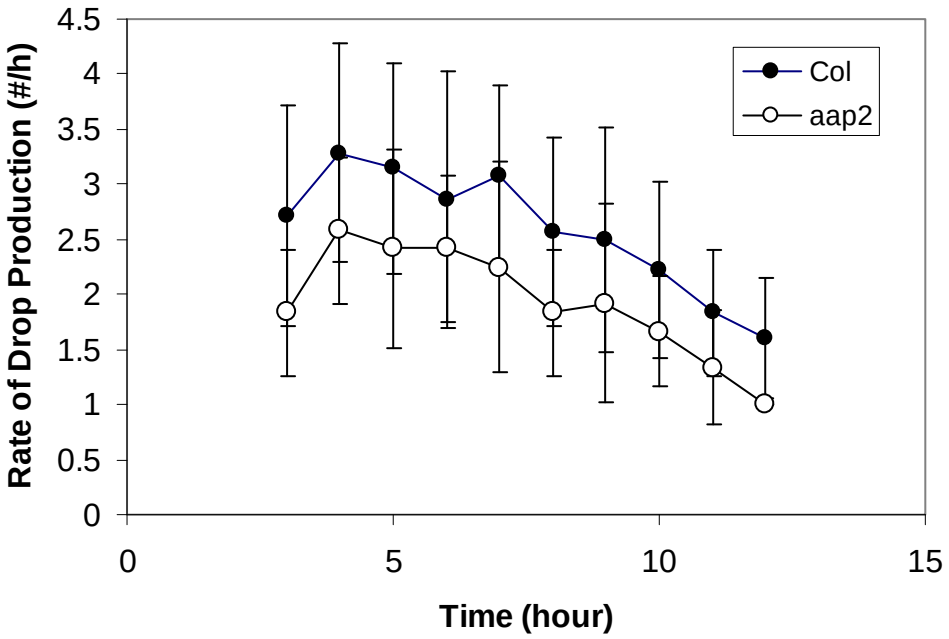

Figure 3.21 - Rate of honeydew production by *M. persicae* feeding on *aap2 mutants* and wildtype plants over a 10 hr period. For wildtype plants each point is the mean of 14 determinations of droplet production rate ± s.e , for *aap2* plants each point is the mean of 12 determinations of droplet production rate ± s.e. A General Linear Model (GLM) ANOVA indicated a significant effect of genotype ($p < 0.000$).

3.5.3.4 Honeydew Production Analysis of *B. brassicae* on *aap2* and wildtype plants

Aphids feeding on wildtype plants produced a mean number of 25.6 (±2.4) honeydew droplets over a 10 hour feeding period. The aphids feeding on *aap2* plants produced a significantly lower mean number of 18 (±1.9) droplets over a 10 hour feeding period ($p < 0.000$). See figure 3.22. A significant effect of time was also observed ($p < 0.000$).

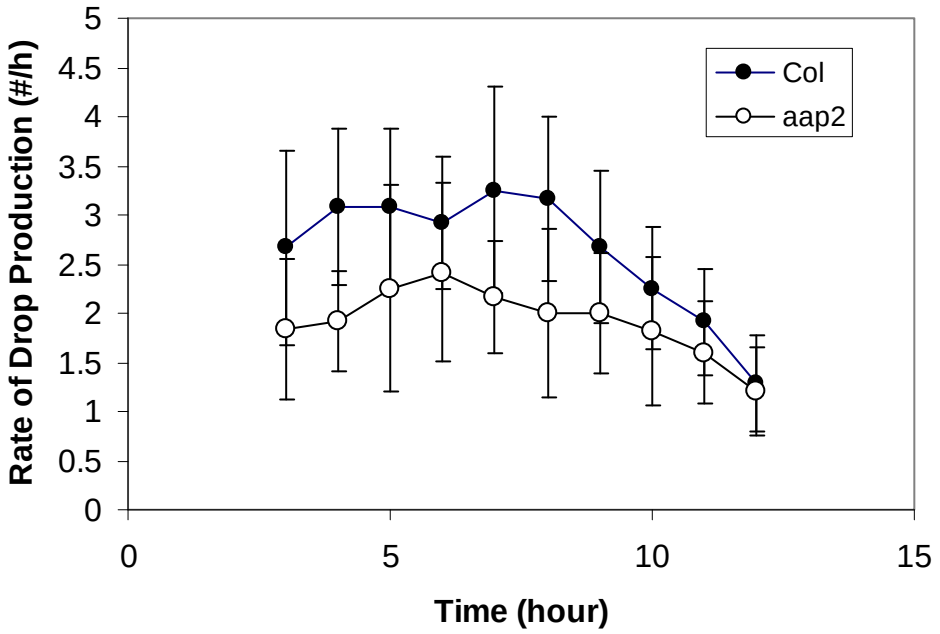

Figure 3.22 - Rate of honeydew production by *B brassicae* feeding on *aap2 mutants* and wildtype plants over a 10 hr period. For wildtype plants each point is the mean of 12 determinations of droplet production rate ± s.e, for *aap1* plants each point is the mean of 12 determinations of droplet production rate ± s.e. A General Linear Model (GLM) ANOVA indicated a significant effect of genotypes ($p < 0.000$).

3.5.4 Honeydew Volume Analysis

3.5.4.1 Honeydew Volume Analysis of *M. persicae* on *aap1,aap2* and wildtype plants

The honeydew drop volumes produced by *M. persicae* did not differ significantly depending on the plant genotype they were feeding on (p = 0.156). See table 3.23. Although the aphids feeding on the *aap1* and *aap2* plants did produce marginally lower honeydew droplet volumes when compared to the aphids feeding on the wildtype plants, this difference was not significant.

Mean Honeydew Droplet Volumes (nl) of feeding *M. persicae*		
aap1	*aap2*	**col**
10.1 (± 0.0606) (n=30)	**10.0 (± 0.0713) (n=30)**	**10.2 (± 0.0683) (n=30)**

Table 3.23 - A table of mean honeydew droplet volumes produced by *M. persicae* feeding on *aap1, aap2* and wildtype plants. Values in brackets refer to ± s.e. For each value n=30, with 10 droplets being measured from 3 aphids on three separate plants of each genotype. Data analysed using a one-way ANOVA indicated there was not a significant effect of genotype on the honeydew droplet volumes (p = 0.156).

3.5.4.2 Honeydew Volume Analysis of *B. brassicae* on *aap1*, *aap2* and wildtype plants

The honeydew drop volumes produced by *B. brassicae* feeding on *aap1* were significantly lower compared to those feeding on wildtype plants (p = 0.026). The honeydew drop volumes produced by *B. brassicae* feeding on *aap2* did not differ significantly when compared to those feeding on wildtype plants (p = 0.357). See table 3.24. The mean honeydew droplet volumes for *B. brassicae* were comparatively lower for all the plant genotypes measured when compared to the values for *M. persicae*. This difference was not significant.

Mean Honeydew Droplet Volumes (nl) of feeding *B. brassicae*		
aap1	*aap2*	**Col**
9.6 (±0.0626) * **(n=30)**	**9.8 (±0.0610)** **(n=30)**	**9.9 (±0.0610)** **(n=30)**

Table 3.24 - A table of mean honeydew droplet volumes produced by *B. brassicae* feeding on *aap1, aap2* and wildtype plants. Values in brackets refer to ± s.e. For each value n=30, with 10 droplets being measured from 3 aphids on three separate plants of each genotype. Data analysed using a one-way ANOVA indicated there was a significant effect of genotype on the honeydew droplet volumes (p = 0.003). An asterisk indicates a statistically significant difference.

3.5.5 Aphid Feeding Rate Estimation

3.5.5.1 Aphid Feeding Rate Analysis (rate of honeydew production) of *M. persicae* on *aap1, aap2* and wildtype plants

Aphids feeding on *aap1* plants produced honeydew at an average rate of 18.2 (±1.9) nl/h compared to 17.8 (±1.7) nl/h on *aap2* plants and 24.1 (±2.6) nl/h on wildtype plants (see figure 3.25). Analysis with a one-way ANOVA indicated the aphid feeding rates of *M. persicae* on both *aap1* and *aap2* were significantly reduced when compared to the feeding rate on wildtype plants ($p < 0.000$).

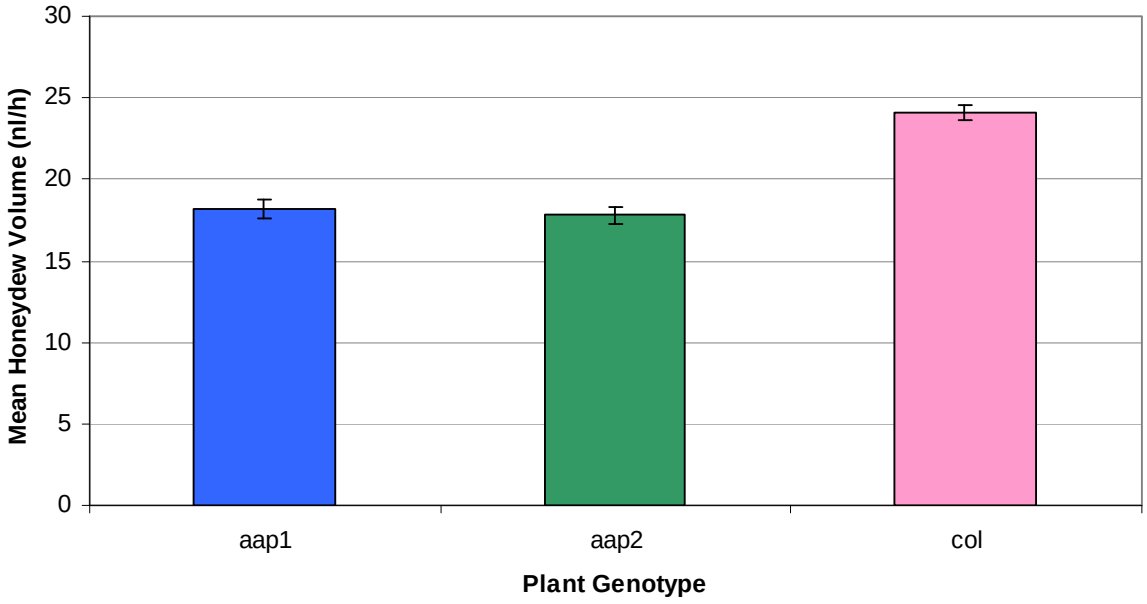

Figure 3.25 – Mean feeding rate of *M. persicae* on *aap1, aap2* and Col plant genotypes. Mean honeydew volume produced per hour by aphids feeding on *aap1* (n=12), *aap2* (n=12) and wildtype plants (n=30). Error bars refer to ± s.e. Data analysed using a one-way ANOVA indicated the a statistically significant effect of genotype ($p < 0.000$).

3.5.5.2 Aphid Feeding Rate Analysis (rate of honeydew production) of *B. brassicae* on *aap1, aap2* and wildtype plants

Aphids feeding on *aap1* plants produced honeydew at an average rate of 16.4 (±1.5) nl/h compared to 17.3 (±1.8) nl/h on *aap2* plants and 23.9 (±2.8) nl/h on wildtype plants (see figure 3.26). Analysis with a one-way ANOVA indicated the aphid feeding rates of *B. brassicae* on both *aap1* and *aap2* were significantly reduced when compared to the feeding rate on wildtype plants ($p < 0.000$).

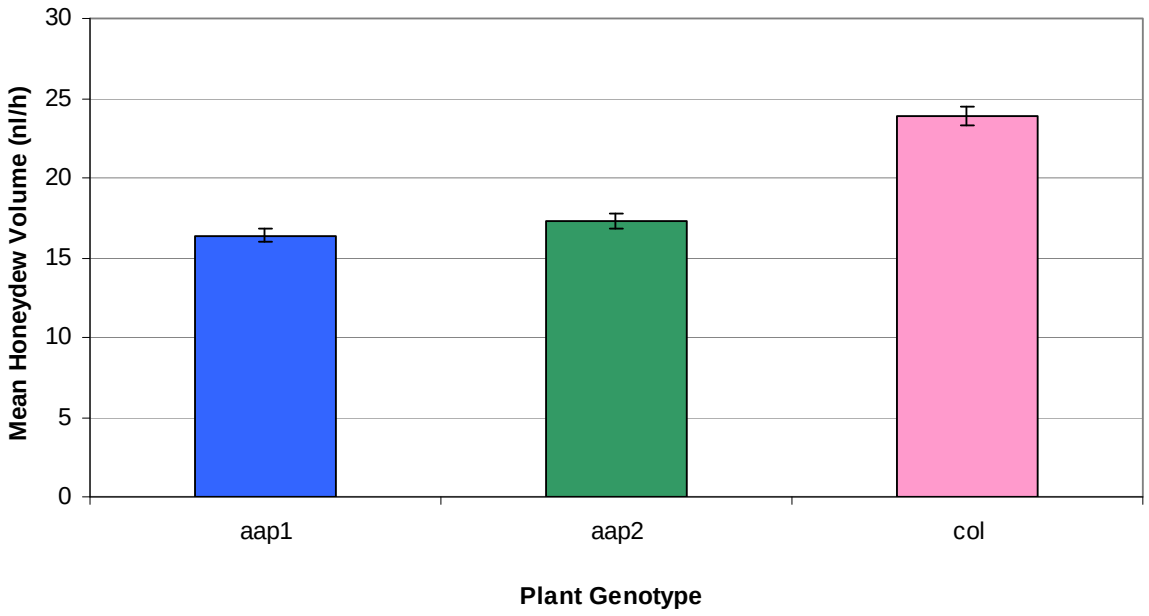

Figure 3.26 – Mean feeding rate of *B. brassicae* on *aap1, aap2* and Col plant genotypes. Mean honeydew volume produced per hour by aphids feeding on *aap1* (n=12), *aap2* (n=12) and wildtype plants (n=23). Error bars refer to ± s.e. Data analysed using a one-way ANOVA indicated a statistically significant effect of genotype ($p < 0.000$).

3.6 Discussion

Experiments were carried out to study the relationship between changes to host plant amino acid transport in *aap1* and *aap2* plants and the consequential effect on aphid feeding behaviour and feeding rate in two different aphid species, *M. persicae* and *B. brassicae.*

3.6.1 Initial Host Preference Analysis

Initial host preference, measured using a choice testing approach, was not found to be significantly affected in either *M. persicae* or *B. brassicae* when feeding on *aap1* and *aap2* compared to wildtype plants ($p < 0.005$).

This result suggests that both *M. persicae* and *B. brassicae* showed no preference over host choice when given free choice to roam over and ultimately settle on either; 1) *aap1* or wildtype plants, 2) *aap2* or wildtype plants or 3) *aap1* or *aap2* plants. In all of these choice testing situations the test aphids were found to be randomly distributed across the plant genotypes at the end of the experimental period. This random distribution suggests that at the initial stages of host plant selection the aphids of both species did not exhibit any preference for either genotype, implying the disruption to AAP1 and AAP2 did not have any effect on the surface cues present in *aap1* and *aap2* plants. Evidence in the literature has shown that aphids actively assess the surface cues present upon a host plant upon landing. The primary assessment of the surface cues involves antennation behaviour (Powell et al., 2006) during which the aphid detects plant volatiles that

lie close to the plant surface by waving of the antennae (Storer et al., 1996; Powell et al., 2006). Aphids are also able to detect other cues using the chemosensary hairs on the antennal tips (Powell et al., 2006). These cues include epicuticular wax composition (Klingauf, 1987), trichome exudates (Neal et al., 1990), surface topology (Ibbotson and Kennedy, 1959), and host colour (Pelletier, 1990). These cues are known to be ultimately less important than internal plant factors, as at this stage once tarsal contact has been made with a hard substratum the aphid will attempt penetration independent of any cues (Powell and Hardie, 2000). This probing behaviour may even occur upon the presence of repellent cues (Griffiths et al., 1982; Phelan and Miller, 1982).

The results suggest that the disruption to both AAP1 and AAP2 did not have a significant effect on the surface cues known to influence aphid behaviour at this initial stage of host plant selection. This observation is supported by the fact that the aphids had settled successfully, yet randomly over the test plant genotypes despite an initially asymmetric distribution (see figure 3.5 for a diagrammatic representation). This successful settling behaviour implies that the disruption to AAP1 and AAP2 had not created mutant *aap1* and *aap2* plants devoid of recognizable surface cues, since a total lack of recognisable surface cues has been reported to result in a restless aphid that will not settle (Wensler, 1962).

It is fair to assume that the epicuticular wax composition (Klingauf, 1987), trichome exudates (Neal et al., 1990), surface topology (Ibbotson and Kennedy, 1959), and host colour (Pelletier, 1990) of the *aap1* and *aap2* plants were not significantly different from that of the wildtype plants. This assumption is supported by the independent observations of random host plant choice witnessed

in both *M. persicae* and *B. brassicae*, aphid species with differing sensitivities to dietary quality (Van Emden et al., 1969).

3.6.2 Honeydew Analysis - Aphid Feeding Rate

Honeydew droplet frequency was significantly reduced for both aphid species when feeding on mutants *aap1* and *aap2* compared to wildtype control plants ($p < 0.000$). In comparison however, honeydew droplet volume was not significantly affected in either aphid species when feeding on *aap1* and *aap2* mutants when compared to wildtype plants ($p > 0.05$).

Using the honeydew drop frequencies and honeydew drop volumes the aphid feeding rates were determined for both aphid species on *aap1, aap2* and control plants. For both aphid species the feeding rates were significantly lower when feeding on *aap1* and *aap2* plants when compared to the feeding rates on control plants ($p < 0.000$).

The reduced aphid feeding rate of both aphid species feeding on *aap1* and *aap2* plants suggests that the composition of the sieve element sap has been significantly altered by the disruption to AAP1 and AAP2. Evidence in the literature shows that diet composition is a contributing factor in determining the aphid's rate of sap ingestion, although should the diet quality fluctuate the aphid is able to vary the rate of ingestion by utilising its cibarial pump (Mittler and Meikle, 1991 and Schoonhoven et al., 1998). This means that aphids are able to selectively regulate the rate at which they ingest sieve element sap according to the presence

of specific cues, including amino acids (Weibull, 1987). Should the nutritional quality of the diet fall below a hypothetical 'limiting' nitrogen threshold, the aphid is able to compensate by increasing the quantity of sap ingested. The increased throughput of sap provides a mechanism allowing the aphid to obtain an increased level of nitrogen. In the case of *aap1* and *aap2* plants both aphid species have independently exhibited a reduced rate of feeding with no effect on the volume of honeydew droplets excreted. This suggests that the total nitrogen content of the sieve element sap was not significantly reduced, if this had happened in *aap1* and *aap2* plants then the aphid feeding rate may be expected to increase significantly in order to maximise throughput and metabolism of the low nitrogen diet. Instead it can be hypothesised that a change, potentially an omission or reduction of certain amino acids, may have occurred as a result of the disruption to AAP1 and AAP2. Early work using artificial diets demonstrated that the amino acid methionine acted as a phagostimulant for aphid feeding. Artificial diets with methionine omitted reduced diet uptake by 50%, with progressive increase in uptake associated with the increasing concentration of methionine (Mittler, 1970). Lee et al., (2007) demonstrated that mutant *aap1* plants are able to survive and grow on normally toxic levels of amino acids, the lack of functional AAP1 transporters reduces amino acid uptake in the roots allowing the *aap1* mutants to survive on these toxic levels. Interestingly *aap1* plants were able to grow on toxic levels of the phagostimulant amino acid, methionine. This implies that the levels of uptake of methionine would be significantly reduced within the plant, potentially explaining the reduced feeding rate observed for both aphid species. Reduced uptake of a known aphid phagostimulant may explain the effects on feeding rate and help to explain the differences observed in the aphid probing

behaviours investigated using the EPG analysis. Phenylalanine and leucine are also known to act as phagostimulants. Studies using artifical diets found they acted to increase the acceptability of the diet (Mittler, 1967; Mittler and Dadd, 1964).

Alternatively, in some situations a feeding aphid is able to reduce its rate of ingestion in order to avoid the negative osmotic effects of excess sucrose ingestion. In cases of extremely high phloem sucrose levels aphids are known to reduce ingestion in an attempt to avoid osmotic stress (Hale et al., 2003).

3.6.3 EPG Analysis

The time taken to reach the first sustained phloem ingestion (E2) was significantly greater for both aphid species feeding on *aap1 and aap2* plants when compared to those feeding on wildtype plants. Also, the time spent in pathway phase was significantly greater for both aphid species feeding on *aap1* and aap2 plants compared to that of wildtype plants. This result represented the reciprocal relationship between time spent in pathway activities and time spent feeding from the sieve element. These results suggest that the disruption to AAP1 and AAP2 has altered the pathway cues normally used by the aphid to locate a suitable sieve element (Powell and Hardie, 2000). The aphids feeding on the mutant plants may not have received the pathway cues that would have normally enabled them to locate a suitable sieve element as quickly as the aphids feeding on the wildtype plants. The pathway cues used by the aphid to locate a suitable sieve element may have been altered, reduced in intensity or entirely absent from the aphid's stylet penetration pathway. Previous studies involving artificial diets indicate that

specific amino acids along with sucrose can act as phagostimulants or cues within the pathway (Mittler, 1967; Srivastava and Auclair, 1974; Srivastava and Auclair, 1975). As previously mentioned in section 3.5.2, the levels of uptake of methionine are significantly reduced in *aap1* mutants, potentially explaining the feeding aphid's delay in locating a suitable sieve element.

An alternative explanation to the delay in E2 feeding observed in both aphid species, may be that the actual sieve element sap quality of *aap1* and *aap2* plants has been adversely affected. Previous evidence within the literature suggests that sieve element sap composition may be a contributing factor to sieve element acceptance, feeding aphids will tend to feed on diets with optimal nitrogen status (Auclair, 1963; Auclair, 1965; Auclair, 1969; Klingauf, 1987). The significant differences in time taken to reach sustained ingestion could therefore be interpreted as being the result of an alteration in the sieve element composition. However EPG data also shows that once the feeding aphids of both species have located a suitable sieve element and reached a level of sustained E2 feeding they continue to feed similarly to aphids on wildtype plants for the rest of the experimental duration. This similar, undisturbed pattern of E2 feeding upon location of a sieve element suggests that the sieve element sap composition has not been significantly affected by the disruption to AAP1 or AAP2. If the sieve element sap was adversely affected in *aap1* and *aap2* plants, the feeding aphid may not have continuously fed from the sieve element, instead probing repeatedly in search of a sieve element with a more acceptable sap concentration. If the sieve element sap had been significantly affected in *aap1* and *aap2* plants this may be reflected in the performance of *M. persicae* and *B. brassicae,* however

performance data for both aphid species also indicates that there is not a significant alteration to sieve element sap, for example measures of reproductive output are similar for both aphid species on both *aap1* and *aap2* compared to wildtype plants (Van Emden, 1966) (see chapter four). Considering this data it can be assumed that the plant cell pathway has been affected in terms of the cues used by the feeding aphid with which to locate a suitable sieve element.

Another tentative conclusion that can be drawn from this study was that the disruption to AAP1 and AAP2 probably do not create any chemical deterrents (Schoonhoven and Derkson-Koppers, 1976). Although a delay in sustained sieve element ingestion may indicate the presence of a deterrent or toxic component within the sieve element sap, the fact that after four hours of feeding on *aap1* and *aap2* plants the aphids reached the same levels of sustained sieve element feeding seen in the aphid of both species on the control plants. This continuous feeding does not indicate the presence of a chemical deterrent. The delay may suggest however that the aphids finally located a weak cue used to locate a sieve element. If a deterrent was present in the knockout plants one could expect the aphids to show significantly lower levels of sieve element feeding as well as taking longer to reach sustained ingestion over many more hours.

3.7 Conclusions

This study highlighted significant differences in the feeding behaviour and feeding rates of both aphid species when feeding on *aap1* and *aap2* mutants. Neither aphid species exhibited any specific choice over preferred host plant. Aphids settled to feed on *aap1* and *aap2* in a random distribution. Following settling, both aphid species showed significant delays in locating a sieve element, spending significantly longer probing within pathway phase before settling to ingest. Once in E2 both *M. persicae* and *B. brassicae* settled and fed almost continuously throughout the experimental period. This continuous E2 feeding implies both aphid species found the diet palatable and acceptable in terms of nitrogen status but perhaps had problems locating a sieve element due to a lack of cues within the pathway. These results are consistent with the aphid honeydew analysis results for *aap1* and *aap2* which demonstrated a significantly lower rate of honeydew production yet no affect on the volume of honeydew excreted in both aphid species. The relationship between rate of sap uptake and the volume of honeydew excreted is interesting considering increased sap ingestion often indicates the aphid is attempting to compensate for reduced amino acid supplies. In this case if the aphid species were attempting to compensate for reduced amino acid supplies it would be expected that the volume of sap ingested (and consequently the volume of honeydew excreted) would have increased proportionally to the reduced feeding rate. This was not observed and instead the volume of honeydew excreted remained unaffected suggesting no changes in sieve element sap ingestion of either aphid species. This supports the idea that the sieve element composition of *aap1* and *aap2* in terms of amino acid levels was acceptable. The fact that both of

these results were observed independently in both aphid species being studied also strengthened this theory.

Although the overall sieve element sap composition appears not to have been affected significantly in terms of its acceptability to feeding aphids, the feeding rate results suggests that subtle yet significant changes in the individual amino acid profile may have occurred. Feeding rates for *M. persicae* and *B. brassicae* on *aap1* and *aap2* were significantly reduced suggesting that levels of certain amino acids which may ordinarily act to encourage feeding and increase palatability of the sieve element sap may be reduced or absent. Examples of these include methionine, leucine and phenylalanine (Mittler, 1967). The observed reduction in feeding rates may also be explained by the ratio of carbon : nitrogen present within the sieve element sap. If the sieve element sap nitrogen levels are reduced in *aap1* and *aap2* plants the relative proportion of carbon would increase. This hypothetical change in the C : N ratio may also explain a reduction in aphid feeding rate, a compensatory strategy employed by the aphid to avoid ingestion of less than ideal carbon levels.

The results from this chapter have shown that it is possible to postulate the reasons behind certain aphid behaviours and measures of feeding rate, however it would be more enlightening if more was known about the sieve element composition of *aap1* and *aap2* plants. Sieve element sap composition needs to be studied further in order to support the theories above and to gain a better idea of how the AAP1 and AAP2 amino acid transporters affect amino acid transport and consequently aphid feeding behaviour.

In the following chapter various techniques are used to compare the performance of *M. persicae* and *B. brassicae* on the *aap1* and *aap2* plants. The aim was to determine whether the disruptions to AAP1 and AAP2 would affect the performance of aphids in terms of their growth and reproductive output, assessing relative differences between the two aphid species.

Chapter 4

How do Loss of function Mutations in At*AAP1* and At*AAP2* Transporter Genes Affect Aphid Performance in two aphid species, *Myzus persicae* and *Brevicoryne brassicae*?

4.1 Abstract

Manipulation of host plant amino acid concentration in the phloem is thought to potentially determine the overall performance of aphid reproductive output and growth rate. Studies were carried out in order to investigate how manipulating amino acid transport by using insertional mutations within the amino acid permease transporter genes *AAP1* and *AAP2* would affect the aphid performance of two comparatively different aphid species, *M. persicae* and *B. brassicae*.

No significant differences in aphid weight were observed for *M. persicae* at 24, 72 and 120 hours when feeding on *aap1* and *aap2* plants compared with wildtype plants ($p > 0.05$). The mean weight of *B. brassicae* raised on *aap1* plants did not differ significantly at 24 hours compared to those raised on wildtype plants ($p > 0.05$), however significant differences were found at 72 and 120 hours ($p < 0.000$). The mean weight of *B. brassicae* raised on *aap2* plants did not differ significantly at 24 and 72 hours ($p > 0.05$), however at 120 hours the mean weight did differ significantly when compared to those

raised on wildtype plants ($p < 0.000$). Both *M. persicae* and *B. brassicae* feeding on *aap1* and *aap2* mutants did not show any significant differences in their bodily dimensions when compared to their growth on wildtype, control plants ($p > 0.05$).

While studying aphid reproductive output, the intrinsic rate of increase of *M. persicae* feeding on *aap1* and *aap2* plants was not significantly different when compared to their intrinsic rate of increase on control plants, $p = 0.2361$ and $p = 0.4089$ respectively. The intrinsic rate of increase of *B. brassicae* feeding on *aap1* and *aap2* was also found not to be significantly different when compared to their intrinsic rate of increase on control plants, $p = 0.7855$ and $p = 0.6453$ respectively.

When assessing aphid performance at a whole population level, *M. persicae* freely infesting *aap1* and *aap2* mutants with control plants showed no significant differences in whole population mass ($p = 0.829$ and $p = 0.767$ respectively). Similarly *B. brassicae* freely infesting *aap1* and *aap2* mutants showed no differences in their total population mass when compared to the total population mass on control plants ($p = 0.666$ and $p = 0.499$ respectively). When studying aphid establishment, both aphid species showed significantly lower levels of establishment on *aap1* and *aap2* plants when compared to establishment on wildtype plants ($p = 0.001$).

4.2 Introduction

4.2.1 Relationships between Aphid Diet and Aphid Performance - Using Aphid Size and Weight as an Indicator of Aphid Performance at Individual and Population Level

The diet of many phytophagous insects is nutritionally inadequate or unbalanced. As previously discussed in chapter one, for many insects nitrogen sequestering processes are generally inefficient. In order to meet dietary requirements of nitrogen from a host plant which is typically nitrogen limited, most herbivores must eat excessive quantities. This compensation for inadequate diet cannot continue indefinitely and therefore the level at which an insect can obtain nitrogen from its host plant will determine the performance of the feeding insect along with the whole population dynamics (McNeill and Southwood, 1978, Mattson, 1980 and Strong et al., 1984). Similarly, the performance of an aphid population is dictated by various factors including the nutritional quality of their diet and the aphid's ability to utilise this diet within their specific plant aphid interaction (Prosser et al., 1992; Abisgold et al., 1994; Girousse and Bournoville 1994; Febvay et al., 1999).

According to previous studies aphids are able to identify the nutritional quality of a host plant by the sieve element amino acid concentration (Nowak and Komor, 2010). It is the manipulation of sieve element amino acid concentration which is thought to dictate whether or not a plant is

accepted by a probing aphid as well as potentially determining the overall performance of the aphid in terms of its reproductive output (Awmack and Leather, 2002) and growth rate. The fact that nitrogen content within the aphid diet influences aphid feeding behaviour and performance has been demonstrated both in plants and in artificial diets (Mittler and Dadd, 1962; Mittler and Dadd, 1963; Dadd and Mittler, 1965; Dadd and Mittler, 1966; Van Emden, 1966; Woolridge and Harrison, 1968; Srivastava and Auclair, 1974; Weibull, 1987).

4.2.2 Intrinsic Rate of Increase as a Measure of Aphid Performance

As previously discussed, the factors affecting the performance of an aphid population are complex. In order to quantify the level of aphid performance the 'rate of increase of an aphid population' can be used as a reliable indicator. Using the rate of increase of a population as an indicator of aphid performance relies upon pre-specified physical conditions and is also more commonly referred to as the rate of natural increase (r_m)(Birch, 1948).

The method advocated by Birch (1948) required two sets of data, the first being a female life table containing survival rates for each age class of the individuals being measured (l_x) and the second being the number of offspring being produced at each age class (M_x). Due to the age specific nature of survival and fecundity, the population being measured must have a stable age structure in order to make an accurate assessment of the rate

of natural increase (Howe, 1953; Birch, 1948). This method of quantifying the rate of natural increase was time consuming and difficult to accurately achieve and so in 1977 a new, more straightforward method was developed by Wyatt and White in order to measure the 'intrinsic rate of increase' (r_m) of aphid populations.

This simplified method does not rely on the use of life tables but instead measures the number of days until an individual aphid begins reproducing and also the number of offspring produced by the test aphid per day. Certain assumptions need to be made in order to use this method including the assumption that the first nymph produced in the first few days of reproduction will have the greatest contribution to its r_m value, deemed to be approximately 95% (Wyatt and White, 1977) because of the aphid's limited lifespan. Other factors that are believed to be accounted for in this method of r_m calculation include survival and fecundity.

This later method developed by Wyatt and White has been used with much success in various aphid studies (Xu and Feng, 2002; Robson et al., 2007) and will be used within this chapter in order to gain a measure of the aphid performance of *M. persicae* and *B. brassicae* on *aap1* and *aap2* plants.

4.2.3 Aphid Establishment Analysis

Although the previously described method of 'r_m analysis' allows for a convenient and precise way of quantifying the rate of increase of an aphid

population this method does not focus on the basic establishment of an aphid population on its host plant. Although the intrinsic rate of increase analysis provides a measure of reproductive output, the aphids that failed to establish could not be included in this method and the fact they could not successfully establish and reproduce on the host plant would be ignored. The establishment analysis therefore provides a way of quantifying the level of reproductive success and failure which is not included by the other methods used. The level of successfully reproducing aphids, those deemed to have established, and the level of aphids that are failing to establish are both considered in this method.

This analysis aims to assess whether or not there was any difference in the level of establishment success of those nymphs left to infest the *aap1 or aap2* plants compared to those on the wildtype plants. This was measured in the ability of the nymph to survive on the genotype and reach a stage where nymphs were being produced daily, allowing for a 'success percentage' to be determined.

4.2.4 Aphid Infestation Analysis – Aphid performance at a Population Level

Previously discussed techniques of quantifying aphid performance have depended upon the periodic manipulation of experimental aphids in order to observe, weigh, measure or count. These actions throughout the course of the experimental period could, although necessary, be considered to be

problematic because of the element of interference which they potentially create within the experimental situation. In order to assess aphid performance with the minimal level of human interference the whole population aphid performance technique was developed. This method involved the undisturbed growth of an aphid population on a test plant before measuring the population performance as a whole in terms of its collective weight. This population level technique allowed for a view of undisturbed aphid performance on the host plant genotype it was colonising, minimising the potentially deleterious effects of manipulating aphids periodically throughout the duration of the experiment. A technique designed to provide a more natural insight into aphid performance on a host plant of a particular genotype.

4.3 Chapter Aims

In the previous chapter various techniques were used to study how mutations to the AtAAP1 and AtAAP2 transporters affected aphid feeding behaviour and metabolism. The results from the feeding behaviour study indicated that plant-aphid interactions in terms of aphid performance and reproductive capacity may be significantly affected by the mutations to the AAP1 and AAP2 transporters.

The first aim of the chapter was to investigate the level of aphid performance in terms of aphid growth. Aphid growth was investigated by measuring weight and body dimensions of *M. persicae* and *B. brassicae* when

raised on *aap1* and *aap2* plants compared to the wildtype control plants. These basic measures of aphid bodily growth were assumed to provide an early measure of aphid performance.

The second aim of the chapter was to investigate the overall reproductive fitness of *M. persicae* and *B. brassicae* when feeding on *aap1* and *aap2* mutants using the 'Intrinsic rate of increase (r_m) technique (Wyatt and White, 1977).

The final aims of the chapter were to determine two other measures of aphid performance by analysing the level of aphid establishment and the total aphid population performance of the two aphid species when feeding on *aap1, aap2* and control plants. The overall objective of the chapter was to quantify all aspects of aphid performance in both aphid species, at all levels from the individual to the population level, when feeding on *aap1, aap2* and control plants.

4.4 Materials and Methods

4.4.1 Aphid Growth Rate – Bodily Dimension and Weight Analysis

4.4.1.1 Plant Material

The wildtype, *aap1* and *aap2* plants were cultivated according to the methods featured in section 2.4.1. These plants were selected for use in the performance analysis when at their optimum stage of growth (25-28 days old from sowing).

4.4.1.2 Aphid Culture

The *M. persicae* and *B. brassicae* used in the aphid growth analysis were produced and used under the methods in section 3.4.1.

4.4.1.3 Body Dimension and Weight Analysis

This experiment was an example of 'destructive sampling', 45 plants in total were used, 15 *aap1* plants, 15 *aap2* plants and 15 wildtype control plant (5 replicates of each plant for each time point of data collection). At the start of the experiment five adult, apterous *M. persicae* were placed on the middle stem region of each plant. Each plant was caged individually using the method described in figure 4.3 (see figure 4.3). These cages were

transferred to the controlled growth room at 20-22ºC with an 18-6 light:dark regime. The plants had a continuous supply of water throughout the duration of the experiment. After 24 hours all adults were removed from each plant. Following the removal of the adult aphids, five *aap1, aap2* and wildtype plants were selected and from these plants all of the first instar nymphs that had been produced were removed with a fine paintbrush and kept separately within culture dishes for one hour to allow honey dew excretion to cease. After an hour the 15 sets of nymphs were photographed using a microscope and a high resolution camera. Photographs were viewed with U-lead Photoexplorer® software. The nymphs within the 15 groups were photographed individually so that their individual relative dimensions could later be derived using Adobe Photoshop®**.** The aphid dimensions that were measured include, 1) width from eye to eye, 2) length from cornicle to forehead, 3) width of body across widest point, 4) width of body across narrowest point and 5) length of body from forehead to cauda (see figure 4.1).

Figure 4.1 – Labelled photograph to illustrate the position of aphid body measurements taken. Measurements included,

1) width from eye to eye (blue)

2) length from cornicle to forehead (red)

3) width of body across widest point (pink)

4) width of body across narrowest point (yellow)

5) length of body from forehead to cauda (black)

After being photographed each group of nymphs was weighed on a balance (sensitivity to 0.00001g/10µg) and the total and average weight for the nymphs recorded. After being weighed the nymphs were disposed of.

After 72 hours another 15 plants were removed from their individual cages, 5 replicates of each genotype, *aap1, aap2* and control plants. As previously described, the groups of nymphs from each plant were removed using a fine paintbrush and contained within separate culture dishes for one hour. After this hour the nymphs were photographed and weighed as described previously before being disposed of. Finally after 120 hours the last 15 plants were removed from their individual cages and the nymphs photographed and weighed according to the previously described method. As before these nymphs were then disposed of after use.

4.4.1.4 Statistical Analysis

Having determined the data was normal using an Anderson-Darling test for normality, the aphid growth weights and bodily dimension measurements were analysed using a General Linear Model (GLM) ANOVA. See appendices 3.1 and 3.2 for details of this statistical analysis.

4.4.2 Intrinsic Rate of Increase Analysis

4.4.2.1 Plant Material

The wildtype, *aap1* and *aap2* plants were cultivated according to the methods featured in section 2.4.1. These plants were selected for use in the performance analysis when at their optimum stage of growth (25-28 days old from sowing).

4.4.2.2 Aphid Culture

The *M. persicae* and *B. brassicae* used in the r_m analysis were produced and used under the methods in section 3.4.1.

4.4.2.3 Assessing Intrinsic Rate of Increase (r_m)

At the start of the experiment two apterous *M. periscae* adults were placed using a fine paintbrush onto the middle section stem region of each test plant. Each of the 56 plants (14 *aap1* plants, 14 *aap2* plants and 28 wildtype control plants aged between 25-28 days old) and their two aphids were contained within the set up shown in figure 4.2.

Figure 4.2 - This photograph illustrates the method of caging used throughout the $\mathbf{r_m}$ experiment. Two plastic pint sized cups were used to form a sealed individual cage for each test plant. One plastic cup was altered by removing the base and replacing with gauze to allow for air flow, the other plastic cup was left intact to form the base of the cage into which the plant was placed and the upturned second cup was taped securely onto using masking tape.

The plants were arranged in a random block design and left in the same controlled growth room. The plants received an initial 10 ml of water to their soil via syringe and were watered every third day with the same

amount. After the initial twenty four hours all adults were removed to leave one first instar nymph on each plant. Excess nymphs produced by either of the two adults were also removed and disposed of. The remaining nymphs were then monitored and examined at the same time each day until the onset of their reproductive stage. The number of days up until this point of (d) was noted and following this point the offspring of the reproductive adults were counted and removed daily at the same time each day for 'd' days (Md). The aphids relative performance was then calculated using Wyatt and Whites (1977) calculation of the 'intrinsic rate of increase' (r_m).

$$r_m = \frac{0.738 \ X \ (\ln Md)}{d}$$

d = the number of days taken for the aphids to reach their reproductive stage from birth

Md = the number of nymph offspring produced during the monitored reproductive stage of 'd' days

Any aphids that did not survive throughout the entirety of the experiment were completely excluded from final analysis. However individual cases of mortality were recorded. The above method was then repeated in its entirety using *B. brassicae* adults in place of *M. periscae*.

4.4.2.4 Statistical Analysis

Using an Anderson-Darling normality test the aphid intrinsic rate of increase data was found not to be normally distributed, therefore a non-parametric statistical test was required. A Mann-Whitney U test was used to analyse the intrinsic rate of increase for both aphid species on *aap1, aap2* and wildtype plants. See appendix 3.3 for this statistical analysis.

4.4.3 Aphid Establishment Analysis

4.4.3.1 Plant Material

The *aap1*, *aap2 and* wildtype control plants were cultivated according to the methods featured in section 2.4.1. These plants were selected for use in the aphid establishment analysis between 25-28 days old from sowing.

4.4.3.2 Aphid Culture

The *B. brassicae* and *M. persicae* used in the aphid establishment analysis were produced and used under the methods in section 3.4.1.

4.4.3.3 Assessing Aphid Establishment

Adult aphids were taken from their individual cultures and were placed onto individually contained wildtype *A. thaliana* leaves. The microcosm culture

technique chosen for this purpose was that of an 'Austin tube' culture (see figure 4.3). The Austin tube set up combined two pieces of hard clear tubing which slotted together to enclose a leaf that had been cut at its petiole and wrapped in this region in moist cotton wool. This cotton wool acted as a wick as it is stood in a honeycomb structure which was then placed into a tray of water. This method kept the individually cultured leaf fresh for up to a week. Once in the Austin tubes the adult aphids would go on to produce nymphs over a 24 hour period. After this 24 hour period the first instar nymphs were relocated to fresh single leaf cultures of either *aap1, aap2 or* wildtype plants where they were monitored and moved accordingly to fresh leaves over the next 15-20 days. During this period of time the first instar nymphs established on their respective culture plant genotype. The nymphs settled to feed, grow, moult and eventually become nymph producing adults themselves.

4.4.3.4 Statistical Analysis

Data was collected in scoring sheets before being tested for normality using an Anderson-Darling test. As the data was found to be normal, it was collectively analysed using a General Linear Model (GLM) ANOVA. Separate interactions of the aphid species and plant genotypes were then analysed using one-way ANOVA. See appendix 3.4 for details of this statistical analysis.

Figure 4.3 - A photograph showing a typical Austin tube culture of aphids reared on individual leaf samples. Individual microcosm cultures of leaf samples and their aphids were supported in an upright fashion within water using a honeycomb weave structure base. This allowed for water to be wicked upwards through the cotton wool supplying the excised leaf.

4.4.4 Aphid Infestation Analysis

4.4.4.1 Plant Material

The *aap1, aap2* and wildtype plants were cultivated according to the methods featured in section 2.4.1. These plants were selected for use in aphid infestation analysis when between 25-28 days old from sowing.

4.4.4.2 Aphid Culture

The *M. persicae* and *B. brassicae* used in the infestation analysis were produced and used under the methods in section 3.3.1.

4.4.4.3 Quantifying Aphid Infestations

Aphids of both species taken from their respective cultures were initially cultured in Austin tubes (see figure 4.3) in order to allow for the production of adult aphids of the same 'synchronised' age to be used for the artificial infestation of caged plants of differing genotypes, *aap1*, *aap2 and* wildtype control plants. Upon harvesting enough adult aphids, plants of both genotypes were caged in the manner described previously and within each cage a single aphid was placed onto the upper surface on the largest rosette leaf. The cage was then sealed with masking tape for the 3 week duration of the experiment (see figure 4.4). The plants were watered by tray, water

being taken up directly by the plant through the irrigation holes of the lower part of the pot cage.

After the infestation period the cages were unsealed one at a time and each plant dissected into smaller parts. These parts were then brushed with a large blusher style cosmetic brush to remove any aphids infesting the plant section. These aphids were brushed into a funnel which was firmly placed into a pre-weighed 1.5ml microfuge tube. This acted as a collection vessel which upon acquiring the entire aphid infestation for a plant would be weighed on a micro-balance to determine the biomass of that plants own aphid infestation. This process of dissecting the plant and brushing the aphids off into a funnel to collect in pre-weighed 1.5 ml eppendorfs was repeated for each plant in the sample.

4.4.4.4 Statistical Analysis

Data was collected in scoring sheets and tested for normality using an Anderson-Darling test. As the data was found to be normal, it was analysed using a General Linear Model (GLM) ANOVA. One-way ANOVAs were used to analyse specific interactions between aphid species and plant genotypes. See appendix 3.5 for the statistical analysis.

Figure 4.4- An image showing a typical infestation set up with the type of caging used to contain single plant infestations. The cages were arranged with a randomised block design.

4.5 Results

4.5.1 Aphid Growth

4.5.1.1 Weight analysis of *M. persicae* raised on *aap1, aap2* and control plants

The mean weight of *M. persicae* raised on *aap1* plants did not differ significantly at 24, 72 and 120 hours when compared to those raised on wildtype plants ($p > 0.05$). Similarly, the mean weight of *M. persicae* raised on *aap2* plants did not differ significantly at 24, 72 and 120 hours when compared to those raised on wildtype plants ($p > 0.05$). See table 4.5.

Plant Genotype	Mean Aphid Weight (µg)		
	24h	72h	120h
aap1 (n=21)	38.4 (±0.590)	97.6 (±0.851)	204.4 (±0.940)
aap2 (n=20)	35.7 (±0.691)	100.1 (±0.596)	213.5 (±2.25)
Col (n=25)	38.2 (±0.605)	112.5 (±1.36)	197.5 (±1.16)

Table 4.5 – A table showing the mean weight of *M. persicae* on *aap1, aap2* and Col0 at three time points, 24, 72 and 120 hours. Values in brackets are ± s.e. Data was analysed using a GLM ANOVA.

4.5.1.2 Weight analysis of *B. brassicae* on *aap1, aap2* and control plants

The mean weight of *B. brassicae* raised on *aap1* plants did not differ significantly at 24 hours compared to those raised on wildtype plants ($p > 0.05$), however significant differences were found at 72 and 120 hours ($p < 0.000$). The mean weight of *B. brassicae* raised on *aap2* plants did not differ significantly at 24 and 72 hours ($p > 0.05$), however at 120 hours the mean weight did differ significantly when compared to those raised on wildtype plants ($p < 0.000$). See table 4.6.

Plant Genotype	Mean Aphid Weight (µg)		
	24h	72h	120h
aap1 (n=25)	31.7 (±0.617)	94.8 * (±0.812)	190.0 * (±1.45)
aap2 (n=23)	304.3 (±0.511)	98.8 (±0.675)	210.1 * (±0.948)
Col (n=20)	32.2 (±0.648)	100.0 (±0.772)	203.8 (±0.842)

Table 4.6 – A table showing the mean weight of *B. brassicae* on *aap1, aap2* and Co0 at three time points, 24, 72 and 120 hours. Data was analysed using a GLM ANOVA. Statistically significant differences are indicated with an asterisk (*). Values in brackets are ± s.e.

4.5.1.3 Body dimension analysis of *M. persicae* on *aap1, aap2* and control plants

After 24 hours *M. persicae* feeding on *aap1* and *aap2* were not significantly different in any of the five dimensions measured when compared to those raised on the control plants ($p > 0.05$) (see figure 4.7). This statistical analysis was performed using a General Linear Model (GLM) ANOVA. For *aap1,* aphid body dimensions 1-5 measured 1.94, 5.32, 2.47, 1.90 and 5.02 units respectively. Dimensions 1-5 for aphids on *aap2* measured 1.92, 5.26, 2.30, 1.87 and 4.99 units respectively. In comparison the measurements for dimensions 1-5 aphids on control plants were 1.89, 5.00, 2.33, 1.78 and 4.75 units respectively.

At 72 hours *M. persicae* feeding on *aap1* and *aap2* were also found not to differ significantly in any of the five dimensions measured when compared to those raised on the control plants ($p > 0.05$) (see figure 4.8). Again, this statistical analysis was performed using a General Linear Model (GLM) ANOVA. For *aap1,* aphid body dimensions 1-5 measured 2.29, 7.61, 3.57, 2.23 and 7.17 units respectively. Dimensions 1-5 for aphids on *aap2* measured 2.28, 7.75, 3.55, 2.45 and 7.39 respectively. In comparison the measurements for dimensions 1-5 for aphids on control plants were 2.30, 7.95, 3.71, 2.27and 7.45 units respectively.

At 120 hours *M. persicae* feeding on *aap1* and *aap2* were also found (when analysed with a General Linear Model (GLM) ANOVA) not to differ significantly in any of the five dimensions measured when compared to

those raised on the control plants ($p > 0.05$) (see figure 4.9). For *aap1,* aphid body dimensions 1-5 measured 2.50, 9.45, 4.46, 2.42 and 8.88 respectively. Dimensions 1-5 for aphids on *aap2* measured 2.53, 9.64, 4.50, 2.42 and 8.77 units respectively. In comparison the measurements for dimensions 1-5 for aphids on control plants were 2.55, 9.82, 4.66, 2.46 and 9.14 units respectively.

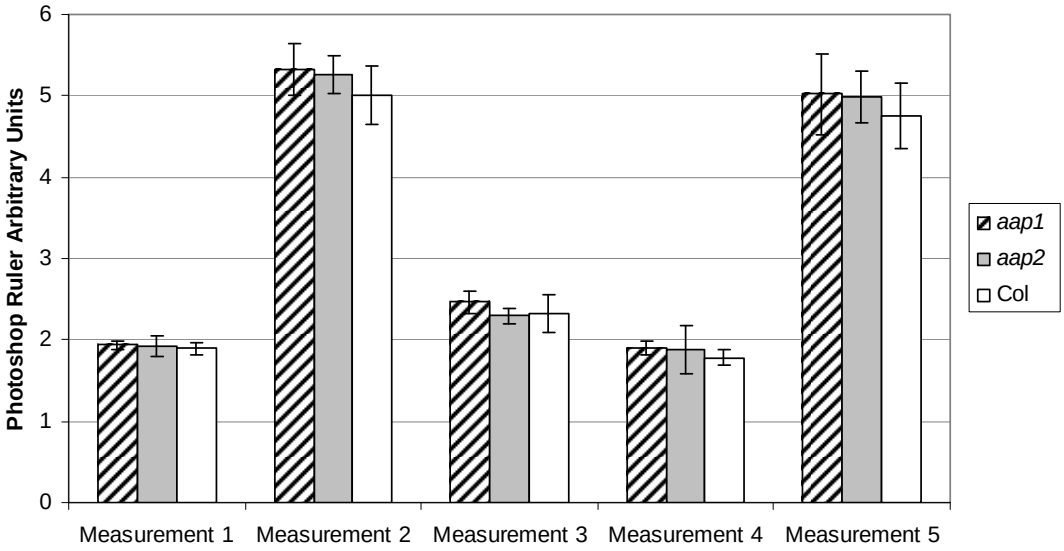

Figure 4.7 – Body Dimensions of *M. persicae* after 24 hours of feeding on *aap1* (n=16), *aap2* (n=16) and wildtype control plans, Col0 (n=21). Error bars represent ± s.e. Data analysed using a General Linear Model (GLM) ANOVA indicated no statistically significant differences ($p > 0.05$). (Refer to figure 4.1 for measurement descriptions).

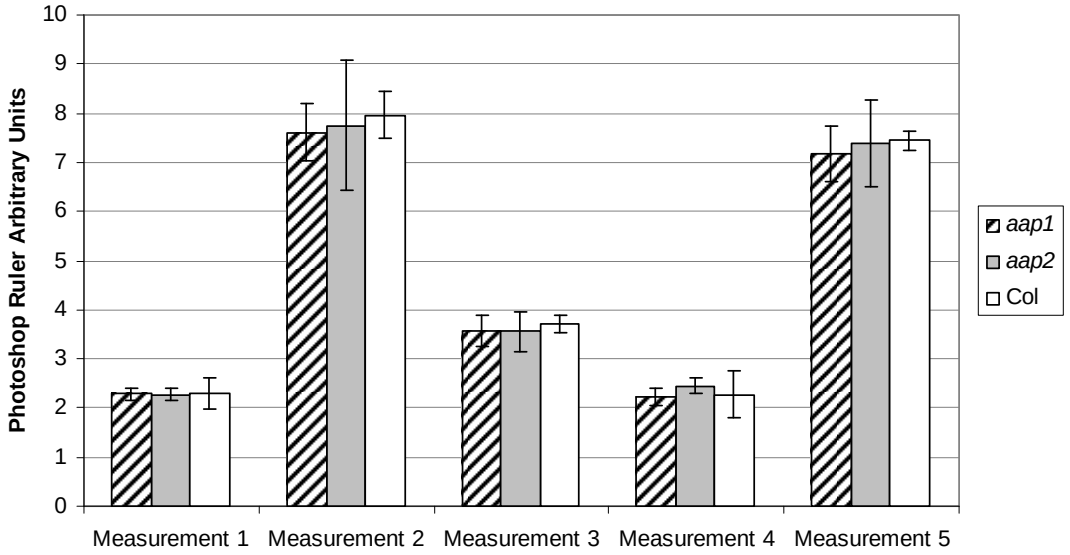

Figure 4.8 – Body Dimensions of *M. persicae* after 72 hours of feeding on *aap1* (n=20), *aap2* (n=20) and wildtype control plants, Col0 (n=17). Error bars represent ± s.e. Data analysed using a General Linear Model (GLM) ANOVA indicated no statistically significant differences ($p > 0.05$). (Refer to figure 4.1 for measurement descriptions).

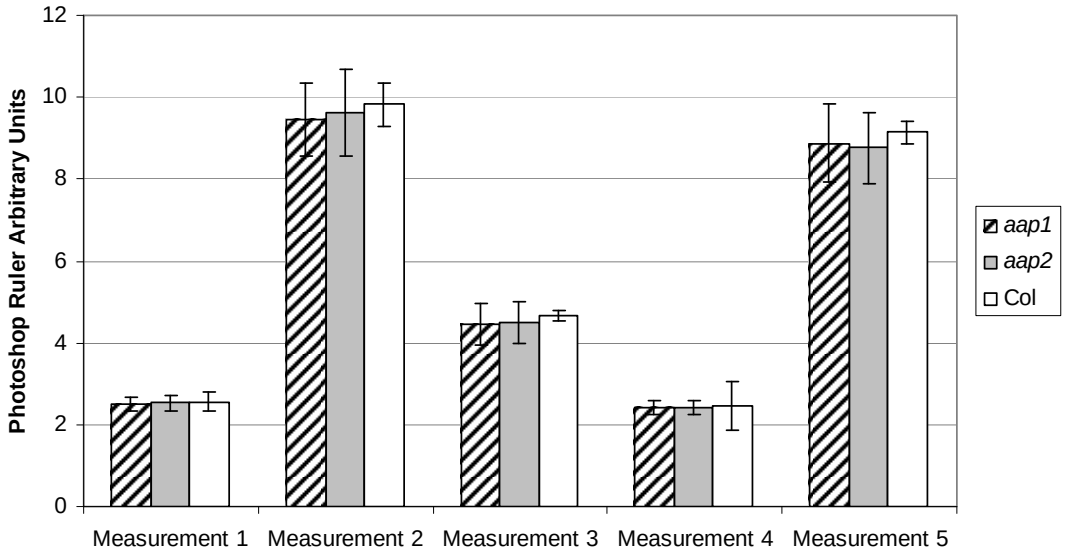

Figure 4.9 – Body Dimensions of *M. persicae* after 120 hours of feeding on *aap1* (n=20), *aap2* (n=20) and wildtype control plants, Col0 (n=17). Error bars represent ± s.e. Data analysed using a General Linear Model (GLM) ANOVA indicated no statistically significant differences ($p > 0.05$). (Refer to figure 4.1 for measurement descriptions).

4.5.1.4 Body dimension analysis of *B. brassicae* on *aap1, aap2* and control plants

After 24 hours *B. brassicae* feeding on *aap1* and *aap2* were not found to be significantly different in any of the five dimensions measured when compared to those raised on the control plants ($p > 0.05$) (see figure 4.10). Data was analysed using a General Linear Model (GLM) ANOVA. For *aap1* aphid body dimensions 1-5 measured 1.96, 5.55, 2.40, 1.92 and 4.79 units respectively. Dimensions 1-5 for aphids on *aap2* measured 1.92, 5.32, 2.32, 1.84 and 4.69 units respectively. In comparison the measurements for dimensions 1-5 aphids on control plants were 1.92, 5.18, 2.44, 1.83 and 4.86 units respectively.

At 72 hours *B. brassicae* feeding on *aap1* and *aap2* were also found not to differ significantly in any of the five dimensions measured when compared to those raised on the control plants ($p > 0.05$). Again, data was analysed using the General Linear Model (GLM) ANOVA. For *aap1* aphid body dimensions 1-5 measured 2.31, 7.59, 3.65, 2.23 and 7.20 units respectively. Dimensions 1-5 for aphids on *aap2* measured 2.29, 7.61, 3.62, 2.40 and 7.22 respectively. In comparison the measurements for dimensions 1-5 for aphids on control plants were 2.36, 7.75, 3.66, 2.54 and 7.21 units respectively.

At 120 hours *B. brassicae* feeding on *aap1* and *aap2* were also found (when analysed using General Linear Model (GLM) ANOVA) not to differ significantly in any of the five dimensions measured when compared to those raised on the control plants ($p > 0.05$). For *aap1* aphid body dimensions 1-5 measured

2.39, 9.59, 4.0, 2.39 and 8.85 respectively. Dimensions 1-5 for aphids on *aap2* measured 2.44, 9.52, 4.50, 2.40 and 8.74 units respectively. In comparison the measurements for dimensions 1-5 for aphids on control plants were 2.49, 9.79, 4.66, 2.37 and 9.10 units respectively.

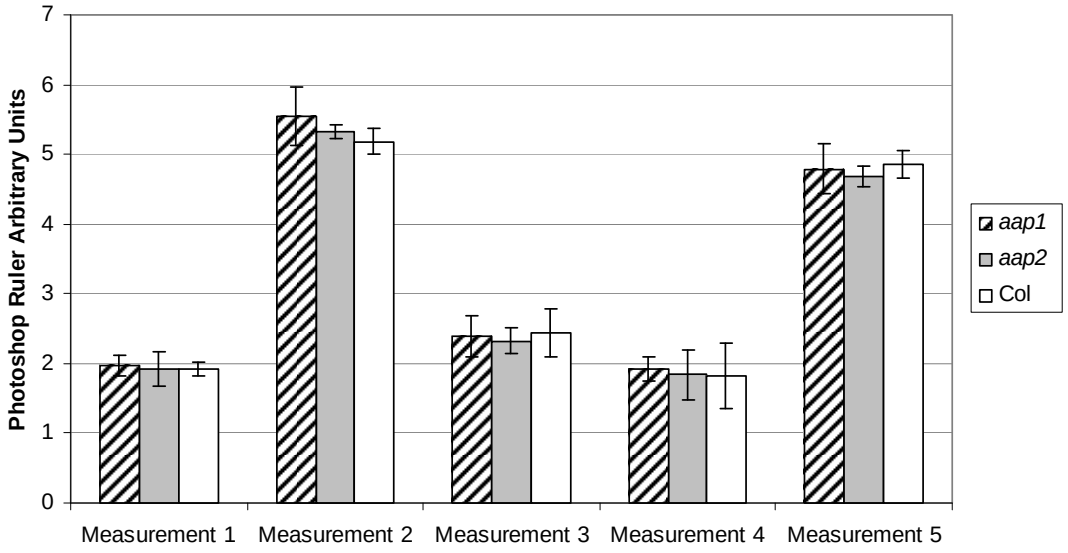

Figure 4.10 – Body Dimensions of *B. brassicae* after 24 hours of feeding on *aap1* (n=19), *aap2* (n=20) and wildtype control plants, Col0 (n=20). Error bars represent ± s.e. Data analysed using a General Linear Model (GLM) ANOVA indicated no statistically significant differences ($p > 0.05$). (Refer to figure 4.1 for measurement descriptions).

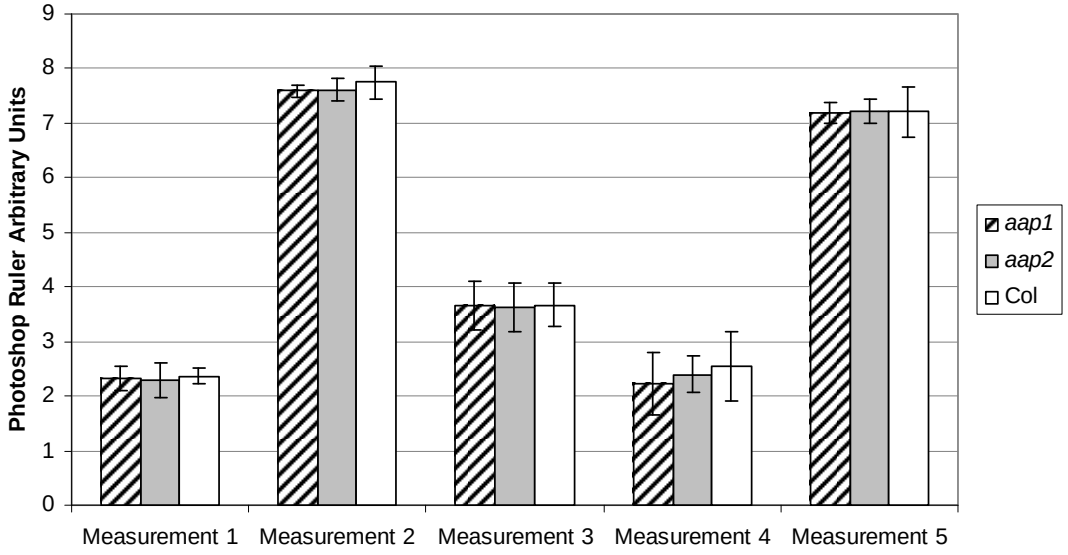

Figure 4.11 – Body Dimensions of *B. brassicae* after 72 hours of feeding on *aap1* (n=20), *aap2* (n=20) and wildtype control plants, Col0 (n=21). Error bars represent ± s.e. Data analysed using a General Linear Model (GLM) ANOVA indicated no statistically significant differences ($p > 0.05$). (Refer to figure 4.1 for measurement descriptions).

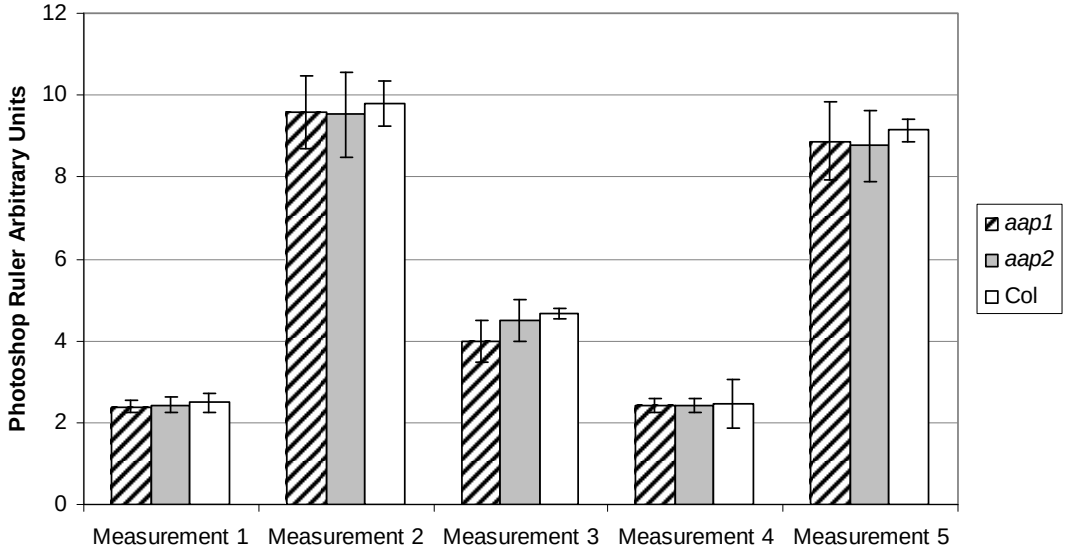

Figure 4.12 – Body Dimensions of *B. brassicae* after 120 hours of feeding on *aap1* (n=20), *aap2* (n=20) and wildtype control plants, Col0 (n=19). Error bars represent ± s.e. Data analysed using a General Linear Model (GLM) ANOVA indicated no statistically significant differences ($p > 0.05$). (Refer to figure 4.1 for measurement descriptions).

4.5.2 Aphid Reproductive Performance

4.5.2.1 Intrinsic Rate of Increase Analysis

The intrinsic rate of increase of *M. persicae* feeding on *aap1* and wildtype plants was 0.295 (SE ± 0.00835) and 0.282 (SE ± 0.00961) respectively. The data was analysed using a Mann Whitney U-test and was found to be not significant ($p = 0.2361$).

The intrinsic rate of increase of *B. brassicae* feeding on *aap1* and wildtype plants was 0.268 (SE ± 0.0109) and 0.276 (SE ± 0.0120) respectively. Again, using a Mann Whitney U-test, these values were also found to be not significant ($p = 0.7855$).

The intrinsic rate of increase of *M. persicae* feeding on *aap2* and control plants was 0.278 (SE ± 0.0105) and 0.287 (SE ± 0.00956) respectively. According to the Mann Whitney U-test these values were not significant ($p = 0.4089$).

The intrinsic rate of increase of *B. brassicae* feeding on *aap2* and control plants was 0.288 (SE ± 0.0115) and 0.297 (SE ± 0.0126) respectively. Similarly, after analysis using the Mann Whitney U-test, these values were also found to be not significant ($p = 0.6453$).

4.5.2.2 Cumulative number of nymphs produced by *M. persicae* feeding on *aap1* and wildtype plants

The mean cumulative number of nymphs produced during the experimental period by *M. persicae* adults feeding on *aap1* mutants did not differ significantly from *M. persicae* adults feeding on wildtype plants ($p = 0.8182$). See figure 4.13.

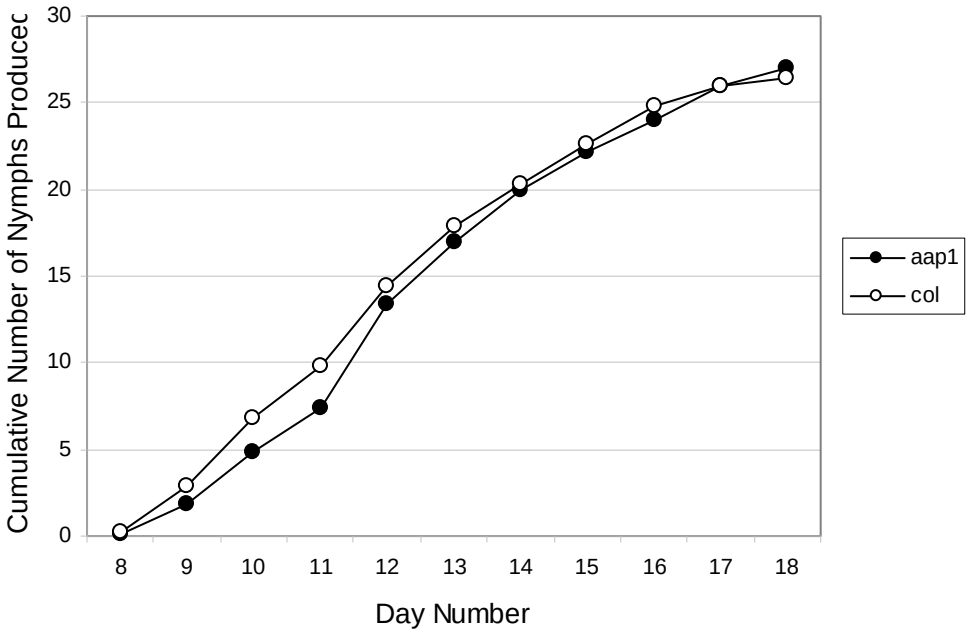

Figure 4.13 – Cumulative number of nymphs produced by *M. persicae* on *aap1 (n=14)* and control plants (n=14) over the 18 days experimental period. When analysed with a Mann Whitney U-test no significant effect of genotype was observed (p=0.8182). Error bars represent ± s.e however are not visible due to the graph scale and symbol size.

4.5.2.3 Cumulative number of nymphs produced by *M. persicae* feeding on *aap2* and wildtype plants

The mean cumulative number of nymphs produced during the experimental period by *M. persicae* adults feeding on *aap2* mutants did not differ significantly from *M. persicae* adults feeding on wildtype plants ($p = 0.7975$). See figure 4.14.

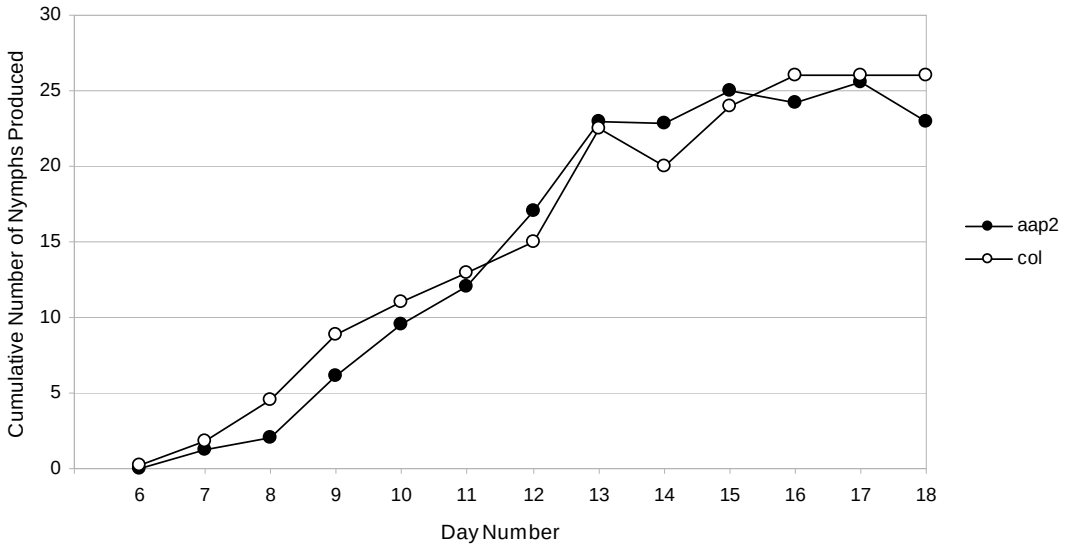

Figure 4.14 – Cumulative number of nymphs produced by *M. persicae* on *aap2* (n=14) and control plants (n=14) over the 18 days experimental period. When analysed with a Mann Whitney U-test no significant effect of genotype was observed (p=0.7975). Error bars represent ± s.e however are not visible due to the graph scale and symbol size.

4.5.2.4 Cumulative number of nymphs produced by *B. brassicae* feeding on *aap1* and wildtype plants

The mean cumulative number of nymphs produced during the experimental period by *B. brassicae* adults feeding on *aap1* mutants did not differ significantly from *B. brassicae* adults feeding on wildtype plants ($p = 0.5993$). See figure 4.15.

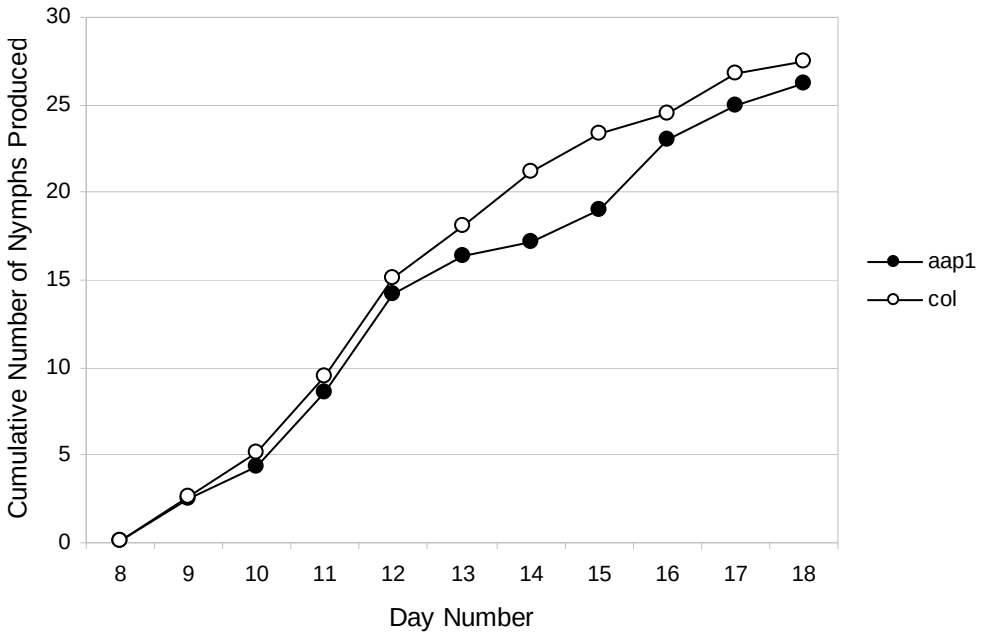

Figure 4.15 – Cumulative number of nymphs produced by *B. brassicae* on *aap1 (n=13)* and control plants (n=14) over the 18 days experimental duration. When analysed with a Mann Whitney U-test no significant effect of genotype was observed (p=0.5993). Error bars represent ± s.e however are not visible due to the graph scale and symbol size.

4.5.2.5 Cumulative number of nymphs produced by *B. brassicae* on *aap2* and wildtype plants

The mean cumulative number of nymphs produced during the experimental period by *B. brassicae* adults feeding on *aap2* mutants did not differ significantly from *B. brassicae* adults feeding on wildtype plants ($p = 0.6032$). See figure 4.16.

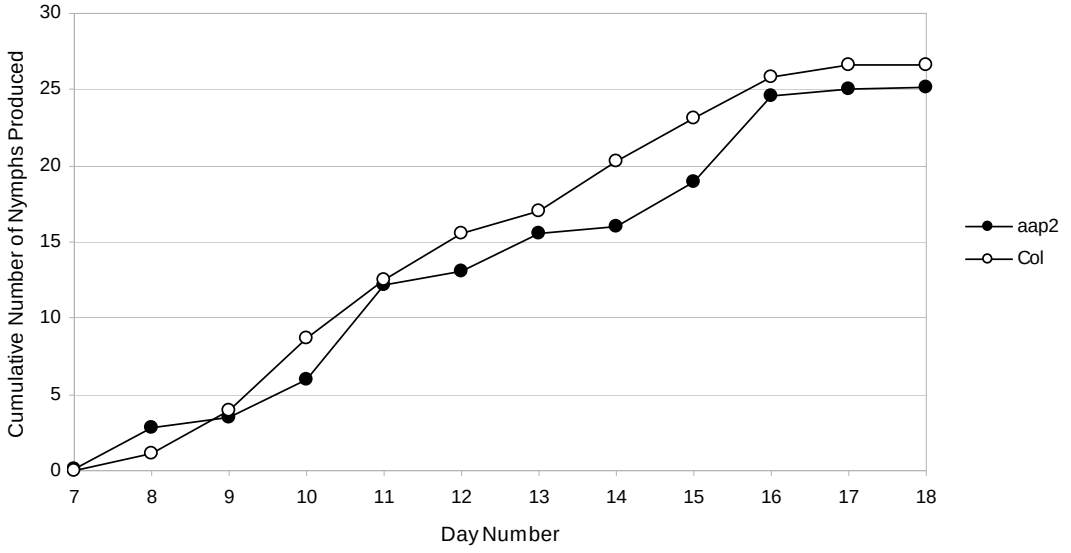

Figure 4.16 – Cumulative number of nymphs produced by *B. brassicae* on *aap2* (n=14) and control plants (n=14) over the 18 days experimental duration. When analysed with a Mann Whitney U-test no significant effect of genotype was observed (p=0.6032). Error bars represent ± s.e however are not visible due to the graph scale and symbol size.

4.5.3 Aphid Establishment of *M. persicae* and *B. brassicae* on *aap1, aap2* and wildtype plants

The mean levels of aphid establishment (the percentage of nymphs successfully able to survive and reach the stage where nymphs were being produced daily) for *M. persicae* on *aap1* plants was determined to be 84.76% (±0.37), compared to 83.10% (SE±0.37) on *aap2* plants and 90.24% (SE±0.26) on wildtype plants. After analysis using a one-way ANOVA the difference in the levels of *M. persicae* establishment between *aap1* and wildtype plants was found to be significantly different ($p = 0.021$). Similarly the difference in the levels of *M. persicae* establishment between *aap2* and wildtype plants was also found to be significantly different ($p = 0.003$). Refer to figure 4.17.

The mean levels of aphid establishment for *B. brassicae* on *aap1* plants was determined to be 85% (SE±0.36), compared to 85.24% (SE±0.41) on *aap2* plants and 90.71% (SE±0.32) on wildtype plants. Similarly, after analysis using a one-way ANOVA the difference in the levels of *B. brassicae* establishment between *aap1* and wildtype plants was found to be significantly different ($p = 0.022$). Similarly the difference in the levels of *B. brassicae* establishment between *aap2* and wildtype plants was also found to be significantly difference ($p = 0.041$). Refer to figure 4.17.

Data analysed using a General Linear Model (GLM) ANOVA indicated a statistically significant difference in the establishment of *M. persicae* and *B. brassicae* on the different plant genotypes *aap1, aap2* and wildtype plants

(p = 0.001). Both aphid species showed significantly lower levels of establishment on *aap1* and *aap2* plants when compared to establishment on Col0 wildtype plants. However, the effect of the aphid species on the levels of establishment was not found to be significant (p = 0.506). Refer to figure 4.17.

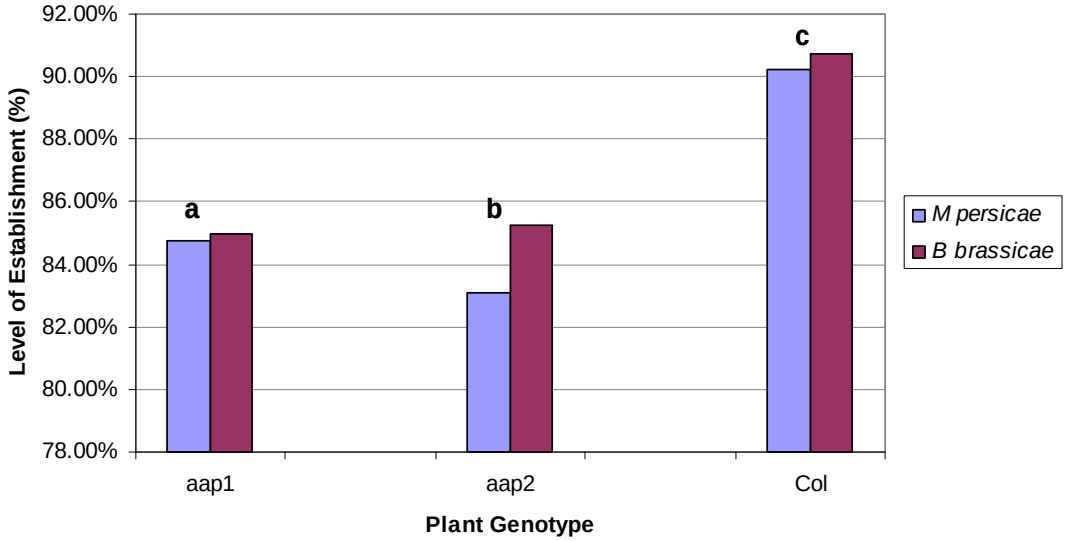

Figure 4.17 – Graph representing the mean levels of *M. persicae* and *B. brassicae* establishment when infesting *aap1* (n=21), *aap2* (n=21) and Col (n=21) plant genotypes. When analysed using a General Linear Model (GLM) ANOVA and one-way ANOVA's statistically significant differences were found. Letters a, b and c refer to ± s.e. (a= *M. persicae* ± 0.37, *B. brassicae* ± 0.36), (b= *M. persicae* ± 0.37, *B. brassicae* ±0.41) and (c= *M. persicae* ± 0.26, *B. brassicae* ±0.32).

4.5.4 Whole Aphid Population Analysis

4.5.4.1 Whole Aphid Population Analysis of *M. persicae* and *B. brassicae* on *aap1, aap2* and wildtype plants

The mean population weights for both *M. persicae* and *B. brassicae* infesting *aap1* plants for a 3 week duration were consistently lower, 10.0 mg (SE±2.5) and 10.4 mg (SE±2.3) respectively, when compared to the mean population weights of *M. persicae* and *B. brassicae* infesting control plants, 10.9 mg (SE±3.1) and 12.2 mg (SE±3.2) respectively. See figure 4.18.

The mean population weights for both *M. persicae* and *B. brassicae* when feeding on *aap1* plants appeared to be comparatively lower than those aphids infesting control plants, however when analysed using a General Linear Model (GLM) ANOVA these results were found not to be significantly different, $p = 0.829$ and $p = 0.666$ respectively. See figure 4.18.

The mean population weights for both *M. persicae* and *B. brassicae* infesting *aap2* plants for a 3 week duration were consistently lower, 9.9 mg (SE±1.1) and 10.0 mg (SE±1.0) respectively, when compared to the mean population weights of *M. persicae* and *B. brassicae* infesting control plants, 10.9 mg (SE±3.1) and 12.2 mg (SE±3.2) respectively. See figure 4.18.

Although the mean population weights for both *M. persicae* and *B. brassicae* when feeding on *aap2* plants showed a trend for comparatively reduced population weights, this data was not significantly different from the

population weights of those aphids infesting control plants when analysed using a General Linear Model (GLM) ANOVA, p = 0.767 and p = 0.499 respectively.

Mean Total Aphid Population Weight (mg)	*aap1*	*aap2*	**Col0**
M. persicae	10.0 (±2.5) n=39	9.9 (±1.1) n=40	10.9 (±3.1) n=40
B. brassicae	10.4 (±2.3) n=40	10.0 (±1.0) n=38	12.2 (±3.2) n=39

Figure 4.18 – Table illustrating the mean total aphid population weights of *M. persicae* and *B. brassicae* when freely infesting *aap1, aap2* and wildtype plants. When analysed using a General Linear Model (GLM) ANOVA no statistically significant differences were found. Values in brackets represent ± s.e.

4.6 Discussion

Aphid growth analysis (measuring aphid weight and body size), r_m analysis (measuring reproductive performance), aphid establishment analysis and whole aphid population analysis were used to assess the effect of *AAP1* and *AAP2* gene knockouts on aphid performance in *M. persicae* and *B. brassicae*. The results of these performance analyses for both aphid species are discussed here.

4.6.1 Aphid Growth – Weight and Body Dimension Analysis

Numerous studies using a wide range of aphid species have shown that the growth of an aphid can be determined by its diet. Previous experiments demonstrate the correlation between reductions in phloem amino acid concentrations and the reduction in performance of the feeding aphid (Chen et al., 1997; Ponder et al., 2000; Weibull, 1988). Other work supports this relationship with aphids shown to perform better on plants with known high phloem amino acid content (Karley et al., 2002; Weibull et al., 1986) This study showed there were no significant effects on *M. persicae* growth rate at 24, 72 and 120 hours. *M. persicae* feeding on *aap1* and *aap2* mutants did not show any significant differences in their bodily dimensions or mass when compared to their growth on wildtype plants ($p > 0.05$). In comparison, *B. brassicae* feeding on *aap1* exhibited significant differences in mass at 72 and 120 hours ($p < 0.05$), however not at 24 hours ($p > 0.05$). On *aap2* plants, *B.*

brassicae exhibited significant differences in mass at 120 hours ($p < 0.05$), but not at 24 or 72 hours ($p > 0.05$).

This outcome suggests that the *app1* and *aap2* plants provided the feeding aphids with a diet that was of sufficient quality to sustain their metabolism allowing for normal growth to occur. Experiments using environmentally manipulated levels of nitrogen supplied in nutrient solutions have seen both positive and negative effects of increased and decreased levels of nitrogen respectively. Increasing levels of nitrogen supplied to *Brassica oleracea* increased the performance of the aphid *M. persicae* (Van Emden, 1966), whereas other studies using nitrogen deficient barley saw a decrease in the performance of the aphid *Rhopalosiphum padi* (Ponder et al., 2000). Aphids on nutritionally poor diets perform poorly in terms of their growth which often leads to secondary effects observed through the reproductive capacity of the aphid (Prosser et al., 1992; Abisgold et al., 1994; Girousse and Bournoville, 1994; Febvay et al., 1988). Studies using artifical diets have been able to manipulate aphid diet in order to understand the thresholds of diet quality that determine aphid performance (Mittler and Dadd, 1962; Mittler and Dadd, 1963; Dadd and Mittler, 1965; Dadd and Mittler, 1966; Van Emden, 1966; Woolridge and Harrison, 1968; Srivastava and Auclair, 1974; Weibull, 1987).

The disruption to the amino acid transporters in *aap1* and *aap2* mutant plants created phenotypic changes within the whole plant morphology, for example reducing the number of rosette leaves, reducing the width of the

rosette leaves and also reducing the number of siliques produced. From what information is already known about AAP1 and AAP2 it is possible to suppose that the decreased uptake, assimilation and translocation of amino acids within the phloem has altered the growth of *aap1* and *aap2* plants, but this level of change in terms of the amino acids available within the phloem sap of the plants is not sufficient to affect the aphid performance of either aphid species investigated. Sieve element sap is considered to be of poor quality due to the low concentrations of essential amino acids. For individual amino acids an inadequate concentration would be enough to significantly impede the growth and performance of the feeding aphid however some evidence suggests that different aphid species show varying sensitivities (Cole, 1997) to lowered essential amino acid concentrations, for instance *M. persicae* is much more sensitive to the specific amino acid composition than the cabbage aphid *B. brassicae* (Van Emden, 1969).

However, although evidence within the literature largely supports the logical correlation between nitrogen availability and performance, there are exceptions to this relationship. Work focusing on the intrinsic rate of increase of *M. persicae* failed to show any correlation between sieve element amino acid concentration and the aphid's reproductive output (Cole, 1997). This work is consistent with the fact that aphids are able to upgrade their diet by utilizing their bacterial symbionts, *Buchnera.* As the growth of both aphid species was significantly unaffected when feeding on *aap1* and *aap2* plants it can be assumed that both aphid species feeding on the mutant plants have been nutritionally 'buffered' from the potential diet

variations by their symbiotic bacteria *Buchnera* (Douglas, 1998; Shigenobu et al., 2000). This supplementary amino acid production by the aphid symbionts allows the aphid to avoid the nutritional threshold below which their performance would be adversely affected in terms of performance.

In order to assess the 'unassisted' aphid performance of both species on *aap1* and *aap2* plants without the symbiotic upgrading and compensation, the elimination of the aphid symbionts, followed by a comparative performance would need to be achieved.

4.6.2 Intrinsic Rate of Increase Analysis

While studying aphid reproductive output, the intrinsic rate of increase of *M. persicae* feeding on *aap1* and *aap2* plants was not significantly different when compared to their intrinsic rate of increase on control plants ($p = 0.2361$ and $p = 0.4089$ respectively). The intrinsic rate of increase of *B. brassicae* feeding on *aap1* and *aap2* was also found not to be significantly different when compared to their intrinsic rate of increase on control plants ($p = 0.7855$ and $p = 0.6453$ respectively).

Evidence within the literature supports the idea that the level of nutrition acquired by the aphids feeding from the *aap1*, *aap2* and wildtype plants may have been similar in order to allow for the very similar levels of reproductive output (Van Emden, 1966; Van Emden, 1971; Awmack and Leather, 2002). This observation is supported by the fact that similar levels

of reproductive output have been observed independently in two different aphid species of varying dietary sensitivities (Van Emden, 1969; Cole, 1997). The fact that the aphids feeding on both plant genotypes showed very little difference in r_m values may suggest that sieve element amino acid composition was not significantly affected by the disruption to AAP1 and AAP2 transporters. This observation supports the aphid growth data which also indicates that the performance of both species remains largely unaffected on *aap1* and *aap2* plants in comparison to wildtype plants. However as previously discussed, phenotypic screening results and aphid feeding behaviour data have highlighted a significant effect of genotype for both *aap1* and *aap2* mutants. As discussed in chapters two and three, changes to plant growth in *aap1* and *aap2* plants along with the delayed time to reach sustained sieve element feeding for both aphid species feeding on *aap1* and *aap2* plants does suggest the disruption to AAP1 and AAP2 has significantly effected amino acid transport within the mutant plants.

Although significant enough to affect plant growth and feeding behaviours of both aphid species, this disruption to normal amino acid transport in *aap1* and *aap2* plants may not have been sufficient enough to affect the performance of the feeding aphid species, even with their varying sensitivities to nutritional quality. As sieve element sap is considered to be of poor quality, due to low concentrations of essential amino acids, aphids are considered to be accustomed to feeding on such a diet. An aphid's ability to upgrade its diet using its symbionts allows the aphid to continue

reproducing while feeding on diets of low concentrations of amino acids. Results in chapter two and three suggest the disruption to *aap1* and *aap2* may have affected the level of amino acids uptake and assimilation into the phloem but this level of disruption may not have been sufficient to alter the reproductive output of either aphid species. The level at which the aphid symbionts are upgrading this altered diet can be assessed in terms of aposymbiotic growth (see chapter five) but the effect on the reproductive output cannot be ascertained with aposymbiotic aphids due to the nature of the method and the rarity of an aposymbiotic aphid producing offspring.

Alternatively, the close r_m values for aphids feeding on both genotypes may be due to the rearing of the adults used to produce the experimental nymphs (Hunt, 2005). The adults that gave birth to the nymphs, which were in turn used as the producing aphids throughout the experiment, were reared on wildtype plants. These aphids could therefore be considered to be 'unaclimatised' to the experimental plant genotype used. These unaclimatised aphids were consequently producing nymphs that were unaffected by the hypothesised altered amino acids status of the *aap1* and *aap2*. This result may be due to the phenomenon known as 'maternal effect'. Immediately after ovulation the oocytes of parthenogenetic female aphids begin development. This means that a female aphid will have individual embryos developing inside her and these embryos will have further embryos developing inside them, a process known as the 'telescoping of generations' (Jones and Jones, 1974, Kindlmann and Dixon, 1989, Dixon, 1992). Therefore as a female aphid feeds from its host plant it

will utilise the nutrients within its diet to sustain and provide for the growth of the three successive generations within itself (Kennedy and Fosbrooke, 1970). The nymphs used for the experiments will therefore have been sustained throughout their initial development by the nutrients acquired by their mother feeding on the wildtype plants, thus gaining the name 'maternal effect'. The nutrient quality experienced by the developing aphid directly determine its size at the time of birth (Dixon and Wratten, 1971) and ultimately influences the age at which it initially begins reproduction (Dixon et al., 1982) and its overall reproductive output. These factors are both essential in determining the intrinsic rate of increase of an aphid (Birch, 1948; Wyatt and White, 1977). This therefore suggests that in this experiment the developmental nutrient provisions that the nymphs experienced during their mothers time of feeding on the wildtype plants may have consequently influenced the reproductive activities of the nymphs used throughout the experiment. This may have accounted for the fact that when raised on *aap1, aap2* and wildtype plants with hypothetically different amino acid compositions this had a minimal effect on their intrinsic rate of increase throughout the experiment. Although this is possible the fact that there were no observed differences in either aphid species with differing dietary sensitivities again suggests that there may have been a sufficient level of dietary compensation provided by the aphids internal bacterial symbionts. These symbiotic bacteria are believed to act as a beneficial force in upgrading the nutritionally poor diet of the feeding aphid, compensating for the low levels of free amino acids by utilising waste ammonia and synthesising essential amino acids for the aphid in order to

allow for consistently stable levels of reproduction and growth when feeding on less than optimal host plants (Douglas, 1998; Douglas, 2001; Douglas, 2003; Koga et al., 2007; Wilkinson et al., 2007). This area of interest is discussed in chapter five.

4.6.3 Aphid Establishment Analysis

The mean levels of aphid establishment for *M. persicae* and B. brassicae on *aap1* mutants were significantly lower when compared to the levels of establishment on wildtype plants, $p = 0.021$ and $p = 0.022$ respectively. Similarly, the difference in the levels of *M. persicae* and *B. brassicae* establishment between *aap2* and wildtype plants was also found to be significantly difference, $p = 0.003$ and $p = 0.041$ respectively.

These results represent the percentage of aphids that were able to establish on their test plant and produce offspring on a daily basis throughout the experimental duration. Aphids that were not deemed to have established were those that survived on the test plant but did not produce any offspring throughout the experimental duration or those aphids that did not survive on the test plant. This analysis aimed to measure what the intrinsic rate of increase could not. The aphids that did not survive or did not produce aphids could not be used in the intrinsic rate of increase analysis, and therefore their response on the plant genotype could not be represented. This analysis included the aphids that did not establish or survive in order to

provide a realistic representation of how many aphids could establish on the *aap1, aap2* and wildtype plants.

This form of performance analysis did highlight significant differences in terms of the percentage of aphids being able to survive and reproduce on the *aap1, aap2* and wildtype plants. In terms of this measure of performance, both species of aphid exhibited significantly reduced levels of performance when feeding on *aap1* and *aap2* plants in comparison to those feeding on wildtype plants. Lack of support from the results of previous analysis of reproductive performance suggests that the intrinsic rate of increase method was more suitable for measuring the level of the reproductive output, however failed to determine the level of establishment success from which a reproductive output can then be measured.

Interestingly, once established and producing offspring the disturbance to AAP1 and AAP2 did not affect the reproductive capacity of either aphid species. This is supported by EPG data which shows aphids of both species take longer to locate a suitable sieve element from which to feed in both *aap1* and *aap2* plants, but upon this location their sieve element feeding patterns are not significantly different to those feeding on wildtype plants. Aphids may initially have difficulty in establishing to feed and settle on the *aap1* and *aap2* plants but once feeding are able to feed similarly and perform similarly in terms of growth and reproductive capacity. Some aphids that do not establish or do not survive clearly cannot feed satisfactorily and will not establish successfully on the plant. This lowered

rate of establishment of both aphid species on *aap1* and *aap2* plants suggests that the feeding process has been affected by the disturbance to AAP1 and AAP2 transporters. The lack of support from other performance analyses implies that the quality of the sieve element sap, in terms of amino acid concentrations and proportions, may not have been affected adversely to the point where aphid performance has been impaired. However the establishment results and support from EPG data suggests that both aphid species have found sieve element location more difficult when feeding on *aap1* and *aap2* plants when compared to wildtype plants. It is believed that amino acid leakage from the sieve element may potentially act as cues that probing aphids will recognise and use in order to find a suitable sieve element from which to feed, an example of which may be the phagostimulant methionine (Mittler et al., 1967). Considering what is known about the function of AAP1 and AAP2 in amino acid uptake and translocation, it is possible that the disruption to these amino acid transporters may have altered the way or level in which these amino acid cues are present within the cell pathway, potentially delaying the time taken for the probing aphid to locate a sieve element from which to feed.

4.6.4 Aphid Whole Population Analysis

When assessing aphid performance at a whole population level the analysis comparing *M. persicae* freely infesting *aap1* and *aap2* mutants with control plants showed no significant differences in whole population mass

($p > 0.05$). Similarly *B. brassicae* freely infesting *aap1* and *aap2* mutants showed no differences in their total population mass when compared to the total population mass on control plants ($p > 0.05$).

This analysis allowed for an undisturbed measure of aphid performance combining both indicators of performance in terms of the aphids reproductive output and the level of aphid growth occurring. Since this analysis involved the undisturbed infestation of a test plant followed by the whole aphid population being collected and weighed, the number of aphids and their level of growth (weight) were clearly an indicator of how well the aphid species had performed on the test plant genotype. The results of this analysis support the previous measures of aphid performance with neither aphid species showing any differences in their performance on either *aap1* or *aap2* when compared to wildtype plants. The level at which amino acid transport had been altered by the disruption to AAP1 and AAP2 plants was not sufficient to create a significant effect on the whole population performance of either aphid species feeding on *aap1* or *aap2* plants when compared to wildtype plants.

4.7 **Conclusions**

The performance of *M. persicae* and *B. brassicae*, in terms of growth and reproductive output, was unaffected in both *aap1* and *aap2* plants. Similar levels of performance were observed in both species suggesting similar dietary quality in terms of sieve element amino acid content. Previous work

has failed to find a correlation with the intrinsic rate of increase for *M. persicae* and varying sieve element amino acid concentration (Cole, 1997) however similar levels of performance for *aap1* and *aap2* have been independently observed in *B. brassicae*. Chosen because of their differing sensitivities to dietary quality, these two aphid species both showed no significant changes in growth or reproductive output. This suggests the sieve element sap composition has remained largely unaffected in *aap1* and *aap2* plants, or that the aphid's symbionts have nutritionally buffered the ingested diet sufficiently to avoid an effect of performance.

After this assessment of aphid performance, chapter five aims to study the relationship between both the aphid species and their symbionts. This next area of work aims to compare aposymbiotic and symbiotic aphid performance on *aap1* and *aap2* mutants compared to that on wildtype plants. A comparison of the level of dietary compensation used in each aphid species will also be attempted.

Chapter 5

How is Aphid Performance of *Myzus persicae* and *Brevicoryne brassicae* affected by the elimination of their primary symbionts?

5.1 Abstract

Previous chapters identified significant changes in the feeding behaviour of both M. *persicae* and *B. brassicae* on *aap1* and *aap2* mutants. Both aphid species took significantly longer to locate a suitable sieve element and reach a sustained level of E2 feeding when feeding on *aap1* and *aap2* plants when compared to those on wildtype plants ($p < 0.05$). These observed effects on aphid feeding behaviour were not supported by alterations in the performance data of either aphid species. Both *M. persicae* and *B. brassicae* showed no effect of performance, in terms of growth or reproductive output, when feeding on the *aap1* and *aap2* plants in comparison to the wildtype plants ($p > 0.05$). This lack of correlation with performance data combined with the measured changes to aphid feeding behaviour potentially suggests that the feeding aphid may be compensating for the altered levels of amino acids and is upgrading its diet sufficiently via its endosymbionts in order to minimise performance deterioration.

Following experiments to determine the correct dosage of rifampicin as 50µg/ml, this dose of antibiotic was administered via artificial diet to synchronised second instar nymphs for 48 hours. Simultaneously second instar nymphs were also left for 48 hours on control artificial diet (lacking rifampicin). Following this treatment the

aposymbiotic and symbiotic aphids were then transferred onto their appropriate plant genotypes, *aap1, aap2* and wildtype and their bodily growth weight measured at hours 96, 120 and 240. Aposymbiotic *M. persicae* were found to show significantly reduced growth rates in terms of average weight ($p < 0.000$) when feeding on *aap1* compared to aposymbiotic aphids feeding on wildtype plants. Similarly, aposymbiotic *B. brassicae* were found to show significantly reduced growth rates in terms of average weight ($p = 0.042$) when feeding on *aap1* compared to aposymbiotic aphids feeding on wildtype plants.

When feeding on *aap2* plants, aposymbiotic *M. persicae* showed significantly lower rates of growth compared to aposymbiotic *M. persicae* that were transferred onto the wildtype plants ($p = 0.004$). Similarly, aposymbiotic *B. brassicae* were found to show significantly reduced growth rates in terms of average weight ($p < 0.000$) when feeding on *aap2* compared to aposymbiotic aphids feeding on wildtype plants.

5.2 Introduction

5.2.1 Mycetocyte Symbiosis

Many insects feed on nutritionally poor and unbalanced diets and in many cases are unable to synthesize certain essential dietary elements themselves. Throughout evolution these insects have developed a 'symbiosis' with intracellular microorganisms. These internalised symbioses work to benefit the insect by upgrading and supplementing the elements typically missing from their diet. A 'mycetocyte symbioses' is an example of a symbiosis which forms within specialised cells or 'mycetocytes' (Douglas, 1998). A mycetocyte symbiosis is defined by the following four characteristics,

1. Intracellular microorganisms remain in the cytoplasm of one specific insect cell type. This cell type is known as a 'mycetocyte'.
2. The intracellular microorganisms are inherited maternally.
3. The relationship is necessary for the survival of both the insect and microorganism.
4. This relationship occurs predominantly within insects that feed on nutritionally inadequate or unbalanced diets.

Mycetocyte symbioses have been identified in a diverse range of insect groups and microorganisms. These insects include the Homoptera, Anobiids, Cimicidae, Diptera Pupiparia, Anoplura, Lygaeidae (Heteroptera) and many more. Although present across a diverse spectrum of insects, a common factor amongst all of these symbiosis-forming insect groups is their diet. Almost all of the insects possessing a

mycetocyte symbiosis feed on diets that are nutritionally inadequate or unbalanced, e.g. vertebrate blood or phloem sap. The symbiosis with a microorganism provides a specialised solution to the host's nutritional problem by upgrading or supplementing a diet lacking in essential amino acids, nutrients, lipids and vitamins. These mycetocyte symbioses are independently evolved, suggesting the nutritional basis behind these relationships. In the case of aphids, the relationship between their endosymbiont is so established, obligate and mutualistic that it is not possible for either of the partners to reproduce separately.

5.2.2 Aphid Symbionts – Using the aphid-symbiont relationship as a means of studying aphid performance on *aap1* and *aap2* plants

Traditionally, the bacteria present in the mycetocyte symbiosis of aphids were referred to as 'primary symbionts'. These intracellular bacteria of the genus *Buchnera* (Munson et al., 1991) are coccoid in form and measure approximately 2.5–4 µm in diameter. They possess a thin, gram negative cell wall, divide by constriction and were identified as γ3-Proteobacteria of the Enterobacteriaceae (Munson et al, 1991). *Buchnera* are known to account for up to 10% of the host aphid's entire biomass, existing in numbers of up to 10^7 bacteria per mg aphid weight (Baumann and Baumann, 1994; Humphreys, 1996). Located within specialised cells known as 'mycetocytes' in the aphid hemocoel, *Buchnera* are

believed to have evolved from a gut inhabiting bacterium once acquired by aphids approximately 160-280 million years ago (Moran et al., 1993).

Buchnera is known to exist in all members of the Aphidoidea apart from the Adelgidae and Phylloxeridídae families, along with some Hormaphididae species. Adelgids possess morphologically distinct bacteria whilst Hormaphidids possess yeasts. Phylloxeridídae lack any form of microbial symbiont.

Transmission of *Buchnera* occurs maternally through each aphid generation via a process known as 'transovarial transmission'. In viviparous aphids the *Buchnera* are passed into the embryo's blastocoel where they are later endocytosed by the embryo's differentiating mycetocytes (Buchner, 1965; Hinde, 1971) In the case of oviparous aphids, *Buchnera* is endocytosed within each ovum as these cells differentiate (Buchner, 1965; Brough, 1990). Following the birth of an aphid the mycetocytes grow in size to accommodate the proliferating *Buchnera* within them, occupying approximately 60% of the cytoplasmic space within the mycetocyte (Whitehead and Douglas, 1993).

Many aphids also bear other bacteria referred to as 'secondary symbionts'. These bacteria are not present in all aphids and are not located within mycetocytes, instead being located within the haemolymph or within sheath cells which surround the mycetocytes. These bacteria can be transmitted horizontally via feeding on a plant which has been covered in infected honeydew and consequently are referred to as infectious agents. Although these infectious agents do not tend to contribute toward the performance of an aphid they have been observed to enhance thermal stress tolerance, enhance resistance to natural enemies and also

enhance or depress the ultilisation of some host plants. Consequently these secondary symbionts are believed to impact on aphid population dynamics.

Recent work on the aphid-*Buchnera* symbiosis reveals the gram negative bacteria work to supply the host aphid with a readily available source of essential amino acids. This form of essential amino acid provisioning is vital due to the nutritionally poor food source with its lack of certain essential amino acids (Douglas, 1993). It is because of the nutritionally poor status of phloem sap that all Homoptera feeding on a phloem sap diet would have some form of microbial symbiosis.

Buchnera's mode of upgrading the aphids diet involves the upgrading of nonessential amino acids, derived from the aphids food source, into vital essential amino acids. The evidence for *Buchnera* upgrading an aphid's diet can be demonstrated using nutritional, metabolic and genomic experiments.

Nutritional experiments involving aposymbiotic aphids, aphids lacking bacteria, clearly demonstrate the need for the symbiotic relationship. Aposymbiotic aphids, that have been created by treating symbiotic aphids (those with bacteria) with oral or injectable antibiotics present slow, minimal bodily growth with very few if any offspring (Sasaki et al., 1991; Douglas, 1992; Douglas, 1996). These indicators of poor performance in aposymbiotic aphids illustrate the level at which *Buchnera* upgrades an aphids dietary intake, allowing the symbiotic aphid to thrive in terms of its own bodily growth and also its reproductive fitness. Aposymbiotic aphids have shown reduced levels of growth when fed on diets omitting individual essential amino acids (Mittler, 1971). In contrast, on the same diets with the same omissions of the essential amino acids symbiotic aphids showed no change in their performance (Dadd and Krieger, 1968). The reduced performance of the

aposymbiotic aphids did not improve with substituted essential amino acids being added to the artificial diet, suggesting that the need for the bacterial symbiosis is essential for the optimally functioning aphid (Douglas, 2003).

Metabolic studies have also definitively demonstrated dietary provisioning within the aphid-*Buchnera* symbiosis. One study quantified the aphid synthesis of methionine by measuring the incorporation of ^{35}S from inorganic sulphate. Short term isolated preparations of *Buchnera* have been shown to use sulphate when synthesising methionine (Douglas, 1990). This study showed that the symbiotic aphids were able to incorporate the ^{35}S into methionine whereas the aposymbiotic aphids were not (Douglas, 1988). The symbiotic aphids were also able to incorporate this labelled methionine into their bodily tissues, identified by traces of the labelled methionine in tissues of the aphid not associated with the *Buchnera*. This study once again highlights the importance of a symbiosis to an aphids nutrient provisioning.

Genome studies have also provided evidence for the importance of the aphid-*Buchnera* symbiosis in nutrient provisioning. Genomic sequencing has shown that *Buchnera* has the necessary genes for enzymes in the essential amino acid biosynthetic pathways however lacks the majority of genes which are necessary for the synthesis of the non-essential amino acids (Latorre et al., 2005; Shigenobu et al., 2000).

The nutritional, metabolic and genomic studies provide firm evidence of *Buchnera's* role in upgrading aphid diet by provisioning essential amino acids. However this provisioning is undoubtedly variable across the different aphid taxa and even between different clones of the same aphid species. In terms of the

variation between aphid taxa, this variation in the capabilities of *Buchnera* may occur due to the rapid evolution of host plant affiliation. Evolutionary shifts in *Buchnera's* metabolic capabilities may allow for differences in the nutritional composition of the host plants phloem sap. In terms of the potential variation between clones of the same species, these differences may be present due to the metabolic versatility of the *Buchnera*. Studies have shown that two clones of the aphid species *A. pisum* were independent of a dietary supply of differing essential amino acids. One clone was independent of isoleucine, valine and phenylalanine, and the second clone was independent of tryptophan, leucine, and lysine (Srivastava et al., 1985). This variation in nutrient provisioning is an example of the extent to which nutrients provided by *Buchnera* may vary in line with the aphid's nutritional demand for those nutrients. This variation could be in terms of the rate of amino acid provision or the composition of the amino acids provided. This metabolic versatility in terms of the amino acid profile provided could be possible if free amino acids could be translocated from the *Buchnera* to the aphid via amino acid specific membrane bound transporters. The activity of these transporters could potentially regulate the demand for specific amino acids, however at present there is no evidence to suggest this type of transport. Instead it is believed that bacteria provide amino acids at rates in excess of the aphid's demand, the excess being excreted via the honeydew, suggesting limited metabolic control.

5.3 Chapter Aims

This chapter aims to investigate the effect of eliminating aphid symbionts in two aphid species and comparing their subsequent performance on *aap1* and *aap2* mutant plants compared to wildtype plants. Selectively eliminating *Buchnera*, the aphid's primary symbiont, using rifampicin treatment has been shown in previous experiments to cause retarded growth and sterility of the cured host aphids. Following this treatment to what extent does the quality of the subsequent food source affect the level of subsequent aphid performance? Do subtle changes in a dietary food source have a more pronounced effect on the performance of an aposymbiotic aphid than an aphid with a full complement of symbiotic bacteria? It is hypothesised by eliminating the aphid symbionts and consequently preventing the upgrading of less than optimal diets the aphids will be exposed to the host plants true dietary quality without the compensation of the symbiotic bacteria.

The first objective of this chapter was to determine a suitable dosage of the antibiotic rifampicin with which to eliminate the aphid symbionts of *M. persicae* and *B. brassicae*.

The second objective was to quantify the performance of these aposymbiotic aphids compared to control symbiotic aphids when transferred to *aap1, aap2* or wildtype plants.

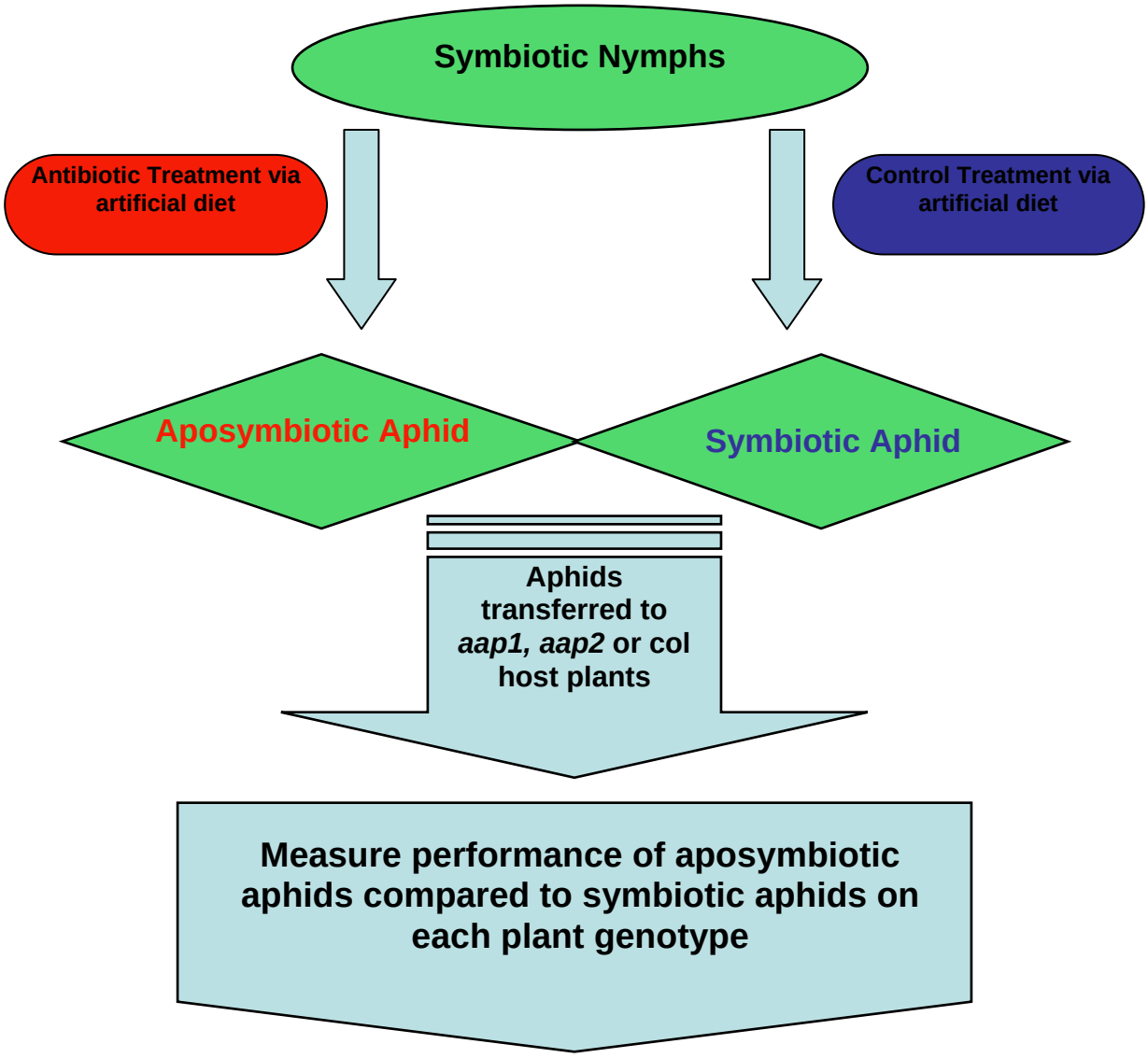

Figure 5.1 – A flowchart illustrating the methodology developed specifically to study the effect of eliminating aphid symbionts in *M. persicae* and *B, brassicae* in order to compare their subsequent performance on *aap1, aap2* and wildtype plants.

5.4 Materials and Methods

5.4 1 Antibiotic Dose Determination Analysis

5.4.1.1 Plant Material

Wildtype plants were cultivated according to the methods featured in section 2.4.1. These plants were selected for use in antibiotic dose determination analysis when at their optimum stage of growth (30-35 days old from sowing).

5.4.1.2 Aphid Culture

In order to be able to measure aphid growth rate following feeding on the artificial diet treatments, synchronised second instar nymphs of both *M. persicae* and *B. brassicae* were used in the antibiotic dose determination analysis (see section 3.4.1 for details).

5.4.1.3 Antibiotic Dose Determination

In order to determine a suitable antibiotic treatment level with which to treat symbiotic nymphs, experiments began by treating symbiotic aphids with a differing levels of the antibiotic rifampicin.

'Artificial Diets' or synthetic liquid diets that simulate an aphids natural diet were used in order to temporarily provide a food source for feeding *M. persicae* or *B. brassicae* so that varying levels of rifampicin could be administered.

Artificial diets stocks were created using 100ml of sterile distilled water, 72.35mg of methionine (5mM) and 20g of sucrose (0.6M). This stock solution was divided up and varying levels of rifampicin added to the different aliquots. Rifampicin at doses of 500µg/ml, 450µg/ml, 400µg/ml, 350µg/ml, 300µg/ml, 250µg/ml, 200µg/ml, 150µg/ml, 100µg/ml, 50µg/ml, 40µg/ml, 30µg/ml, 20µg/ml, 10µg/ml, 5µg/ml and 0µg/ml were added to the separate aliquots of artifical diet to form the test diets. The methionine, sucrose and rifampicin were thoroughly dissolved into solution using a bench top mixer. Once fully dissolved the solutions were frozen at -80^{o}C in aliquots of 2ml to ensure fresh solution aliquots ready for experimentation.

When setting up artificial diet rings small, plastic vials were used to form the structural basis for the diet sachet (see figures 5.2-5.4). Within a sterilised flowhood the vials were sterilised with alcohol and previously UV sterilised strips of parafilm (3cm by 3cm) were stretched as thinly as possibly across the open end of the vial to form a 'drum skin' surface. Onto this surface 2ml of artificial diet which was previously sterilised using a 4mm Acrodisc® syringe filter was pipetted. Another strip of UV sterilised parafilm was then stretched over the liquid to form a sandwich or sachet of artificial diet solution secured to the rim of the vial (see figures 5.2-5.4).

Test second instar nymphs (10 per diet ring) were previously collected and starved within a petri dish for one hour prior to application to the diets, with 20 aphids being fed to each dose level of rifampicin treatment. The aphids were introduced into the plastic vials prior to the sachet being applied so that they were effectively 'caged' onto the diet and would therefore have no other food source to feed on, increasing the chance of successful feeding and therefore the treatments being administered. Following 48 hours of feeding on the artificial diets the aphids were removed and observations made. At this point the number of dead aphids was recorded and the remaining, living aphids were transferred to wildtype plant hosts on which they would remain for the following weeks, eventually providing a measure of their sterility (and therefore a measure of their symbiotic bacteria levels) in terms of number of nymphs produced.

5.4.2 Comparing performance of aposymbiotic and symbiotic aphids on *aap1, aap2* and wildtype plants

5.4.2.1 Plant Material

Both *aap1, aap2* and wildtype plants were cultivated according to the methods described in section 2.4.1. These plants were selected for use in selective elimination of aphid endosymbiont analysis when at their optimum stage of growth (30-35 days old from sowing).

5.4.2.2 Aphid Culture

In order to measure aphid growth rate following feeding on the artificial diet treatments, synchronised second instar nymphs of both *M. persicae* and *B. brassicae* were used in the selective elimination of aphid endosymbiont analysis (see section 3.4.1 for details).

5.4.2.3 A comparative analysis of aposymbiotic and symbiotic performance on *aap1, aap2* and wildtype plants

'Artificial Diets' or synthetic liquid diets that simulate an aphids natural diet were used in order to temporarily provide a food source for feeding *M. persicae* or *B. brassicae* so that rifampicin could be administered.

Two artificial diets stocks were created using 100ml of sterile distilled water, 72.35mg of methionine (5mM) and 20g of sucrose (0.6M). Into one of these solutions rifampicin at a dose of 50µg/ml was added to form the treatment diet. The other diet acted as a control measure. The methionine, sucrose and rifampicin (in the case of the treatment diet) were thoroughly dissolved into solution using a whirly mixer. Once fully dissolved the stock solution was frozen at -80ºC in aliquots of 2ml to ensure fresh solution aliquots ready for experimentation.

When setting up artificial diet rings, small plastic vials were used to form the structural basis for the diet sachet. Within a sterilised flowhood the vials were sterilised with alcohol and previously UV sterilised strips of parafilm (3cm by 3cm)

were stretched as thinly as possibly across the open end of the vial to form a 'drum skin' surface. Onto this surface 2ml of artificial diet which was previously sterilised using a 4mm Acrodisc® syringe filter was pipetted. Another strip of UV sterilised parafilm was then stretched over the liquid to form a sandwich or sachet of artificial diet solution secured to the rim of the vial (see figures 5.2-5.4).

Test aphids (10 per diet ring) were previously collected and starved within a petri dish for one hour prior to application to the diets, with half the aphids being fed the diet with the rifampicin treatment and half the aphids being fed the control diet simply so that any effect of the artificial diet method itself could be taken into account. The aphids were introduced into the plastic vials prior to the sachet being applied so that they were effectively 'caged' onto the diet and would therefore have no other food source to feed on, increasing the chance of successful feeding and therefore the treatments being administered. Following 48 hours of feeding on the artificial diets the aphids were removed and placed onto individual test plants, either *aap1, aap2* or control plants, where they would remain for the following 6 days, being removed, weighed and photographed under microscope at 48 hourly intervals.

5.4.2.4 Statistical Analysis

The performance data of aposymbiotic and symbiotic *M. persicae* and *B. brassicae* on *aap1, aap2* and wildtype plants was tested for normality with an Anderson-Darling test. As the data was found to be normal, it was analysed using two-way ANOVAs. See appendix 4 for details of this statistical analysis.

Figure 5.2 – A photo illustrating the plastic vial used as a device to contain feeding aphids while administering artifical diet via the parafilm sachet method.

Figure 5.3 – A photo illustrating the position of the parafilm sachet bottom layer. This bottom layer of parafilm serves a dual purpose by containing the aphids within the plastic vial, acting as a feeding chamber, while also acting as the bottom of the parafilm sachet onto which artifical diet solution can be administered and the contained aphids will feed through.

Figure 5.4 – A photo illustrating the finished artificial diet sachet secured to the plastic vial feeding chamber. For visual purposes the artificial diet has been stained blue and feeding aphids have been placed on top of the sachet when in reality they would be contained within the vial underneath the sachet, ensuring they feed from the artificial diet.

5.5 Results

5.5.1 Antibiotic Dose Determination

5.5.1.1 Antibiotic Dose Determination for *M. persicae* and *B. brassicae*

The rifampicin dose level deemed suitable for *M. persicae* and *B. brassicae* was determined to be 50µg/ml. This figure was derived using the percentage of aphids producing nymphs post treatment along with the percentage of post treatment aphid mortality. The point at which post-treatment aphid mortality reached 0% and the percentage of aphids producing nymphs post-treatment reached 0% was the point at which the dose of rifampicin was deemed optimal, at 50µg/ml (see figure 5.5).

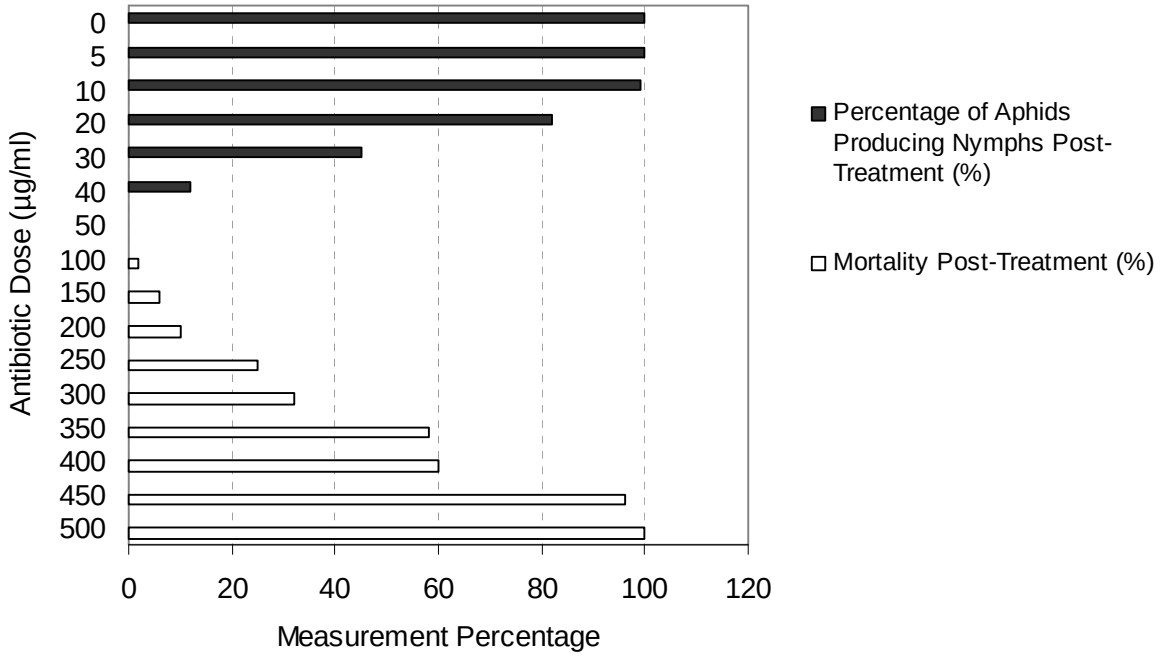

Figure 5.5 – A graph used for the determination of optimal rifampicin dose for later use in the performance analysis of aposymbiotic and symbiotic *M. persicae* and *B. brassicae* on *aap1, aap2* and wildtype plants. Optimal rifampicin doseage was determined to be 50µg/ml (n=21).

5.5.2 Aposymbiotic aphid performance on *aap1* and *aap2* plants

5.5.2.1 Performance of aposymbiotic and symbiotic *M. persicae* on *aap1* and wildtype plants

The mean growth rate (µg/h) of aposymbiotic *M. persicae* on *aap1* plants was significantly lower ($p < 0.000$) than aposymbiotic *M. persicae* feeding on wildtype plants with mean growth rates of 0.199 µg/h (±0.0867) and 1.046 µg/h (±0.15) respectively (see figure 5.6). The significant interaction between plant genotype and symbiosis ($p = 0.003$) suggests that antibiotic treatment significantly decreases aphid performance on the mutant plants to a greater extent than it does on wildtype plants.

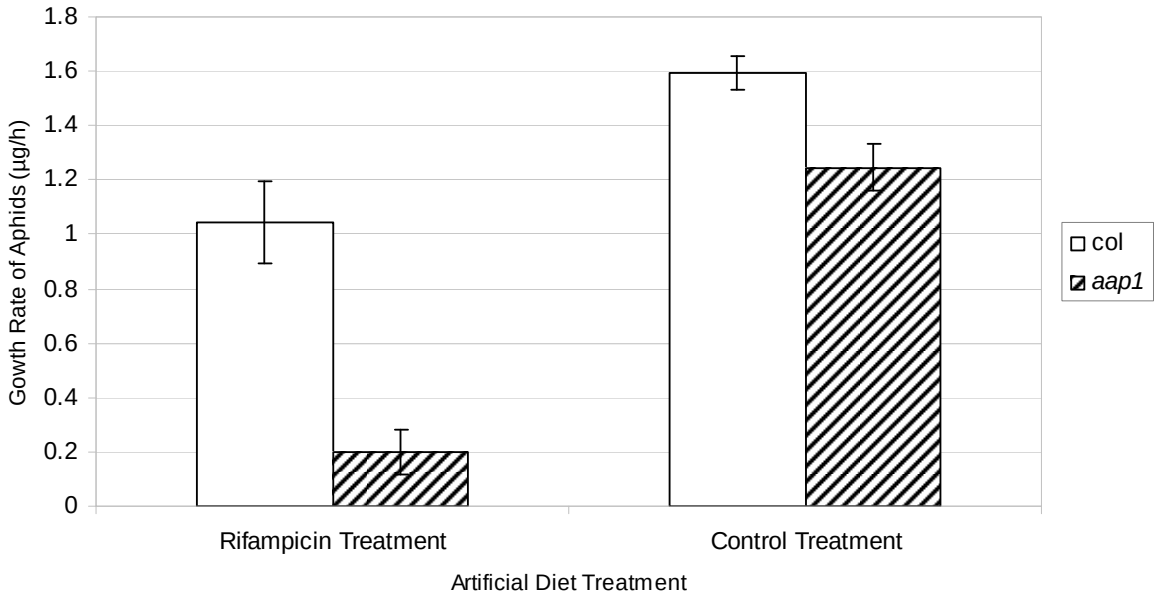

Figure 5.6 Mean growth rate (µg/h) of aposymbiotic and symbiotic second instar *M. persicae* on *aap1* (n=12) and wildtype plants (n=12). Data was analysed using a two-way ANOVA. Error bars represent ± s.e.

5.5.2.2 Performance of aposymbiotic and symbiotic *M. persicae* on *aap2* and wildtype plants

The mean growth rate (µg/h) of aposymbiotic *M. persicae* on *aap2* plants was significantly lower ($p < 0.000$) than aposymbiotic *M. persicae* feeding on wildtype plants with mean growth rates 0.196 µg/h (±0.04167) and 1.126 µg/h (±0.0867) respectively (see figure 5.7). The significant interaction between plant genotype and symbiosis ($p = 0.000$) suggests that antibiotic treatment significantly decreases aphid performance on the mutant plants to a greater extent than it does on wildtype plants.

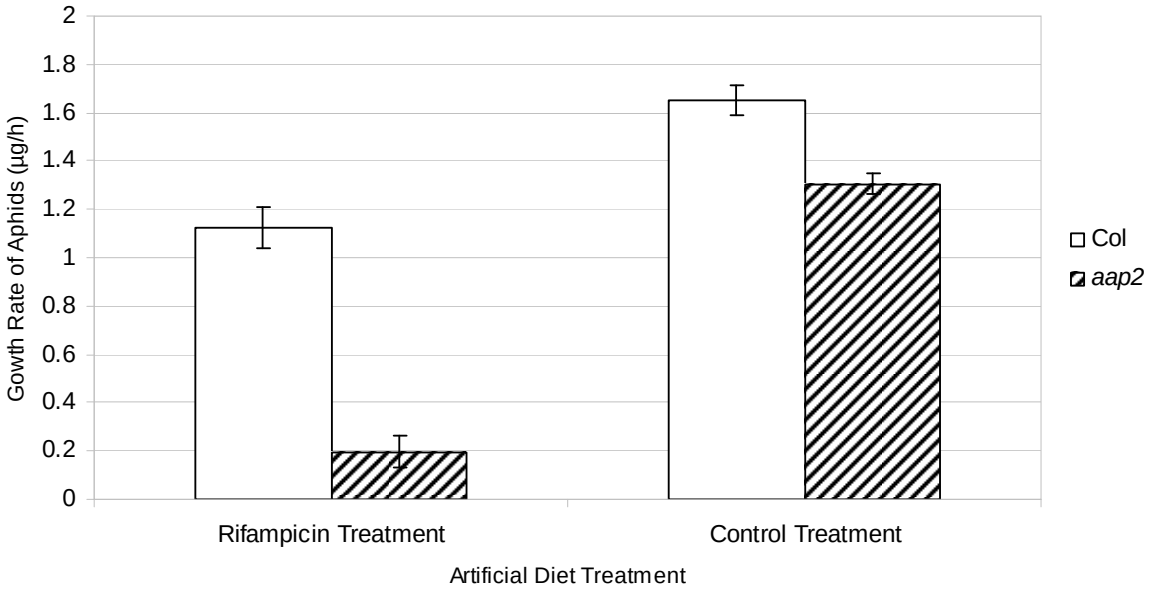

Figure 5.7 – Mean growth rate (µg/h) of aposymbiotic and symbiotic second instar *M. persicae* on *aap2* (n=12) and wildtype plants (n=12). Data was analysed using a two-way ANOVA. Error bars represent ± s.e.

5.5.2.3 Performance of aposymbiotic and symbiotic *B. brassicae* on *aap1* and wildtype plants

The mean growth rate (µg/h) of aposymbiotic *B. brassicae* on *aap1* plants was significantly lower (p = 0.042) than aposymbiotic *B. brassicae* feeding on wildtype plants with mean growth rates 0.213 µg/h (±0.0502) and 0.77 µg/h (±0.356573) respectively (see figure 5.8). The significant interaction between plant genotype and symbiosis (p = 0.042) suggests that antibiotic treatment significantly decreases aphid performance on the mutant plants to a greater extent than it does on wildtype plants.

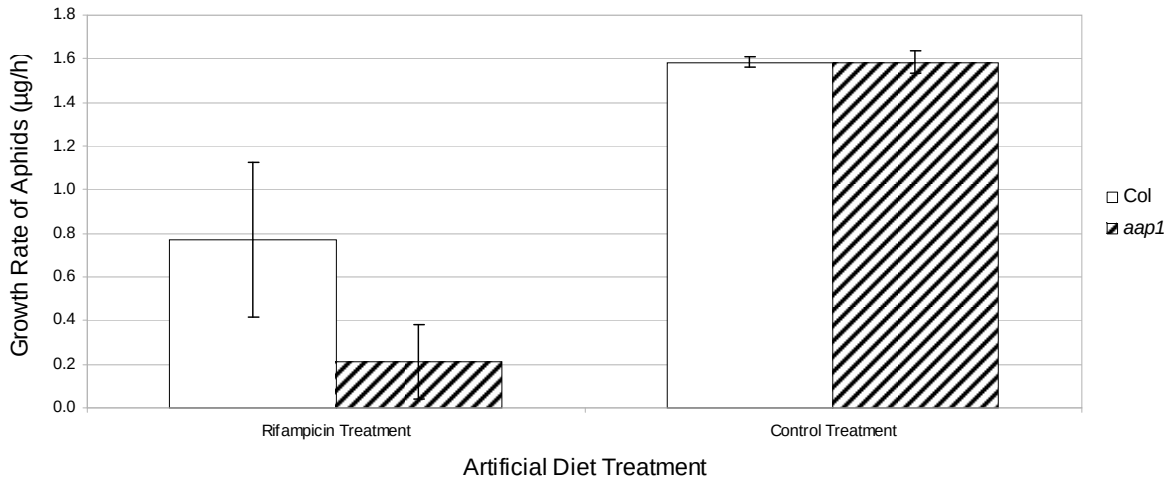

Figure 5.8 – A graph comparing the mean growth rate (µg/h) of apopsymbiotic and symbiotic second instar *B. brassicae* on *aap1* (n=12) and wildtype plants (n=12). Data was analysed using a two-way ANOVA. Error bars represent ± s.e.

5.5.2.4 Performance of aposymbiotic and symbiotic *B. brassicae* on *aap2* and wildtype plants

The mean growth rate (µg/h) of aposymbiotic *B. brassicae* on *aap2* plants was significantly lower (p = 0.004) than aposymbiotic *B. brassicae* feeding on wildtype plants with mean growth rates 0.2 µg/h (± 0.0417) and 0.783 µg/h (±0.341) respectively (see figure 5.9). The interaction between plant genotype and symbiosis was not significant (p = 0.157). This suggests antibiotic treatment did not significantly decrease aphid performance on the mutant plants to a greater extent than it does on wildtype plants.

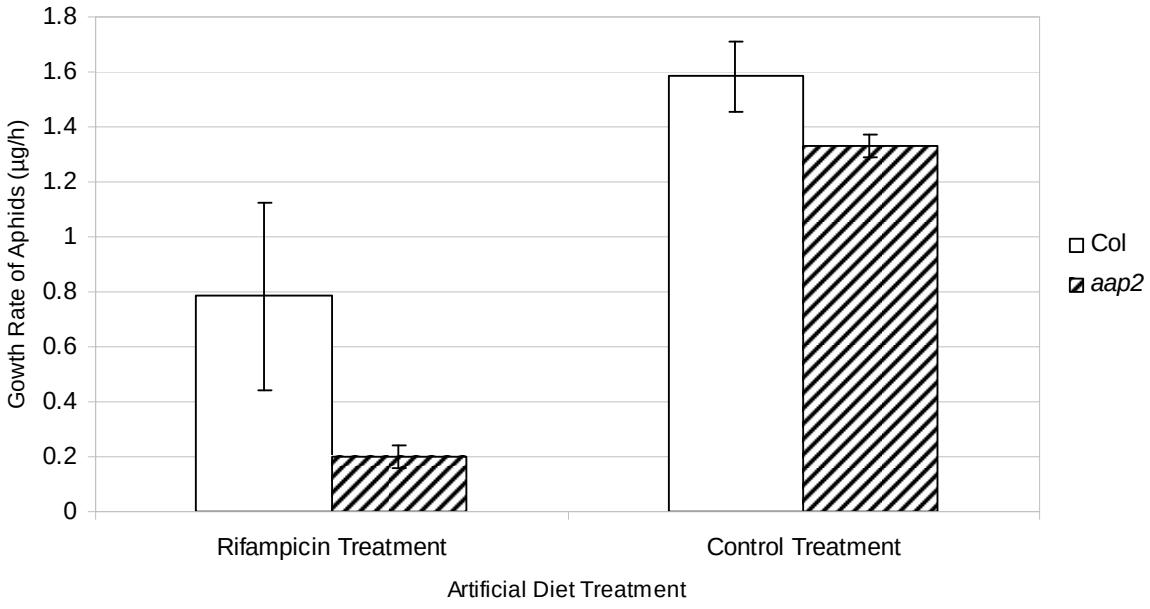

Figure 5.9 – A graph comparing the mean growth rate (µg/h) of apopsymbiotic and symbiotic second instar *B. brassicae* on *aap2* (n=12) and wildtype plants (n=12). Data was analysed using a two-way ANOVA. Error bars represent ± s.e.

5.6 Discussion

Previous chapters identified significant changes in the aphid feeding behaviour and metabolism of both *M. persicae* and *B. brassicae* when feeding on both *aap1* and *aap2* mutants. These effects on aphid feeding behaviour were not paralleled by the performance data of either aphid species, which indicated overall no significant effect in either aphid species. One explanation is that the feeding aphids have been nutritionally buffering their diet, utilising their symbionts in order to compensate for the nutritional alterations or deficits potentially present in *aap1* and *aap2* plants. This explanation was investigated within chapter five and the results of these experiments are reviewed in this discussion.

5.6.1 Antibiotic Dose Determination

The rifampicin dose level deemed suitable for *M. persicae* and *B. brassicae* was determined to be 50µg/ml. This figure was derived using the percentage of nymph producing aphids treatment along with the percentage of post treatment aphid mortality. This dose determination result is supported by previous studies that have also used a 50µg/ml dose of rifampicin for the successful elimination of aphid symbionts (Rahbe et al., 1994, Wilkinson et al., 1997). These studies have shown that this dose level of rifampicin, administered via the artifical diet method, is adequate for the successful elimination of aphid symbionts. This evidence along with the dose determination results allowed for the confident production of aposymbiotic aphids.

5.6.2 Comparing performance of aposymbiotic and symbiotic aphids on *aap1, aap2* and wildtype plants

Aposymbiotic *M. persicae* showed significantly reduced growth rates in terms of average weight ($p < 0.000$) when feeding on *aap1* and *aap2* compared to aposymbiotic aphids feeding on wildtype plants. Similarly aposymbiotic *B. brassicae* had significantly reduced growth rates in terms of average weight ($p < 0.000$) when feeding on *aap1* and *aap2* plants compared to aposymbiotic aphids feeding on wildtype plants.

The growth rate of aposymbiotic aphids is significantly lower than that of symbiotic aphids. It is known that the use of antibiotics, at a suitable dosage, selected for the elimination of aphid symbionts has a minimal effect on the treated aphids behaviour and metabolism (Griffiths et al., 1974; Douglas, 1988; Wilkinson and Douglas, 1995), however aposymbiotic aphids have also been shown to exhibit a slower rate of growth, developing into smaller adults which tend not to produce any offspring (Douglas, 1992; Douglas, 1996). In this analysis, a slower rate of growth was observed for both aposymbiotic *M. persicae* and *B. brassicae* however this slower rate of growth was found to be more pronounced in the aposymbiotic aphids feeding on the *aap1* and *aap2* mutant compared to the wildtype plants. This meant the aposymbiotic nymphs of both aphid species grew more slowly when raised on *aap1* and *aap2* plants compared to when they were raised on wildtype plants. This slower growth rate acts as an indicator of comparatively poor performance in the aposymbiotic aphids feeding on the *aap1* and *aap2* mutants. Work in chapter four indicated no effect on the growth and

reproductive performance of either aphid species when feeding on *aap1* and *aap2* plants when compared to control plants. However, upon eliminating the aphid symbionts the growth rate of both aphid species on *aap1* and *aap2* had been significantly reduced. This suggests that when an aphid is unable to compensate for deleterious changes to its diet, by utilising its bacterial symbionts to supplement the food source, it will exhibit a significant reduction in performance in terms of its growth rate. This indicates that the composition of the phloem sap has been altered enough to affect the performance of both aposymbiotic aphid species on *aap1* and *aap2*. Results also suggest however the composition is not altered enough to affect the performance of the symbiotic aphids with a full complement of symbiotic bacteria working to compensate for nutritional deficits. This observation supports behavioural data in chapter three to suggest that levels of the amino acids that act as phagostimulants (working to increase the palatability and overall the acceptance of the diet) may potentially be lowered or absent in the *aap1* and *aap2* plants.

It is reasonable to assume that sieve element sap composition has been affected due to disruption in the uptake and translocation of amino acids within *aap1* and *aap2* plants. The EPG data for *M. persicae* and *B. brassicae* showed both species of aphid took significantly longer to reach a sustained level of E2 ingestion on *aap1* and *aap2* plants compared to wildtype plants. Once the aphids reach the sustained E2 feeding they continue to feed similarly to that of an aphid on a wildtype plant. This result is congruent with the unaffected performance, in terms of aphid growth and reproductive output, of both aphid species. The alterations to the amino acid transport throughout the *aap1* and *aap2* plants is sufficient enough to delay

sustained sieve element feeding in both aphid species but not sufficient enough to produce an effect on the overall aphid performance.

Only when the symbiotic bacteria are eliminated can an effect on the growth rate of both aphid species be seen. These results imply that the alterations in the transport of amino acids observed in *aap1* and *aap2* may potentially reduce the level or supply of cues used by aphids with which to locate a suitable sieve element, however these changes do not alter the nitrogen content of the sieve element sufficiently in order to affect aphid performance while their diet is nutritionally buffered by their symbiont bacteria.

5.7 Conclusions

The data presented in this chapter represents the growth rate of *M. persicae* and *B. brassicae* feeding on *aap1* and *aap2* plants without nutritional support from their symbionts. In the previous chapter, earlier performance work indicated that neither aphid species performance was significantly affected when feeding on *aap1* and *aap2* plants. When considering the significant feeding behaviour results similarly observed in two aphid species, and the plant phenotypic changes observed in *aap1* and *aap2* plants, it is fair to assume that the disruption to AAP1 and AAP2 may potentially affect the sieve element sap composition. The lack of effects on aphid performance indicate that this alteration to the sieve element sap composition may be so subtle that the constitutive level of symbiont upgrading of the aphids diet buffers any changes. Consequently, no effect of performance is seen in either aphid species being observed. Following symbiont elimination, the

results showed reduced growth rates for both aphid species feeding on both mutant plants in comparison to aposymbiotic aphids on wildtype plants. This evidence supports the theory that a subtle change in sieve element composition may have occurred in *aap1* and *aap2* plants and it is only once we remove the compensatory effects of the aphid symbionts that we are able to observe the effect of this subtle change on aphid performance.

Chapter 6

General Discussion

The aim of this study was to investigate the effect of loss of function mutations in At*AAP1* and At*AAP2* on whole plant phenology and the feeding behaviours and performance of two ecologically important aphid species, *M. persicae* and *B. brassicae*. A study of this sort required an approach with techniques that facilitated quantitative comparisons of aphid feeding behaviour and performance in two aphid species feeding on two plant genotypes. It is the first study to compare the aphid-plant interactions occurring between At*aap1* and At*aap2* and *M. persicae* and *B. brassicae*. This study also includes the first quantitative analysis of aposymbiotic performance, in terms of aphid growth rate, of both *M. persicae* and *B. brassicae* on At*aap1* and At*aap2* plants.

6.1 Interactions between plant amino acid transporters AtAAP1 and AtAAP2 and the aphids *M. persicae* and *B. brassicae.*

This study demonstrated that the two amino acid transporters of interest do indeed have a significant effect on the interactions between plant and aphid. Firstly, the disruption to AAP1 and AAP2, and the hypothesised effect on the amino acid transport around the plant, was significant enough to affect the

whole plant phenotype of both *aap1* and *aap2* plants. The overall conclusion from chapter two strongly implies that both AAP1 and AAP2 play an important role in resource allocation to the developing seed. This conclusion is heavily supported by very recent literature studying AtAAP1 and its role in regulating the import of amino acids into developing embryos (Sanders et al., 2009).

Work in chapter two highlighted a significant affect in behaviour of both aphid species when feeding on *aap1* and *aap2* plants. Both *M. persicae* and *B. brassicae* exhibited a delay in their ability to locate a sieve element when feeding on *aap1* and *aap2* plants. Upon locating a sieve element and settling, the feeding rate of both aphid species was found to be significantly reduced in *aap1* and *aap2* plants. These results indicate the mutations in *AAP1* and *AAP2* reduced the levels of locatory cues present within the cell pathway. These cues, potentially amino acids that have leaked into cells surrounding the sieve element, do appear to have been reduced by the change in amino acid transport in the *aap1* and *aap2* plants.

In terms of the performance of both aphid species, results from chapter four suggest that the disruption to AAP1 and AAP2 and its consequent effects on plant amino acid transport was not significant enough to affect aphid development or reproductive capacity in either aphid species. Although this effect in the amino acid transport of *aap1* and *aap2* plants was too subtle to affect performance of symbiotic aphids, chapter five demonstrated that the change in amino acid transport, and potentially the sieve element sap composition, in *aap1* and *aap2* plants did significantly reduce the aphid

growth rate of aposymbiotic aphids of both species. This was an important result that indicated the disruption to AAP1 and AAP2 may potentially have affected the transport of essential amino acids into the sieve element of *aap1* and *aap2* plants. Considering it is the concentrations of essential amino acids within the sieve element sap that ultimately determines aphid performance, the reduced performance of aposymbiotic aphids on the *aap1* and *aap2* plants suggests that because the aphid can no longer synthesise these essential amino acids using their symbionts, they are required to ingest them from their diet. As expected the performance of all aposymbiotic aphids in the experiment did decrease however the aposymbiotic aphids feeding on the *aap1* and *aap2* plants did exhibit dramatically lower rates of growth than aposymbiotic aphids feeding on wildtype plants. This strongly implies that the transport of some or all of the nine essential amino acids into the sieve element was reduced in *aap1* and *aap2* plants.

In conclusion, work throughout this investigation has suggested the ways in which amino acid transporters, AAP1 and AAP2, influence the complex relationship between aphid and plant. Evidence within this study suggests AAP1 and AAP2 both play a similarly significant role in the transport of amino acid within *A. thaliana*. These potential effects on amino acid transport were independently identified using the two aphid species, *M. persicae* and *B. brassicae*.

6.2 Future Work

As discussed previously, aspects of whole plant growth, aphid feeding behaviour and aphid performance were affected in *aap1* and *aap2* plants. If this investigation was to be explored further, new areas of the investigation would be primarily focused on determining the composition of the sieve element sap in *aap1* and *aap2* plants. For a long time it has been known that the nitrogen content within the sieve element sap of a host plant influences the feeding aphid's overall performance, however the relationship as indicated within this study is not a straightforward one. Previous studies have shown it is the actual amino acid composition, or nitrogen quality, rather than the amount of nitrogen within the sieve element sap that ultimately affects the feeding aphid's performance. The essential amino acids, methionine, histidine, threonine, tryptophan, valine, leucine, isoleucine, lysine and phenylalanine are the key determinants of diet quality. Determining the amino acid profile and the relative proportions of these essential amino acids in *aap1, aap2* and wildtype plants may allow for more informed explanations of the observations made so far within this study. In order to create an amino acid profile of the *aap1*, *aap2* and wildtype plants, sieve element sap from each plant genotype would need to be sampled. This process of sampling the sieve element would involve the use of aphid stylectomy, a technique which involves sampling exuding sieve element sap from the tip of the aphid's severed stylet (Kennedy and Mittler, 1953). This method is favoured above others due to the purity of the sample collected, providing a sample of sap from individual sieve tubes rather than a sample

taken from whole phloem tissue. These samples could then be analysed using laser-induced fluorescent capillary electrophoresis (CE-LIF) which would provide an accurate measurement of the amino acid content within the sieve element sap. Using the amino acid profiles of the sieve element sap would allow for an accurate assessment of the quality of the aphid's diet, allowing a comparison to be made between *aap1, aap2* and wildtype plants. This analysis could allow for greater discussion of the results observed in the two aphid species with regards to their similarly lowered performance on the mutant plants upon elimination of their symbionts. It could also allow for further speculation regarding both aphid species delayed ability to feed from the sieve element on the mutant plants. Further to this extension of work, it would also be valuable to sample the honeydew of the feeding aphids, both symbiotic and aposymbiotic, on *aap1, aap2* and wildtype plants. These samples would be analysed in a similar manner using the CE-LIF technique, so that that an amino acid profile could be determined for sieve element sap post-digestion. This could potentially help to determine the level of demand of specific amino acids in the two aphid species, both symbiotic and aposymbiotic. Consequently, these results may further help to understand the observed performance and behaviour effects of both aphid species in context to the amino acid profile of the pre-ingested sieve element sap of *aap1, aap2* and wildtype plants.

Lastly, further work could be undertaken in which to continue the analysis of the effect on silique and seed production in *aap1* and *aap2* plants. The reduced mean seed weights observed in *aap2* plants and the reduced total

seed weights for individual siliques in both *aap1* and *aap2* are results which are consistent with the literature (Sanders et al., 2009). This study strongly suggest that both AAP1 and AAP2 are important in the loading of amino acids into the developing embryo, therefore it would be interesting to further this study by analysing the amino acid composition of *aap1* and *aap2* seeds in comparison with wildtype seeds. A similar form of amino acid profile could be determined for the seeds further supporting the results in this study which suggest both AAP1 and AAP2 play an important role in embryo loading.

Chapter 7

Bibliography

Abisgold, J. D., Simpson, S. J. and Douglas, A. E. (1994) "Nutrient regulation in the pea aphid *Acyrthosiphon pisum*: application of a novel geometric framework to sugar and amino acid consumption." Physiological Entomology. Volume 19, Issue 2, pages 95–102

Annan, I. B., Schaefers, G. A., Tingey, W. M. and Tjallingii, W. F. (1997) "Effects of treatments for electrical penetration graph recordings on behaviour and biology of *Aphis craccivora* (Aphididae)." Physiological Entomology, 22: 95-101.

Auclair, J. L. (1963) "Aphid feeding and nutrition." Annual Review of Entomology 8: 439-451.

Auclair, J. L. (1965) "Feeding and nutrition of the pea aphid, *Acyrthosiphon pisum* (Homoptera: Aphididae), on chemically defined diets of various pH and nutrient levels." Annals of the Entomological Society of America 58: 855-875.

Auclair, J. L. (1969) "Nutrition of plant-sucking insects on chemically defined diets." Entomologia Experimentalis et Applicata 12: 623-641.

Awmack, C. S. and Leather, S.R. (2002) "Host plant quality and fecundity in herbivorous insects." Annual Review of Entomology 47: 817-844.

Azpiroz-Leehan, R. and Feldmann, K.A. (1997) "T-DNA insertion mutagenesis in *Arabidopsis:* going back and forth." Trends in Genetics 13: 152-156.

Baldwin, I. T., Halitschke, R., Kessler, A. and Schittko, U. (2001) "Merging molecular and ecological approaches in plant-insect interactions." Current Opinion in Plant Biology 4: 351-355.

Banks, C. J. and MacAulay, E. D. M. (1964) "The feeding, growth and reproduction of *Aphis fabae Scop*. on *Vicia faba* under experimental conditions." Annals of Applied Biology 53: 229-242.

Barker, J. S. and. Tauber, O. E. (1954) "Fecundity of the pea aphid on garden pea under various combinations of light, moisture and nutrients." Journal of Economic Entomology 47: 113-117.

Barlow, P.W. (2002) "The root cap: cell dynamics, cell differentiation and cap function." Journal of Plant Growth Regulation. 21, 261–286.

Baumann, L. and Baumann, P. (1994) "Growth kinetics of the endosymbiont *Buchnera aphidicola* in the aphid *Schizaphis graminum*." Appl. Environ. Microbiol. 60: 3440-3443.

Bernays, E. A. and Funk, D. J. (1999) "Specialists make faster decisions than generalists: experiments with aphids." Proceedings of the Royal Society of London Series B. vol. 266 no. 1415 151-156.

Bevan, M., Mayer, K., White, O., Eisen, J. A, Preuss, D., Bureau, T., Salzberg, S. L. and Mewes, H.W. (2001) "Sequence and analysis of the *Arabidopsis* genome." Current Opinion in Plant Biology 4: 105-110.

Birch, L. C. (1948) "The intrinsic rate of natural increase of an insect population." Journal of Animal Ecology 17: 15-26.

Blackman, R. L. (1974) "Life-cycle variation of *Myzus persicae* (Sulz.) (Hom., Aphididae) in different parts of the world, in relation to genotype and environment." Bulletin of Entomological Research. 63: 595–607.

Boggess, S. F. and Stewart, C. R (1976) "Effect of Water Stress on Proline Synthesis from Radioactive Precursors." Plant Physiology. (1976) 58, 398-401

Boorer, K. J., Frommer, W. E., Bush, D. R., Kreman, M., Loo, D. D. F. and Wright, E. M. (1996) "Kinetics and specificity of a H+/amino acid transporter from *Arabidopsis thaliana*." The Journal of Biological Chemistry 271: 2213-2220.

Bouché, N. and Bouchez, D. (2001) "*Arabidopsis* gene knockout: phenotypes wanted." Current Opinion in Plant Biology 4: 111-117.

Bouchez, D. and Höfte, H. (1998) "Functional genomics in plants." Plant Physiology 118: 725-732.

Boyes, D. C., Zayed, A. M., Ascenzi, R., McCaskill, A. J., Hoffman, N. E., Davis, K. R. and Gorlach, J. (2001) "Growth stage-based phenotypic analysis of *Arabidopsis*; A model for high throughput functional genomics in plants." Plant Cell 13: 1499-1510.

Bradley, R. H. E. (1952) "Studies on the aphid transmission of a strain of henbane mosaic virus." Annals of Applied Biology 39: 78-97.

Bradley, R. H. E., Sylvester, E. S, Wade, C.V. (1962) "Note on the movements of the mandibular and maxillary stylets of the aphid vector *Myzus persicae* (Sulzer)." The Canadian Entomologist. 94: 653-654.

Broadbent, L. (1951) "Aphid excretion." Proceedings of the Royal Entomological Society of London 26: 97-103.

Brough, D. A. (1990) "Ultrastructural features of egg development in oviparae of the vetch aphid, *Megoura viciae*." Tissue Cell 22: 51-63.

Buchner, P. (1965) "Endosymbiosis of Animals with Plant Microorganisms." Interscience Publishers (New York), Rev. Eng. edition, xvii, 909 p.

Bush, D. R. (1993) "Proton-coupled sugar and amino-acid transporters in plants." Annual Review of Plant Physiology. Plant Molecular Biology. 44, 513–542.

Bush, D. R. (1999) "Amino acid transport. In Plant Amino Acids: Biochemistry and Biotechnology" (Singh, B.K., ed.). New York/Basel/Hong Kong: Marcel Dekker, pp. 305–318.

Caillaud, M. C., Via, S. (2000) "Specialized feeding behavior influences both ecological specialization and assortative mating in sympatric host races of pea aphids." American Naturalist. 156: 606-621.

Cakmak, I. and Engels, C. (1999) "Role of mineral nutrients in photosynthesis and yield formation." Crop Nutrition 141-168.

Calabrese, E. J., Edwards, L. J. (1976) "Light and gravity in leaf-side selection by the green peach aphid, *Myzus persicae*." Annals of the Entomological Society of America. 69: 1145-1146.

Caputo, C. and Barneix A. J. (1997) "Export of amino acids to the phloem in relation to N supply in wheat." Physiologica Plantarum 101: 853-860.

Chaffei, C., Pageau, K., Suzuki, A., Gouia, H., Ghorbel, M. H. and Masclaux-Daubrese, C. (2004) "Cadmium toxicity induced changes in nitrogen management in *Lycopersicon esculentum* leading to a metabolic safeguard through an amino acid storage strategy. ." Plant and Cell Physiology 45: 1681-1693.

Chang, H. C. and Bush, D. R (1997) " Membrane topology of a neutral amino acid transporter (NAT2/AAPl) from *Arabidopsis thaliana*." Plant Physiology 114: 549.

Chapin, F. S., Moilanen, L. and Kielland, K. (1993) "Preferential use of organic nitrogen for growth by a non-mycorrhizal arctic sedge." Nature, 361, 150–153.

Chapman, R. F., Bernays, E. A, Simpson, S. J. (1981) "Attraction and repulsion of the aphid, *Cavariella-aegopodii*, by plant odors." Journal of Chemical Ecology. 7: 881-888.

Chen, D. and Purcell, A. H. (1997) "Occurrence and Transmission of Facultative Endosymbionts in Aphids." Current Microbiology. Volume 34, Number 4, 220-225.

Cherqui, A. and Tjallingii**,** W. F. (2000) "Salivary proteins of aphids, a pilot study on identification, separation and immunolocalisation." Journal of Insect Physiology 46: 1177-1186.

Chinery, M. (1993) "Insects of Britain & Northern Europe." HarperCollins UK.

Clough, S. J. and Bent, A. F. (1998) "Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*." Plant Journal 16, 735–743.

Cole, R. A. (1997) "Comparison of feeding behaviour of two Brassica pests *Brevicoryne brassicae* and *Myzus persicae* on wild and cultivated brassica species." Entomologia Experimentalis et Applicata 85: 135-143.

Corbesier, L., Bernier G., Perilleux, C. (2002) "C:N ratio increases in the phloem sap during floral transition of the long-day plants *Sinapis alba* and *Arabidopsis thaliana*. ." Plant and cell Physiology 43: 684-688.

Dadd, R., Krieger, D. L. (1968) "Dietary requirements of the aphid *Myzus persicae*." Journal of Insect Physiology. 14: 741-764.

Dadd, R. and Krieger, D. L. (1968) "Dietary amino acid requirements of the aphid *Myzus persicae*." Journal of Insect Physiology 14: 741-764.

Dadd, R. and Mittler, T. E. (1966) "Permanent culture of an aphid on a totally synthetic diet." Experientia 12: 832-833.

Dadd, R. and Mittler, T. E. (1965) "Studies on the artificial feeding of the aphid *Myzus persicae* (Sulzer) - Ill. Some major nutritional requirements. ." Journal of Insect Physiology 11: 714-743.

De Loach, C. J. (1974) "Rate of increase of populations of cabbage, green peach and turnip aphids at constant temperatures." Annals of the Entomological Society of America 67: 332-340.

Delrot, S., Atanassova, R., Gomes, E. and Coutos-Thevenot, P. (2001) Plasma membrane transporters: a machinery for uptake of organic solutes and stress resistance. Plant Science. 161, 391–404.

Dixon, A. F. G. (1985) "Structure of aphid populations." Annual Review of Entomology 30: 155-174.

Dixon, A. F. G. (1987) "Aphids: Their Biology, Natural Enemies and Control." Elsevier.

Dixon, A. F. G. (1992) "Constraints on the rate of parthenogenetic reproduction and pest status of aphids." Invertebrate Reproduction and Development 22: 159-163.

Dixon, A. F. G. (1998) "Aphid Ecology: an optimisation approach." Chapman & Hall.

Dixon, A. F. G., Horth, S, and Kindlmann, P. (1993) "Migration in insects-cost and strategies." Journal of Animal Ecology 62: 182-190.

Dixon, A. F. G. and Wellings, P.W. (1982) "Seasonality and reproduction in aphids." International Journal of Invertebrate Reproduction." Vol. 5, no. 2, pp. 83-89.

Dixon, A. F. G. and Wratten, S. D. (1971) "Laboratory studies on aggregation, size and fecundity in the black bean aphid, *Aphis fabae* Scop." Bulletin of Entomological Research. 61: 97-111.

Dolan, L. (2001) "How and where to build a root hair." Current Opinion in Plant Biology. 4, 550–554.

Dolan, L., Janmaat, K., Willemsen, V., Linstead, P., Poethig, S., Roberts, K. and Scheres, B. (1993) "Cellular organization of the *Arabidopsis thaliana* root hair." Development, 119, 71–84.

Douglas, A. E. (1988) "Sulphate utilisation in an aphid symbiosis." Insect Biochemistry. 18: 599-605.

Douglas, A. E. (1990) "Nutritional interactions between *Myzus persicae* and its symbionts. In *Aphid-Plant Genotype Interactions*" ed. RK Campbell, RD Eikenbary, pp. 319–27. Amsterdam: Elsevier

Douglas, A. E. (1992) "Requirement of pea aphids (*Acyrthosiphon pisum*) for their symbiotic bacteria." Entomologia Experimentalis et Applicata. 65: 195-198.

Douglas, A. E. (1993) "The nutritional quality of phloem sap utilized by natural aphid populations." Ecological Entomology. 18: 31-38.

Douglas, A. E. (1996) "Reproductive failure and the amino acid pools in pea aphids (*Acyrthosiphon pisum*) lacking symbiotic bacteria." Journal of Insect Physiology. 42: 247-255.

Douglas, A. E. (1998) "Nutritional interactions in insect-microbial symbioses:aphids and their symbiotic bacteria *Buchnera*." Annual Review of Entomology 43: 17-37.

Douglas, A. E. (2003) "The nutritional physiology of aphids." Advances in Insect Physiology 31: 73-140.

Douglas, A. E., Minto, L. B. and Wilkinson, T. L. (2001) "Quantifying nutrient production by the microbial symbionts in an aphid." Journal of Experimental Biology, 204, 349-358.

Douglas, A. E. and Prosser, W. A. (1992) "Synthesis of the essential amino acid tryptophan in the pea aphid (*Acyrthosiphon pisum*) symbiosis." Journal of Insect Physiology 38: 565–568.

Febvay, G., Rahbe, Y., Rynkiewicz, M., Guillard, J. and Bonnot, G. (1999) "Fate of dietary sucrose and neosynthesis of amino acids in the pea aphid, *Acyrthosiphon pisum*, reared on different diets." Journal of Experimental Biology. 19: 2639–2652.

Feldmann, K. A. (1991) "T -DNA insertion mutagenesis in *Arabidopsis*: mutational spectrum." Plant Journal 1: 71-82.

Fischer, W. N., Andre, E., Rentsch, D., Krolkiewicz, S. and Tegeder, M. (1998) "Amino acid transport in plants." Trends in Plant Science 3: 188-195.

Fischer, W. N., Kwart, M., Hummel, S. and Frommer, W. E. (1995) "Substrate specificity and expression profile of amino acid transporters (AAPs) in *Arabidopsis*." The Journal of Biological Chemistry 270: 16315-16320.

Fischer, W. N., Loo, D. D. F., Koch, W., Ludewig, U., Boorer, K. J., Tegeder, M., Rentsch, D., Wright, E. M. and Frommer, W. E. (2002) "Low and high affinity amino acid H+-cotransporters for cellular import of neutral and charged amino acids." Plant Journal 29: 717-731.

Forbes, A. R. (1969) "The stylets of the green peach aphid, *Myzus persicae* (Homoptera: Aphididae)." The Canadian Entomologist. 101: 31-41.

Forbes, A. R. (1977) "Aphids as Virus Vectors." New York Academic.

Frommer, W. B., Hummel, S. and Riesmeier, J. W. (1993) "Expression cloning in yeast of a cDNA encoding a broad specificity amino acid permease from *Arabidopsis thaliana*. ." Proceedings of the National Academy of Science of the United States of America 90: 5944-5948.

Gamalei, Y. (1991) "Phloem loading and its development related to plant evolution from trees to herbs." Trees. 5:50-64.

Girousse, C. and Bournville, R. (1994) "Role of phloem sap quality and exudation characteristics on performance of pea aphid grown on lucerne genotypes". Entomologia Experimentalis et Applicata. Volume 70, Issue 3, pages 227–235

Gould, N., Thorpe, M. R., Minchin, P. E. H., Pritchard, J. and White, P. J. (2004) "Solute is imported to elongating root cells of barley as a pressure driven flow of solution." Functional Plant Biology 31: 391-397.

Griffiths, D. C., Pickett, J. A. and Woodcock, C. (1982) "Behaviour of alatae of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) on chemically-treated surfaces after tethered flight." Bulletin of Entomological Research. 72: 687-693.

Griffiths, G. and Beck, S. D. (1974) "Effects of antibiotics on intracellular symbiotes in the pea aphid *Acyrthosiphon pisum*." Cell Tissue Research. 48: 287-300.

Hale, B. K., Bale, J. S., Pritchard, J., Masters, G. J. and Brown, V. K. (2003) "Effects of host plant drought stress on the performance of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.): a mechanistic analysis." Ecological Entomology. Volume 28, Issue 6, pages 666–677

Hardie, J. (1989) "Spectral specificity for targeted flight in the black bean aphid, *Aphis fabae*." Journal of Insect Physiology. 35: 619-626.

Hardie, J. H., Taylor, N. J, and Griffiths, D. C. (1992) "The combination of electronic monitoring and video-assisted observations of plant penetration by aphids and behavioral effects of polygodial." Entomologia Experimentalis et Applicata 62: 233-239.

Harrewijn, P. (1970) "Reproduction of the aphid *Myzus persicae* related to the mineral nutrition of potato plants." Entomologia Experimentalis et Applicata 13: 307-319.

Harrewijn, P. and Noordink, J. Ph. W. (1971) "Taste perception of *Myzus persicae* in relation to food uptake and developmental processes." Entomologia Experimentalis et Applicata 14: 413-419.

Harrington, R., Katis, N. and Gibson, R. W. (1986) "Field assessment of the relative importance of different aphid species in the transmission of potato virus Y." Potato Research. 29: 67-76.

Hayashi, H., Fukuda, A., Suzui, N. and Fujimaki, S. (2000) "Proteins in the sieve element-companion cell complexes: their detection, localisation and possible functions." Australian Journal of Plant Physiology 27: 489-496.

Heisswolf, A., Obermaier, E. and Poethke, H-J. (2005) "Selection of large host plants for oviposition by a monaphagous leaf beetle: nutritional quality or enemy-free space?" Ecological Entomology. 30, 3 : 299-306.

Herrera-Rodriguez, M. B., Maldonado, J. M. and Perez-Vicente R. (2006) "Role of asparagines and asparagines synthetase genes in sunflower (*Helianthus annuus*) germination and natural senescence." Journal of Plant Physiology 163: 1061-1070.

Hinde, R. (1971) "The control of the mycetocyte symbiotes of the aphids *Brevicoryne brassicae, Myzus persicae* and *Macrosiphum rosae*." Journal of Insect Physiology 17: 1791-1800.

Hodgson, C. J., and Elbakhiet, I. B. (1985) "Effect of colour and shape of 'target' hosts on,the orientation of emigrating adult apterous *Myzus persicae* in the laboratory." Entomologia Experimentalis et Applicata 38: 267-272.

Humphreys, N. J. (1996) "Symbiosis and embryo production in the aphid symbiosis." PhD thesis. The University of York, United Kingdom**.** 162 pp**.**

Hunt, E. J. (2005) "Investigating Aphid-Plant Interactions by Manipulating Sieve Element Composition: a Functional Genomics Approach." PhD Thesis. School of Biosciences. Birmingham, The University of Birmingham.

Ibbotson, A. and Kennedy, J. S. (1959) "Interaction between walking and probing in *Aphis fabae* Scop." Journal of Experimental Biology 36: 377-390.

Jaffe, M. J., Leopold, A. C. and Staples, R. C. (2002) "Thigmo responses in plants and fungi." American Journal of Botany 89: 375-382.

Jeffrey, S. W. and Humphrey, G. F. (1975) "New Spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae, and natural phytoplankton." Biochem. Physiol. Pflanz. 167: 191-194.

Jones, F. G. W., and Jones, M. G. (1974) "Pests of Field Crops." London, Edward Arnold.

Karley, A. E., Douglas, A. E. and Parker, W. E. (2002) "Amino acid composition and nutritional quality of potato leaf phloem sap for aphids." Journal of Experimental Biology. 205, 3009-3018.

Kennedy, J. S., Ibbotson, A. and Booth, C. O. (1950) "The distribution of aphid infestation in relation to leaf age. *Myzus persicae* (Sulzer) and *Aphis fabae* Scop. on spindle trees and sugar-beet plants." Annals of Applied Biology 37: 651-679.

Kennedy, J. S. and Mittler, T. E. (1953) "A method of obtaining phloem sap via the mouth-parts of aphids." Nature 171: 528.

Kennedy, J. S. and Stroyan, H. L. G. (1959) "Biology of aphids." Annual Review of entomology. 4: 136-160.

Kennedy, J. S., Booth, C. O. and Kershaw, W. J. S. (1961) "Host finding by aphids in the field. Visual attraction." Annals of Applied Biology 49: 1-21.

Kichey, T., Hirel, B., Heumez, E., Dubois, F. and Le Gouis, J. (2007) "In winter wheat (*Triticum aestivum L.*), post-anthesis nitrogen uptake and remobilisation to the grain correlates with agronomic traits and nitrogen physiological markers." Field Crop Research 102: 22-32.

Kielland, K. (1994) "Amino acid absorption by arctic plants: implications for plant nutrition and nitrogen cycling." Ecology. 75, 2373–2383.

Kindlmann, P. and Dixon, A. F. G. (1989) "Developmental constraints in the evolution of reproductive strategies: telescoping of generations in parthenogenetic aphids." Functional Ecology. Vol. 3, No. 5 (1989), pp. 531-537

Klingauf, F. A. (1987) "Aphids, their Biology, Natural Enemies and Control." Amsterdam, Elsevier.

Kobayashi , M., Ishikawa, H. (1993) "Breakdown of indirect flight muscles of alate aphids (*Acyrthosiphon pisum*) in relation to their flight, feeding and reproductive behaviour." Journal of Insect Physiology 39: 549-554.

Koga, R., Tsuchida, T., Sakurai, M, and Fukatsu, T. (2007) "Selective elimination of aphid endosymbionts: effects of antibiotic dose and host genotype, and fitness consequences." FEMS microbiology ecology 60: 229-239.

Komor, E., Orlich, G., Weig, A., and Kockenberger, W. (1996) "Phloem loading -

Not metaphysical, only complex: Toward a unified model of phloem loading." Journal of Experimental Botany. 47:1115-1164.

Krysan, P. J., Young, J. C. and Sussman, M. R. (1999) "T-DNA as an insertional mutagen in *Arabidopsis.*" The Plant Cell 11: 2283-2290.

Kühn. C., Franceschi, V.R., Schulz. A., Lemoine, R. and Frommer, W.B. (1997) "Macromolecular trafficking indicated by localization and turnover of sucrose transporters in enucleate sieve elements." Science. 275(5304):1298–1300.

Lalonde, T. M., Throne-Holst, M., Frommer, W. B., and Patrick J. W. (2003) "Phloem loading and unloading of sugars and amino acids." Plant Cell and Environment 26: 37-56.

Latorre, L., Gil, R., Silva, F. J. and and Moya, A. (2005) "Chromosomal stasis versus plasmid plasticity in aphid endosymbiont *Buchnera aphidicola*." Heredity. 95, 339–347.

Liadouze, I., Febvay, G., Guillaud, J. and Bonnot, G. (1996) "Metabolic fate of energetic amino acids in the aposymbiotic pea aphid *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae)." Symbiosis. 21: 115–127.

Liu, X. and Bush, D. R. (2006) "Expression and transcriptional regulation of amino acid transporters in plants." Amino Acids. 30, 113–120.

Loomis, W. E. (1955) "Resistance of plants to herbicides. In Origin of Resistance to Toxic Agents." New York: Academic Press. pp. 99–121.

Mann, J. A., Harrington, R., Carter, N., and Plumb, R.T. (1997) "Control of aphids and barley yellow dwarf virus in spring-sown cereals." Crop Protection 16: 81-87.

Marschner, H. (1989) "Mineral Nutrition of Higher Plants." London, Academic Press.

Martienssen, R. A. (1998) "Functional genomics: probing plant gene function and expression with transposons." Proceedings of the National Academy of Science of the United States of America 95: 2021-2026.

Martin, B, C. J., Tjallingii, W. F. and Fereres, A. (1997) "Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of non-persistently transmitted plant viruses." Journal of General Virology 78: 2701-2705.

Masclaux-Daubresse, C., Reisdorf-Cren. M., Pageau, K., Lelandais, M., Grandjean, O., Kronenberger, J., Valadier, M-H., Feraud, M., Jouglet, T. and Suzuki, A. (2006). "Glutamine sythetase-glutamate synthase pathway and glutamate dehydrogenase play distinct roles in the sink source-source nitrogen cycle in tobacco." Plant Physiology 140: 444-456.

Mattson, W. J. (1980) "Herbivory in Relation to Plant Nitrogen Content." Annual

Review of Ecology and Systematics. Vol. 11: 119-161.

McLean, D. L. and Kinsey, M. G. (1964) "A technique for electronically recording aphid feeding and salivation." Nature 202: 1358-1359.

McNeill, S. and Southwood, T. R. E. (1978) "The role of nitrogen in the development of insect/plant relationships." In Biochemical Aspects of Plant and Animal Coevolution. pp 77– 98.

Miles, P. W. (1999) "Aphid saliva." Biological Reviews 74: 41-85.

Minks, A. K., Harrewijn P (1987) "Aphids: Their Biology, Natural Enemies and Control." Amsterdam, Elsevier.

Mittler, T. E. (1967) "Effect of amino acid and sugar concentrations on the food uptake of the aphid *Myzus persicae*." Entomologia Experimentalis et Applicata 10: 3951.

Mittler, T. E. (1967) "Effect on aphid feeding of dietary methionine." Nature 214: 386.

Mittler, T. E. (1967) "Gustation of dietary amino acids by the aphid *Myzus persicae*." Entomologia Experimentalis et Applicata 10: 87-96.

Mittler, T. E. (1970) "Effects of dietary amino acids on the feeding rate of the aphid *Myzus persicae*." Entomologia Experimentalis et Applicata 13: 432-437.

Mittler, T. (1971) "Dietary amino acid requirement of the aphid *Myzus persicae* affected by antibiotic uptake." Journal of Nutrition 101: 1023-1028.

Mittler, T. E. and Dadd, R. H. (1962) "Artificial feeding and rearing of the aphid, *Myzus persicae* (Sulzer), on a completely defined synthetic diet." Nature 195: 404.

Mittler, T. E. and Dadd, R. H. (1963) "Studies on the artificial feeding of the aphid *Myzus persicae* (Sulzer) - Relative survival, development and larviposition on different diets." Journal of Insect Physiology 9: 741-757.

Mittler, T. E. and Dadd, R. H. (1964) "Gustatory discrimination between liquids by the aphid *Myzus persicae* (Sulzer)." Entomologia Experimentalis et Applicata 7: 315-328.

Mittler, T. E. and Dadd, R. H. (1965) "Differences in the probing responses of *Myzus persicae* (Sulzer) elicited by different feeding solutions behind a parafilm membrane." Entomologia Experimentalis et Applicata 8: 107-122.

Mittler, T. E., Dadd, R. H. and Daniels, S. C. (1970). "Utilisation of different sugars by the aphid *Myzus persicae*." Journal of Insect Physiology 16: 1873-1890.

Mittler, T. E. and Meikle, T. (1991) "Effects of dietary sucrose concentration on aphid honeydew carbohydrate levels and rates of excretion." Entomologia Experimentalis et Applicata. Volume 59, Issue 1, pages 1–7

Mittler, T. E. and Sylvester, E. S. (1961) "A Comparison of the Injury to Alfalfa by the Aphids, *Therioaphis maculata* and *Macrosiphum sp.*" Journal of Economic Entomology, Volume 54, Number 4,pp. 615-622(8)

Moran, N. A. (1992) "The evolution of aphid lifecycles." Annual Review of Entomology. 37: 321–348.

Moran, N., Munson, M. A, Baumann, P. and Ishikawa, H. (1993) "A molecular clock in endosymbiotic bacteria is calibrated using the insect hosts." Proceedings of the Royal Society, London. 253: 167-171.

Moran, P. and Thompson, G. A. (2001) "Molecular responses to aphid feeding in *Arabidopsis* in relation to plant defence pathways." Plant Physiology 125: 1074-1085.

Mueller, C., Williams, I. S. and Hardie, J. (2001) "The role of nutrition, crowding and interspecific interactions in the development of winged aphids." Ecological Entomology 26: 330-340.

Münch, E. (1930) "Die Stoffbewegungen in der Pflanze." (Jena, Germany: G. Fischer).

Munson, M., Baumann, P., Clark, M. A., Baumann, L. and Moran, N. A. (1991) "Evidence for the establishment of aphid-eubacterium endosymbiosis in an ancestor of four aphid families." The Journal of Bacteriology. 173: 6321-6324.

Munson, M., Baumann, P. and Kinsey, M. G. (1991) "*Buchnera* gen. nov. and *Buchnera aphidicola* sp. nov., a taxon consisting of the mycetocyte-associated, primary endosymbionts of aphids." International Journal of Systematic Bacteriology 41: 566-568.

Nault, L. R. and Gyrisco, G.G. (1966) "Relation of the feeding process of the pea aphid to the inoculation of pea enation mosaic virus." Annals of the Entomological Society of America 59: 1185-1197.

Nault, L. R. and Styer, W. E. (1972) "Effects of sinigrin on host selection by aphids." Entomologia Experimentalis et Applicata. 15: 423-437.

Neal, J. J., Tingey, W. M. and Steffens, J. C. (1990) "Sucrose esters of carboxylic-acids in glandular trichomes of *Solanum berthaultii* deter settling and probing by green peach aphid. ." Journal of Chemical Ecology. 16: 487-497.

Nottingham, S. F. and Hardie, J. (1993) "Flight behavior of the black bean aphid, *Aphis fabae*, and the cabbage aphid, *Brevicoryne brassicae*, in host and nonhost plant odor." Physiological Entomology 18: 389-394.

Nowak, H. and Komor, E. (2010) "How aphids decide what is good for them: experiments to test aphid feeding behaviour on *Tanacetum vulgare* (L.) using different nitrogen regimes." Oecologia. 163(4):973-84.

Okumoto, S., Koch, W., Tegeder, M., Fischer, W. N., Biehl, A., Leister, D., Stierhof, Y. D. and Frommer, W. B. (2004) "Root phloem-specific expression of the plasma membrane amino acid proton co-transporter AAP3." Journal of Experimental Botany 55: 2155-2168.

Oparkaa, K. J. and Turgeon, R. (1999) "Sieve Elements and Companion Cells-Traffic Control Centres of the Phloem." Plant Cell, Vol. 11, 739-750.

Ortiz-Lopez, A., Chang, H. C. and Bush, D. R. (2000) "Amino acid transporters in plants." Biochimica Et Biophysica Acta-Biomembranes 1465: 275-280.

Parinov, S. and Sundaresan, V. (2000) "Functional genomics in *Arabidopsis*: large-scale insertiona1 mutagenesis complements the genome sequencing project." Current Opinion in Plant Biotechnology 11: 157-161.

Park, K. C. and Hardie, J. (2004) "Electrophysiological characterisation of olfactory sensilla in the black bean aphid, *Aphis fabae*." Journal of Insect Physiology 50: 647-655.

Pate, J. S. (1980) "Transport and partitioning of nitrogenous solutes." Annual Review of Plant Physiology 31: 313-340.

Pelletier, Y. (1990) "The role of the colour of the substratum on the initiation of the probing behavior in *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae*." Annual Review of Entomology: 309-330.

Phelan, P. L. and Miller, J. R. (1982) "Post-landing behavior of alate *Myzus persicae* as altered by (E)-B-farnesene and three carboxylic acids." Entomologia Experimentalis et Applicata 32: 46-53.

Ponder, K. L., Pritchard, J., Harrington, R. and Bale, J. S. (2000) "Difficulties in location and acceptance of phloem sap combined with reduced concentration of phloem amino acids explain lowered performance of the aphid *Rhopalosiphum padi* on nitrogen deficient barley (*Hordeum vulgare*) seedlings." Entomologia Experimentalis et Applicata 97: 203-210.

Powell, G. (1991) "Cell membrane punctures during epidermal penetrations by aphids: consequences for the transmission of two potyviruses." Annals of Applied Biology 119: 313-321.

Powell, G. and Hardie, J. (2000) "Host-selection behaviour by genetically identical aphids with different plant preferences." Physiological Entomology 25: 54-62.

Powell, G., Pirone, T. and Hardie J. (1995) "Aphid stylet activities during potyvirus acquisition from plants and an in vitro system that correlate with subsequent transmission." European Journal of Plant Pathology. 101: 411-420.

Powell, G. (2005) "Intracellular salivation is the aphid activity associated with inoculation of non-persistently transmitted viruses." Journal of General Virology. 86: 469-472.

Powell, G., Tosh, C. R., Hardie, J. (2006) "Host Plant Selection by Aphids: Behavioural, Evolutionary and Applied Perspectives." Annual Review of Entomology. 51: 309-330.

Prado, E., and Tjallingii, W. F. (1994) "Aphid activities during sieve element punctures." Entomologia Experimentalis et Applicata 72: 157-165.

Price, P. W. (1991) "The Plant Vigor Hypothesis and Herbivore Attack." Oikos, Vol. 62, No. 2 (Nov., 1991), pp. 244-251

Prosser, W. A., Simpson, S. G. and Douglas, A. E. (1992) "How an aphid responds to variation in nitrogen." Journal of Insect Physiology 38: 301-307.

Pyati, P., Bandani, A. R., Fitches, E. and Gatehouse, J.A. (2011) "Protein digestion in cereal aphids (*Sitobion avenae*) as a target for plant defence by endogenous proteinase inhibitors." Journal of Insect Physiology. 57(7):881-91.

Raab, T.K., Lipson, D.A. and Monson, R.K. (1996) "Non-mycorrhizal uptake of amino acids by roots of the alpine sedge *Kobresia myosuroides* : implications for the alpine nitrogen cycle." Oecologia. 108, 488–494.

Rahbe, Y., Delobel, B., Febvay, G. and Chantegrel, B. (1994) "Aphid-specific triglycerides in symbiotic and aposymbiotic *Acyrthosiphon pisum*." Insect Biochemistry and Molecular Biology. Volume 24, Issue 1,Pages 95-101

Ramsey, J. S., Rider, D. S., Walsh, T., De Vos, M., Gordon, K., Ponnala, L., Macmil, S. L, Roe, B. A. and Jander, G.(2010) "Comparative analysis of detoxification enzymes in *Acrythosiphon pisum* and *Myzus persicae*." Insect Molecular Biology. 19 (Suppl. 2): 177–186.

Raven, J. A. (1991) "Long-term functioning of enucleate sieve elements - Possible mechanisms of damage avoidance and damage repair." Plant Cell Environment. 14:139-146.

Raven, P. H., Evert, R. F. and Eichhom, S. E. (1999) "Biology of Plants." Worth Publishers.

Rentsch, D., Boorer, K. J. and Frommer, W. B. (1998) "Structure and function of plasma membrane amino acid, oligopeptide and sucrose transporters from higher plants." Journal of Membrane Biology 162: 177-190.

Rentsch, D., Schimdt, S. and Tegeder, M. (2007) "Transporters for uptake and allocation of organic nitrogen compounds in plants." FEBS Letters 581: 2281-2289.

Robson, J. D., Wright, M. G., and Almeida, R. P. (2007) "Biology of *Pentalonia nigronervosa* (Hemiptera, Aphididae) on Banana Using Different Rearing Methods." Environmental Entomology, Volume 36, Number 1,pp. 46-52(7)

Rolletschek, H., Hosein, F., Miranda, M., Heim, U., Gotz, K. P., Schlereth, A., Borisjuk, L., Saalbach, I., Wobus, U. and Weber, H. (2005) "Ectopic expression of an amino acid transporter (VfAAP1) in seeds of *Vicia narbonensis* and pea increases storage proteins." Plant Physiology 137: 1236-1249.

Sanders, A., Collier, R., Trethewy, A., Gould, G., Sieker, R. and Tegeder, M. (2009) "AAP1 regulates import of amino acids into developing *Arabidopsis* embryos." The Plant Journal 59:540-552.

Sasaki, T., Hayashi, H. and Ishikawa, H. (1991) "Growth and reproduction of the symbiotic and aposymbiotic pea aphids, *Acyrthosiphon pisum* maintained on artificial diets." Journal of Insect Physiology. 37: 749-756.

Sauge, M. H., Kervella, J. and Rahbe, Y. (1998) "Probing behaviour of the green peach aphid *Myzus persicae* on resistant Prunus genotypes." Entomologia Experimentalis et Applicata 89: 223-232.

Schmidt, R., Stransky, H. and Koch, W. (2007) "The amino acid permease AAP8 is important for early seed development in *Arabidopsis thaliana*." Planta 226: 805.

Schobert, C. and Komor, E. (1989) "Amino-acid-uptake by *Ricinus communis* roots - characterization and physiological significance." Plant Cell Environ. 10, 493–500.

Schmidt, S. and Stewart, G. R. (1999) Glycine metabolism by plant roots and its occurrence in Australian plant communities. Australian Journal of Plant Physiology. 26, 253–264.

Schoonhoven, L. M., Jermy, T. and van Loon, J. J. A. (1998) "Insect-Plant Biology" Chapman & Hall.

Schoonhoven, L. M. and Derkson-Koppers, I. (1976) "Effects of some allelochemicals on food uptake and survival of a ployphagous aphid, *Myzus persicae*." Entomologia Experimentalis et Applicata 19: 53-56.

Scriber, J. M. and Slansky, F. (1981) The nutritional ecology of immature insects. Annual Review of Entomology. 26:183–211.

Shigenobu, S., Watanabe, H., Hattori, M., Sakaki, Y. and Ishikawa, H. (2000) "Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp." Nature 407, 81-86.

Sjolund, R. D. (1997) "The Phloem Sieve Element: A River Runs through It." Plant Cell 9(7): 1137–1146.

Smith, H. G. and Hallsworth, P. B. (1990) "The effects of yellowing viruses on yield of sugar beet in field trials, 1985 and 1987." Annals of Applied Biology 116: 503-511.

Smith, C., Andrew, J. and Milburn, J. A. (1980) "Phloem turgor and the regulation of sucrose loading in *Ricinus communis* L. Planta." Planta 148: 42-48.

Srivastava, P. N. (1987). "Nutritional physiology - Aphids, their Biology, Natural Enemies and Control." Amsterdam, Elsevier.

Srivastava, P. N. and Auclair, J. L. (1971). "Influence of sucrose concentration on diet uptake and performance by the pea aphid, *Acyrthosiphon pisum*." Annals of the Entomological Society of America 64: 739-743.

Srivastava, P. N. and Auclair, J. L. (1974). "Effect of amino acid concentration on diet uptake and performance by the pea aphid *Acyrthosiphon pisum* (Homoptera: Aphididae)." The Canadian Entomologist. 106: 149-156.

Srivastava, P. N. and Auclair, J. L. (1975) "Role of single amino acids in phagostimulation, growth and survival of *Acyrthosiphon pisum*." Journal of Insect Physiology 21: 1865-1871.

Srivastava, P. N., Gao, Y., Levesque, J. and Auclair JL. (1985) "Differences in amino acid requirement between two biotypes of the pea aphid, *Acyrthosiphon pisum*." Canadian. Journal of Zoology. 63:603–6

Storer, J. R., Powell, G. and Hardie J. (1996) "Settling responses of aphids in air permeated with non-host plant volatiles." Entomologia Experimentalis et Applicata 80: 76-78.

Strong, D. R., Lawton, J. H. and Southwood, T. R. E. (1984) "Insects on Plants Community Patterns and Mechanisms." Oxford: Blackwell Sci. 313 pp.

TAGI (2000) "Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*." Nature 408: 796-815.

Taiz, L. and Zeiger, E. (1991) "Plant Physiology." California, Benjamin Cummings.

Thomas, H. and. Stoddart, J. L. (1980) "Leaf senescence." Annual Review of Plant Physiology 31: 83-111.

Tilsner, J., Kassner, N., Struck, C. and Lohaus, G. (2005) "Amino acid content and transport in oilseed rape (*Brassica napus* L.) under different conditions." Planta 221: 328-338.

Tjallingii, W. F. (1978) "Electronic recording of penetration behaviour by aphids." Entomologia Experimentalis et Applicata 24: 521-530.

Tjallingii, W. F. (1978) "Mechano receptors of the aphid labium." Entomologia Experimentalis et Applicata 24: 731-737.

Tjallingii, W. F. (1985) "Electrical nature of recorded signals during stylet penetration by aphids." Entomologia Experimentalis et Applicata 38: 177-186.

Tjallingii, W. F. (1985) "Membrane potentials as an indication for plant- cell penetration by aphid stylets." Entomologia Experimentalis et Applicata 38: 187-193.

Tjallingii, W. F. (1987) "Electrical recording of stylet penetration activities - Aphids, their Biology, Natural Enemies and Control." Amsterdam, Elsevier.

Tjallingii, W. F. (1990) "Continuous recording of stylet penetration activities by aphids - Aphid- Plant Genotype Interactions." Amsterdam, Elsevier Science Publishers.

Tjallingii, W. F. (1994) "Sieve element acceptance by aphids." European Journal of Entomology 91: 47-52.

Tjallingii, W. F. and Gabrys, B. (1999) "Anomalous stylet punctures of phloem sieve elements by aphids." Entomologia Experimentalis et Applicata. Volume 91, Number 1, 97-103,

Tjallingii, W. F. and Hogan Esch, T. (1993) "Fine structure of aphid stylet route in plant tissues in correlation with EPG signals." Physiological Entomology 18: 317-328.

Tsay, Y. F., Chiu, C. C., Tsai, C. B., Ho, C. H. and Hsu, P. K. (2007) "Nitrate transporters and peptide transporters." FEBS Letters 581: 2290-2300.

Turgeon, R. (1991) "Symplastic phloem loading and the sink–source transition in leaves: A model. In Recent Advances in Phloem Transport and Assimilate Compartmentation. Nantes, France: Ouest Editions. pp. 18–22.

Turgeon, R. (1995) "The selection of raffinose oligosaccharides as translocates in higher plants. In Carbon Partitioning and Source - Sink Interactions in Plants." American Society of Plant Physiologists, pp. 195–203.

Turgeon, R. (1996) "Phloem loading and plasmodesmata." Trends Plant Science.1:403 - 441.

Turgeon, R., Beebe, D.U., and Gowan, E. (1993) "The intermediary cell: Minor-vein anatomy and raffinose oligosaccharide synthesis in the Scrophulariaceae." Planta 191:446-456.

Turgeon, R., and Medville, R. (1998) "The absence of phloem loading in willow leaves." Proceedings of the National academy of Sciences of the United States of America. 95:12055-12060.

Uzest, M., Gargani, D., Drucker, M., Hébrard, E., Garzo, E., Candresse, T., Fereres, A. and Blanc, S. (2007) "A protein key to plant virus transmission at the tip of the insect vector stylet." Proceedings of the National Academy of Sciences of the United States of America. vol. 104 no. 46 17959-17964.

Van Bel, A. J. E., Ehlers, K. and Knoblauch, M. (2002) "Sieve elements caught in the act." Trends in Plant Science 7(126-132).

Van Emden, H. F. (1966) "Studies on the relations of insect and host plant. III. A comparison of the reproduction of *Brevicoryne brassicae* and *Myzus persicae* (Homoptera: Aphididae) on Brussel's sprout plants supplied with different rates of nitrogen and potassium." Entomologia Experimentalis et Applicata 9: 444-460.

Van Emden, H. F. (1969) "The differing reactions of *Breyicoryne* and *Myzus* to leaf age, turgidity and soluble nitrogen in brassicas." Annals of Applied Biology 63: 324-326.

Van Emden, H. F., Eastop, V. F., Hughes, R. D. and Way, M. J. (1969) "The Ecology of *Myzus persicae*." Annual Review of Entomology 14: 197-270.

Van Emden, H. F. and Bashford., M. A. (1971) "The performance of *Brevicoryne brassicae* and *Myzus persicae* in relation to plant age and leaf amino acids." Entomologia experimentalis et applicata 14: 349-360.

Via, S. (1991) "The genetic structure of host plant adaptation in a spatial patchwork-demographic variability among reciprocally transplanted pea aphid clones." Evolution 45: 827-852.

Visser, J., Piron, P. G. M. and Hardie, J. (1996) "The aphids' peripheral perception of plant volatiles." Entomologia Experimentalis et Applicata 80: 35-38.

von Wiren, N., Gazzarrini, S. and Frommer, W.B. (1997) "Regulation of mineral nitrogen uptake in plants." Plant and Soil 196: 191-199.

Walling, L. L. (2000) "The myriad plant responses to herbivores." Journal of Plant Growth Regulation 19: 195-216.

Ward, S., Leather, S. R, Pickup, J. and Harrington, R. (1998) "Mortality during dispersal and the cost of host-specificity in parasites: How many aphids find hosts?" Journal of Animal Ecology. 67: 763-773.

Weibull, J. (1987) "Seasonal changes in the free amino acids of oat and barley phloem sap in relation to plant growth stage and growth of *Rhopalosiphum padi*." Annals of Applied Biology 111: 729-737.

Weibull, J. (1988) "Free amino acids in the phloem sap from oats and barley resistant to *Rhopalosiphum padi*." Phytochemistry 27: 2069-2072.

Wensler, R. (1977) "The fine structure of distal receptors on the labium of the aphid." Annual Review of Entomology 51: 309-330.

Wensler, R. J. D. (1962) "Mode of host selection by an aphid." Nature 195: 830-831.

Wensler, R. J. D. (1974) "Sensory innervation monitoring movement and position in the mandibular stylets of the aphid, *Brevicoryne brassicae*." Journal of Morphology. 143: 349-364.

Wensler, R. J. D. and Filshie, B. K. (1969) "Gustatory sense organs in the food canal of aphids." Journal of Morphology. 129: 473-492.

Whitehead, L. F. and Douglas, A.E. (1993) "A metabolic study of *Buchnera*, the intracellular bacterial symbionts of the pea aphid *Acyrthosiphon pisum*." Journal of Genetic Microbiology. 139, 821-826.

Wilkinson, T. and Douglas, A. E. (1995) "Aphid feeding, as influenced by the disruption of the symbiotic bacteria." Journal of Insect Physiology. 41: 635-640.

Wilkinson, T. L., Koga, R. and Fukatsu, T. (2007) "Role of host nutrition in symbiont regulation: impact of dietary nitrogen on proliferation of obligate and facultative bacterial endosymbionts of the pea aphid *Acyrthosiphon pisum*." Applied and environmental microbiology 4: 1362-1366.

Williams, L. E. and Miller, A. J. (2001) "Transporters responsible for the uptake and partitioning of nitrogenous solutes." Annual Review of Plant Physiology and Plant Molecular Biology 52: 659-688.

Woolridge, A. W. and Harrison, F. P. (1968) "Effects of soil fertility on abundance of green peach aphids on Maryland tobacco." Journal of Economic Entomology 61: 387-391.

Wyatt, J. and White, P.F. (1977) "Simple estimation of intrinsic increase rates for aphids and tetranychid mites." Journal of Applied Ecology 14: 757-766.

Zimmermann, S. (1960) "Transport in the phloem." Annual Review of Plant Physiology 11: 167-190.

Appendices

Appendix 1.1

Plant Characteristics Analysis

One-way ANOVA: Comparison of *aap1* and Col0 Rosette Leaf Number

```
Source  DF      SS      MS      F      P
Factor   1  209.76  209.76  29.87  0.000
Error   72  505.61    7.02
Total   73  715.36

S = 2.650   R-Sq = 29.32%   R-Sq(adj) = 28.34%
```

One-way ANOVA: Comparison of *aap1* and Col0 Rosette Leaf Width

```
Source  DF      SS    MS     F      P
Factor   1    9.22  9.22  2.37  0.128
Error   72  280.52  3.90
Total   73  289.74

S = 1.974   R-Sq = 3.18%   R-Sq(adj) = 1.84%
```

One-way ANOVA: Comparison of *aap1* and Col0 Above Root Biomass

```
Source  DF      SS      MS     F      P
Factor   1  0.0619  0.0619  1.10  0.298
Error   72  4.0535  0.0563
Total   73  4.1154

S = 0.2373   R-Sq = 1.50%   R-Sq(adj) = 0.14%
```

One-way ANOVA: Comparison of *aap1* and Col0 Plant Height

```
Source  DF      SS     MS     F      P
Factor   1   171.4  171.4  6.43  0.013
Error   72  1920.1   26.7
Total   73  2091.5

S = 5.164   R-Sq = 8.20%   R-Sq(adj) = 6.92%
```

One-way ANOVA: Comparison of *aap2* and Col0 Rosette Leaf Number

Source	DF	SS	MS	F	P
Factor	1	288.10	288.10	41.68	0.000
Error	73	504.57	6.91		
Total	74	792.67			

S = 2.629 R-Sq = 36.35% R-Sq(adj) = 35.47%

One-way ANOVA: Comparison of *aap2* and Col0 Rosette Leaf Width

Source	DF	SS	MS	F	P
Factor	1	40.06	40.06	9.54	0.003
Error	73	306.50	4.20		
Total	74	346.56			

S = 2.049 R-Sq = 11.56% R-Sq(adj) = 10.35%

One-way ANOVA: Comparison of *aap2* and Col0 Above Root Biomass

Source	DF	SS	MS	F	P
Factor	1	0.0638	0.0638	1.28	0.262
Error	73	3.6455	0.0499		
Total	74	3.7093			

S = 0.2235 R-Sq = 1.72% R-Sq(adj) = 0.37%

One-way ANOVA: Comparison of *aap2* and Col0 Plant Height

Source	DF	SS	MS	F	P
Factor	1	475.3	475.3	17.98	0.000
Error	73	1929.9	26.4		
Total	74	2405.2			

S = 5.142 R-Sq = 19.76% R-Sq(adj) = 18.66%

One-way ANOVA: Plant height versus Plant Genotype

Source	DF	SS	MS	F	P
Plant Genotype	1	475.3	475.3	17.98	0.000
Error	73	1929.9	26.4		
Total	74	2405.2			

S = 5.142 R-Sq = 19.76% R-Sq(adj) = 18.66%

Descriptive Statistics: Rosette Leaf Number of Col0 Plants

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Rosette No_1	35	0	17.629	0.494	2.921	12.000	15.000	17.000	20.000	24.000

Descriptive Statistics: Rosette Leaf Width of Col0 Plants

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Rosette width_1	35	0	9.220	0.264	1.560	6.000	8.200	9.400	10.600	12.000

Descriptive Statistics: Above Soil Plant Mass of Col0 Plants

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Rosette weight_1	35	0	0.5497	0.0376	0.2226	0.2200	0.3400	0.5100	0.6900	1.1100

Descriptive Statistics: Plant Height of Col0 Plants

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Height_1	35	0	37.249	0.900	5.322	25.900	33.200	37.300	41.700	47.300

Descriptive Statistics: Rosette Leaf Number of *aap1* Plants

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Rosette No aap1	39	0	14.256	0.381	2.381	10.000	13.000	14.000	16.000	20.000

Descriptive Statistics: Rosette Leaf Width of *aap1* Plants

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Rosette width aap1	39	0	8.513	0.365	2.282	4.000	7.400	8.200	10.100	13.000

Descriptive Statistics: Above Soil Plant Mass of *aap1* Plants

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Rosette weight aap1	39	0	0.4918	0.0400	0.2497	0.1100	0.2500	0.4700	0.7000	0.9700

Descriptive Statistics: Plant Height of *aap1* Plants

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Height aap1	39	0	34.200	0.804	5.018	19.000	32.000	34.900	37.000	44.100

Descriptive Statistics: Rosette Leaf Number of *aap2* Plants

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Rosette No	38	0	13.658	0.245	1.512	11.000	13.000	13.000	15.000	17.000

Descriptive Statistics: Rosette Leaf Width of aap2 Plants

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Rosette width	38	0	7.753	0.308	1.901	4.600	6.500	7.300	9.075	12.000

Descriptive Statistics: Above Soil Plant Mass of aap2 Plants

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Rosette weight	38	0	0.4874	0.0332	0.2045	0.1500	0.3200	0.4600	0.6700	0.8900

Descriptive Statistics: Plant Height of *aap2* Plants

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Height	38	0	32.187	0.828	5.106	23.800	28.200	33.000	36.100	42.600

Appendix 1.2

Whole Silique Analysis

General Linear Model: Analysis of Total Dry Seed Mass in *aap1, aap2* and Col0

```
Factor             Type   Levels  Values
Plant Genotype     fixed       3  1, 2, 3
Position (strata)  fixed       3  1, 2, 3


Analysis of Variance for Total Seed Weight, using Adjusted SS for Tests

Source             DF     Seq SS     Adj SS     Adj MS      F      P
Plant Genotype      2  0.0000019  0.0000015  0.0000008  17.34  0.000
Position (strata)   2  0.0000035  0.0000035  0.0000018  40.20  0.000
Error              74  0.0000033  0.0000033  0.0000000
Total              78  0.0000087


S = 0.000209807   R-Sq = 62.43%   R-Sq(adj) = 60.40%
```

General Linear Model: Analysis of Mean Dry Seed Mass - Comparison between *aap1* and Col0

```
Factor             Type   Levels  Values
Plant Genotype     fixed       2  1, 2
Position (strata)  fixed       3  1, 2, 3


Analysis of Variance for Total Seed Weight, using Adjusted SS for Tests

Source             DF     Seq SS     Adj SS     Adj MS      F      P
Plant Genotype      1  0.0000011  0.0000007  0.0000007  12.66  0.001
Position (strata)   2  0.0000016  0.0000016  0.0000008  14.40  0.000
Error              30  0.0000017  0.0000017  0.0000001
Total              33  0.0000044


S = 0.000237452   R-Sq = 61.70%   R-Sq(adj) = 57.86%
```

General Linear Model: Analysis of Total Dry Seed Mass in *aap2* and Col0

```
Factor               Type   Levels  Values
Plant Genotype 2     fixed       2  1, 2
Position (strata)    fixed       3  1, 2, 3


Analysis of Variance for Total Seed Weight 1, using Adjusted SS for Tests

Source               DF     Seq SS     Adj SS     Adj MS      F      P
Plant Genotype 2      1  0.0000007  0.0000007  0.0000007  19.27  0.000
Position (strata)     2  0.0000019  0.0000019  0.0000010  25.66  0.000
Error                41  0.0000015  0.0000015  0.0000000
Total                44  0.0000042
```

```
S = 0.000194177   R-Sq = 62.97%   R-Sq(adj) = 60.26%
```

One-way ANOVA: Bottom strata comparison of *aap1* and Col0

```
Source             DF         SS         MS     F      P
Plant Genotype ID   1  0.0000001  0.0000001  1.23  0.293
Error              10  0.0000007  0.0000001
Total              11  0.0000008

S = 0.0002707   R-Sq = 10.95%   R-Sq(adj) = 2.05%
```

One-way ANOVA: Middle strata comparison of *aap1* and Col0

```
Source             DF         SS         MS      F      P
Plant Genotype ID   1  0.0000007  0.0000007  15.74  0.003
Error               9  0.0000004  0.0000000
Total              10  0.0000011

S = 0.0002062   R-Sq = 63.62%   R-Sq(adj) = 59.58%
```

One-way ANOVA: Upper strata comparison of *aap1* and Col0

```
Source             DF         SS         MS     F      P
Plant Genotype ID   1  0.0000001  0.0000001  2.71  0.134
Error               9  0.0000004  0.0000000
Total              10  0.0000005

S = 0.0002129   R-Sq = 23.14%   R-Sq(adj) = 14.60%


Level  N       Mean      StDev
1      7  0.0002429  0.0001349
2      4  0.0004625  0.0003155
```

One-way ANOVA: Bottom strata comparison of *aap2* and Col0

```
Source           DF         SS         MS     F      P
Plant Genotypes   1  0.0000001  0.0000001  3.48  0.083
Error            14  0.0000003  0.0000000
Total            15  0.0000004

S = 0.0001566   R-Sq = 19.89%   R-Sq(adj) = 14.17%
```

One-way ANOVA: Middle strata comparison of *aap2* and Col0

Source	DF	SS	MS	F	P
Plant Genotypes	1	0.0000009	0.0000009	13.40	0.003
Error	13	0.0000008	0.0000001		
Total	14	0.0000017			

S = 0.0002521 R-Sq = 50.75% R-Sq(adj) = 46.97%

One-way ANOVA: Upper strata comparison of *aap2* and Col0

Source	DF	SS	MS	F	P
Plant Genotypes	1	0.0000001	0.0000001	8.40	0.013
Error	12	0.0000001	0.0000000		
Total	13	0.0000002			

S = 0.00009036 R-Sq = 41.18% R-Sq(adj) = 36.28%

Descriptive Statistics: Bottom Strata Col0

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Bottom Col	6	0	0.000902	0.000119	0.000291	0.00042	0.000697	0.00090	0.001195	0.001210

Descriptive Statistics: Middle Strata Col0

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Middle Col	6	0	0.001093	0.000068	0.000167	0.00088	0.000925	0.00109	0.001270	0.001300

Descriptive Statistics: Upper Strata Col0

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Upper Col	4	0	0.000463	0.000158	0.000316	0.000250	0.000262	0.000335	0.000790	0.000930

Descriptive Statistics: Bottom Strata *aap1*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Bottom aap1	6	0	0.000728	0.000102	0.000249	0.00046	0.000498	0.000675

```
Variable           Q3   Maximum
Bottom aap1  0.000993  0.001090
```

Descriptive Statistics: Middle Strata *aap1*

```
Variable     N  N*      Mean   SE Mean     StDev   Minimum    Q1    Median
Middle aap1  5   0  0.000598  0.000110  0.000246  0.00040  0.000425 0.000540

Variable           Q3   Maximum
Middle aap1  0.000800  0.001020
```

Descriptive Statistics: Upper Strata *aap1*

```
Variable    N  N*      Mean   SE Mean     StDev   Minimum    Q1    Median
Upper aap1  7   0  0.000243  0.000051  0.000135  0.000130  0.00013 0.000220

Variable          Q3   Maximum
Upper aap1  0.000350  0.000490
```

Descriptive Statistics: Bottom Strata *aap2*

```
Variable  N  N*      Mean   SE Mean     StDev   Minimum      Q1    Median
Bottom    8   4  0.000811  0.000056  0.000157  0.000570  0.000662  0.000830

Variable        Q3   Maximum
Bottom    0.000938  0.001040
```

Descriptive Statistics: Middle Strata *aap2*

```
Variable  N  N*      Mean   SE Mean     StDev   Minimum       Q1    Median
Middle    8   0  0.000642  0.000049  0.000138  0.000410  0.000562  0.000630

Variable        Q3   Maximum
Middle    0.000765  0.000840
```

Descriptive Statistics: Upper Strata *aap2*

```
Variable  N  N*      Mean   SE Mean     StDev   Minimum       Q1    Median
Upper     7   0  0.000361  0.000036  0.000094  0.000250  0.000280  0.000360

Variable        Q3   Maximum
Upper     0.000470  0.000490
```

Descriptive Statistics: Bottom Strata Col0

```
Variable  N  N*      Mean   SE Mean     StDev   Minimum       Q1    Median
Bottom_1  8   0  0.000958  0.000055  0.000156  0.000780  0.000840  0.000920

Variable        Q3   Maximum
Bottom_1  0.001085  0.001240
```

Descriptive Statistics: Middle Strata Col0

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Middle_1	7	0	0.001120	0.000128	0.000340	0.000770	0.000910	0.000970	0.001600	0.001600

Descriptive Statistics: Upper Strata Col0

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Upper_1	7	0	0.000501	0.000033	0.000086	0.000370	0.000420	0.000510	0.000580	0.000620

Appendix 1.3

Seed Analysis

General Linear Model: Analysis of Seed Weight of All Three Developmental Strata of Col0, *aap1* and *aap2*

```
Factor          Type   Levels  Values
Plant Genotype  fixed       3  1, 2, 3
Position        fixed       3  1, 2, 3


Analysis of Variance for Seed Weight, using Adjusted SS for Tests

Source          DF     Seq SS     Adj SS     Adj MS      F      P
Plant Genotype   2  0.0001185  0.0001360  0.0000680   4.47  0.014
Position         2  0.0028824  0.0028824  0.0014412  94.78  0.000
Error           82  0.0012469  0.0012469  0.0000152
Total           86  0.0042478


S = 0.00389948   R-Sq = 70.65%   R-Sq(adj) = 69.21%
```

General Linear Model: Analysis of Seed Weights - Comparison of Col0 and *aap1* at all Strata

```
Factor            Type   Levels  Values
Plant genotype 1  fixed       2  1, 2
Position 1        fixed       3  1, 2, 3


Analysis of Variance for Seed Weight 1, using Adjusted SS for Tests

Source            DF     Seq SS     Adj SS     Adj MS      F      P
Plant genotype 1   1  0.0000616  0.0000605  0.0000605   3.52  0.066
Position 1         2  0.0020661  0.0020661  0.0010331  60.06  0.000
Error             57  0.0009805  0.0009805  0.0000172
Total             60  0.0031082


S = 0.00414743   R-Sq = 68.46%   R-Sq(adj) = 66.80%
```

General Linear Model: Analysis of Seed Weights - Comparison of Col0 and *aap2* at all Strata

```
Factor            Type   Levels  Values
Plant Genotype 2  fixed       2  1, 3
Position 2        fixed       3  1, 2, 3


Analysis of Variance for Seed Weight 2, using Adjusted SS for Tests
```

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Plant Genotype 2	1	0.0001101	0.0001262	0.0001262	13.38	0.001
Position 2	2	0.0014726	0.0014726	0.0007363	78.06	0.000
Error	50	0.0004716	0.0004716	0.0000094		
Total	53	0.0020543				

S = 0.00307117 R-Sq = 77.04% R-Sq(adj) = 75.67%

General Linear Model: Analysis of Seed Weights – Comparison of *aap1* and *aap2* at all Strata

Factor	Type	Levels	Values
Plant Genotype 3	fixed	2	2, 3
Position 3	fixed	3	1, 2, 3

Analysis of Variance for Seed Weight 3, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Plant Genotype 3	1	0.0000103	0.0000186	0.0000186	1.15	0.288
Position 3	2	0.0023776	0.0023776	0.0011888	73.44	0.000
Error	55	0.0008903	0.0008903	0.0000162		
Total	58	0.0032781				

S = 0.00402324 R-Sq = 72.84% R-Sq(adj) = 71.36%

One-way ANOVA: Analysis of Bottom Strata Seed Weights – Comparison of aap2 and Col0

Source	DF	SS	MS	F	P
Plant Genotype	1	0.0000003	0.0000003	0.05	0.834
Error	17	0.0001151	0.0000068		
Total	18	0.0001154			

S = 0.002602 R-Sq = 0.27% R-Sq(adj) = 0.00%

Descriptive Statistics: Seed Analysis - Bottom Strata Col0

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Bottom Col	9	0	0.022158	0.000701	0.002104	0.018960	0.020390	0.021610	0.024210	0.024790

Descriptive Statistics: Seed Analysis - Middle Strata Col0

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Middle Col	10	0	0.021333	0.000556	0.001757	0.018080	0.020317	0.021030

Variable	Q3	Maximum
Middle Col	0.023350	0.023480

Descriptive Statistics: Seed Analysis - Upper Strata Col0

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3
Upper Col	9	0	0.01109	0.00120	0.00361	0.00811	0.00876	0.00946	0.01397

Variable	Maximum
Upper Col	0.01789

Descriptive Statistics: Seed Analysis - Bottom Strata *aap1*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Bottom aap1	11	0	0.02571	0.00176	0.00585	0.01778	0.01912	0.02564

Variable	Q3	Maximum
Bottom aap1	0.03141	0.03539

Descriptive Statistics: Seed Analysis - Middle Strata *aap1*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Middle aap1	11	0	0.014484	0.000694	0.002303	0.011540	0.013250	0.013630

Variable	Q3	Maximum
Middle aap1	0.015740	0.020000

Descriptive Statistics: Seed Analysis - Upper Strata *aap1*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Upper aap1	11	0	0.00868	0.00113	0.00376	0.00378	0.00701	0.00854

Variable	Q3	Maximum
Upper aap1	0.00926	0.01885

Descriptive Statistics: Seed Analysis - Bottom Strata *aap2*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Bottom aap2	9	0	0.02147	0.00103	0.00308	0.01741	0.01898	0.02106

Variable	Q3	Maximum
Bottom aap2	0.02476	0.02612

Descriptive Statistics: Seed Analysis - Middle Strata *aap2*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Middle aap2	9	0	0.01637	0.00110	0.00331	0.01121	0.01334	0.01689

Variable	Q3	Maximum
Middle aap2	0.01832	0.02214

Descriptive Statistics: Seed Analysis - Upper Strata *aap2*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Upper aap2	8	0	0.00764	0.00132	0.00373	0.00215	0.00430	0.00819

Variable	Q3	Maximum
Upper aap2	0.00914	0.01421

Descriptive Statistics: Seed Analysis Bottom Strata Col0

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Bottom Col	10	0	0.021722	0.000661	0.002091	0.018740	0.019765	0.021425

Variable	Q3	Maximum
Bottom Col	0.023473	0.025140

Descriptive Statistics: Seed Analysis - Middle Strata Col0

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Middle Col	10	0	0.021299	0.000616	0.001948	0.017840	0.019748	0.021715

Variable	Q3	Maximum
Middle Col	0.023342	0.023510

Descriptive Statistics: Seed Analysis - Upper Strata Col0

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Upper Col	10	0	0.01410	0.00119	0.00375	0.00812	0.00999	0.01482

Variable	Q3	Maximum
Upper Col	0.01695	0.01988

Appendix 1.4

Chlorophyll Analysis of *aap1, aap2* and Col0 Plants

Two-Sample T-Test and CI: Chlorophyll 'a' analysis – comparing *aap1* and Col0

```
Two-sample T for A - AAP1 vs COL

           N    Mean   StDev  SE Mean
A - AAP1  10  0.0880  0.0287   0.0091
COL       20  0.0907  0.0414   0.0093


Difference = mu (A - AAP1) - mu (COL)
Estimate for difference:  -0.0027
95% CI for difference:  (-0.0294, 0.0241)
T-Test of difference = 0 (vs not =): T-Value = -0.20  P-Value = 0.840  DF = 24
```

Two-Sample T-Test and CI: Chlorophyll 'b' analysis – comparing *aap1* and Col0

```
Two-sample T for B- aap1 vs b - col

          N    Mean   StDev  SE Mean
B- aap1  10  0.0224  0.0109   0.0034
b - col  20  0.0404  0.0294   0.0066


Difference = mu (B- aap1) - mu (b - col)
Estimate for difference:  -0.01799
95% CI for difference:  (-0.03326, -0.00272)
T-Test of difference = 0 (vs not =): T-Value = -2.42  P-Value = 0.023  DF = 26
```

Two-Sample T-Test and CI: Chlorophyll 'a+b' analysis – comparing *aap1* and Col0

```
Two-sample T for a+b aap1 vs a+b col

           N    Mean   StDev  SE Mean
a+b aap1  10  0.1104  0.0367    0.012
a+b col   20  0.1230  0.0488    0.011


Difference = mu (a+b aap1) - mu (a+b col)
Estimate for difference:  -0.0126
95% CI for difference:  (-0.0456, 0.0203)
T-Test of difference = 0 (vs not =): T-Value = -0.79  P-Value = 0.435  DF = 23
```

Two-Sample T-Test and CI: Chlorophyll ‘a’ analysis – comparing *aap2* and Col0

```
Two-sample T for a - aap2 vs aap2 col

           N    Mean   StDev  SE Mean
a - aap2  10  0.0822  0.0271   0.0086
aap2 col  20  0.0907  0.0414   0.0093


Difference = mu (a - aap2) - mu (aap2 col)
Estimate for difference:  -0.0085
95% CI for difference:  (-0.0345, 0.0175)
T-Test of difference = 0 (vs not =): T-Value = -0.67  P-Value = 0.508  DF = 25
```

Two-Sample T-Test and CI: Chlorophyll ‘b’ analysis – comparing *aap2* and Col0

```
Two-sample T for b - aap2 vs b col aap2

             N     Mean    StDev  SE Mean
b - aap2    10  0.02148  0.00863   0.0027
b col aap2  20   0.0404   0.0294   0.0066


Difference = mu (b - aap2) - mu (b col aap2)
Estimate for difference:  -0.01888
95% CI for difference:  (-0.03358, -0.00418)
T-Test of difference = 0 (vs not =): T-Value = -2.65  P-Value = 0.014  DF = 24
```

Two-Sample T-Test and CI: Chlorophyll ‘a+b’ analysis - comparing *aap2* and Col0

```
Two-sample T for a+b aap2 vs a+b col2

           N    Mean   StDev  SE Mean
a+b aap2  10  0.0973  0.0270   0.0086
a+b col2  20  0.1230  0.0488    0.011


Difference = mu (a+b aap2) - mu (a+b col2)
Estimate for difference:  -0.0258
95% CI for difference:  (-0.0542, 0.0026)
T-Test of difference = 0 (vs not =): T-Value = -1.86  P-Value = 0.074  DF = 27
```

Appendix 1.5

Analysis of Plant Characteristics Following Nitrogen Treatment

Descriptive Statistics: Rosette Leaf Number of *aap2* after Nitrogen Treatment

```
Variable     N   N*  Mean  SE Mean  StDev  Minimum   Q1    Median    Q3
Rosette No  40   0  13.450  0.343  2.171   10.000  12.000  13.500  15.000

Variable    Maximum
Rosette No   19.000
```

Descriptive Statistics: Rosette Leaf Width of *aap2* after Nitrogen Treatment

```
Variable        N   N*  Mean   SE Mean  StDev  Minimum   Q1  Median    Q3
Rosette width  40   0  7.645    0.329  2.079    3.700  6.025   7.450  8.975

Variable       Maximum
Rosette width   12.000
```

Descriptive Statistics: Above Soil Plant Mass of *aap2* after Nitrogen Treatment

```
Variable         N  N*  Mean  SE Mean   StDev  Minimum    Q1    Median  Q3
Rosette weight  40  0  0.4953   0.0340  0.2151  0.1900  0.3350 0.4450 0.6775

Variable        Maximum
Rosette weight  0.9200
```

Descriptive Statistics: Plant Height of *aap2* after Nitrogen Treatment

```
Variable   N   N*  Mean   SE Mean  StDev  Minimum    Q1    Median    Q3
Height    40   0  31.068    0.776  4.908   19.000  28.100  31.200  34.100

Variable  Maximum
Height     44.000
```

Descriptive Statistics: Rossette Leaf Number of Col0 after Nitrogen Treatment

```
Variable         N   N*  Mean  SE Mean  StDev  Minimum   Q1   Median  Q3
Rosette No_col  35   0  20.971  0.632  3.738   15.000  18.000  20.000 24.0


Variable        Maximum
Rosette No_col   29.000
```

Descriptive Statistics: Rosette Leaf Width of Col0 after Nitrogen Treatment

```
Variable            N   N* Mean   SE Mean StDev  Minimum  Q1    Median   Q3
Rosette width_col  35   0  11.783  0.335  1.980   8.000  10.000 12.000  13.5
```

```
Variable           Maximum
Rosette width_col  16.500
```

Descriptive Statistics: Above Soil Mass of Col0 after Nitrogen Treatment

```
Variable            N   N*  Mean  SE Mean   StDev  Minimum   Q1  Median  Q3
Rosette weight_col  35   0  0.6280  0.0293  0.1734  0.3300  0.470 0.640 0.74

Variable            Maximum
Rosette weight_col  0.9900
```

Descriptive Statistics: Plant Height of Col0 after Nitrogen Treatment

```
Variable     N  N*    Mean  SE Mean  StDev  Minimum      Q1  Median      Q3
Height_col  35   0  39.820    0.986  5.831   23.800  37.000  40.100  44.500

Variable    Maximum
Height_col   48.600
```

Descriptive Statistics: Rosette Leaf Number of *aap1* after Nitrogen Treatment

```
Variable       N  N*  Mean  SE Mean StDev  Minimum    Q1  Median     Q3
Rosette No_1   39  0 14.026  0.361   2.254  10.000  12.000 14.000  16.000

Variable      Maximum
Rosette No_1   19.000
```

Descriptive Statistics: Rosette Leaf Width of *aap1* after Nitrogen Treatment

```
Variable          N   N*  Mean  SE Mean  StDev  Minimum   Q1  Median    Q3
Rosette width_1  39   0  8.374    0.359  2.241    3.800  6.800   8.30 9.60

Variable         Maximum
Rosette width_1   13.000
```

Descriptive Statistics: Above Soil Plant Mass of *aap1* after Nitrogen Treatment

```
Variable           N  N*  Mean  SE Mean   StDev  Minimum    Q1  Median   Q3
Rosette weight_1  39  0  0.5077  0.0407  0.2540   0.1200  0.290 0.4900  0.74

Variable             Maximum
Rosette weight_1     0.9600
```

Descriptive Statistics: Plant Height of *aap1* after Nitrogen Treatment

```
Variable   N  N*   Mean  SE Mean  StDev  Minimum    Q1   Median    Q3
Height_1   39 0  33.982   0.835   5.216   20.000  30.800 35.000  37.000

Variable  Maximum
Height_1   45.000
```

One-way ANOVA: Analysis of Rosette Leaf Number in *aap1* and Col0 Following Nitrogen Treatment

Source	DF	SS	MS	F	P
Factor	1	889.91	889.91	95.93	0.000
Error	72	667.95	9.28		
Total	73	1557.85			

S = 3.046 R-Sq = 57.12% R-Sq(adj) = 56.53%

One-way ANOVA: Analysis of Rosette Leaf Width in *aap1* and Col0 Following Nitrogen Treatment

Source	DF	SS	MS	F	P
Factor	1	214.30	214.30	47.60	0.000
Error	72	324.18	4.50		
Total	73	538.49			

S = 2.122 R-Sq = 39.80% R-Sq(adj) = 38.96%

One-way ANOVA: Analysis of Above Root Plant Mass in *aap1* and Col0 Following Nitrogen Treatment

Source	DF	SS	MS	F	P
Factor	1	0.2670	0.2670	5.53	0.021
Error	72	3.4753	0.0483		
Total	73	3.7422			

S = 0.2197 R-Sq = 7.13% R-Sq(adj) = 5.84%

One-way ANOVA: Analysis of Plant Height in *aap1* and Col0 Following Nitrogen Treatment

Source	DF	SS	MS	F	P
Factor	1	628.7	628.7	20.67	0.000
Error	72	2190.1	30.4		
Total	73	2818.8			

S = 5.515 R-Sq = 22.30% R-Sq(adj) = 21.22%

One-way ANOVA: Analysis of Rosette Leaf Number in *aap2* and Col0 following Nitrogen Treatment

Source	DF	SS	MS	F	P
Factor	1	1056.01	1056.01	117.00	0.000
Error	73	658.87	9.03		
Total	74	1714.88			

S = 3.004 R-Sq = 61.58% R-Sq(adj) = 61.05%

One-way ANOVA: Analysis of Rosette Leaf Width in *aap2* and Col0 following Nitrogen Treatment

Source	DF	SS	MS	F	P
Factor	1	319.61	319.61	77.27	0.000
Error	73	301.93	4.14		
Total	74	621.54			

S = 2.034 R-Sq = 51.42% R-Sq(adj) = 50.76%

One-way ANOVA: Analysis of Above Root Weight in *aap2* Col0 Following Nitrogen Treatment

Source	DF	SS	MS	F	P
Factor	1	0.3290	0.3290	8.49	0.005
Error	73	2.8276	0.0387		
Total	74	3.1565			

S = 0.1968 R-Sq = 10.42% R-Sq(adj) = 9.19%

One-way ANOVA: Analysis of Plant Height in *aap2* and Col0 Following Nitrogen Treatment

Source	DF	SS	MS	F	P
Factor	1	1430.0	1430.0	49.81	0.000
Error	73	2095.7	28.7		
Total	74	3525.7			

S = 5.358 R-Sq = 40.56% R-Sq(adj) = 39.74%

One-way ANOVA: Analysis of Rosette Leaf Number in Col0 (water treatment) and Col0 (nitrogen treatment)

Source	DF	SS	MS	F	P
Factor	1	195.6	195.6	17.38	0.000
Error	68	765.1	11.3		
Total	69	960.7			

S = 3.354 R-Sq = 20.36% R-Sq(adj) = 19.18%

One-way ANOVA: Analysis of Rosette Leaf Width in Col0 (water treatment) and Col0 (Nitrogen Treatment)

Source	DF	SS	MS	F	P
Factor	1	114.94	114.94	36.18	0.000
Error	68	216.03	3.18		
Total	69	330.97			

S = 1.782 R-Sq = 34.73% R-Sq(adj) = 33.77%

One-way ANOVA: Analysis of Above Root Plant Mass in Col0 (water treatment) and Col0 (nitrogen treatment)

Source	DF	SS	MS	F	P
Factor	1	0.1073	0.1073	2.69	0.105
Error	68	2.7079	0.0398		
Total	69	2.8151			

S = 0.1996 R-Sq = 3.81% R-Sq(adj) = 2.40%

One-way ANOVA: Analysis of Plant Height in Col0 (Water Treatment) and Col0 (nitrogen treatment)

Source	DF	SS	MS	F	P
Factor	1	115.7	115.7	3.71	0.058
Error	68	2119.2	31.2		
Total	69	2234.9			

S = 5.582 R-Sq = 5.18% R-Sq(adj) = 3.78%

Appendix 1.6

Analysis of Plant Characteristics in *aap1, aap2* and Col0 Plants Following Infestation by *M. persicae*

Descriptive Statistics: Rosette leaf number of *aap2* plants following infestation by *M. persicae*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
aap2 Rosette No	40	0	14.175	0.299	1.893	10.000	13.000	14.000	15.000	19.000

Descriptive Statistics: Rosette leaf width of *aap2* plants following infestation by *M. persicae*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
aap2 Rosette width	40	0	7.410	0.301	1.904	3.700	6.000	6.950	8.975	11.400

Descriptive Statistics: Above soil plant mass of *aap2* plants following infestation by *M. persicae*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
aap2 Rosette weight	40	0	0.4822	0.0307	0.1940	0.2000	0.3600	0.4450	0.6475	0.8600

Descriptive Statistics: Plant height of *aap2* plants following infestation by *M. persicae*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
aap2 Height	40	0	33.148	0.756	4.780	20.900	29.950	32.550	37.275	44.000

Descriptive Statistics: Rosette leaf number of Col0 plants following infestation by *M. persicae*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
col0 Rosette No	40	0	18.300	0.353	2.233	14.000	16.250	18.000	20.000	23.000

```
Variable             Q3  Maximum
col0 Rosette No  20.000   23.000
```

Descriptive Statistics: Rosette leaf width of Col0 plants following infestation by *M. persicae*

```
Variable             N  N*   Mean  SE Mean  StDev  Minimum     Q1  Median
col0 Rosette width  40   0  8.880    0.275  1.739    6.000  7.225   8.950

Variable               Q3  Maximum
col0 Rosette width  10.300   12.300
```

Descriptive Statistics: Above soil plant mass of Col0 plants following infestation by *M. persicae*

```
Variable              N   N*  Mean   SE Mean   StDev  Minimum    Q1  Median
col0 Rosette weight  40   0  0.5347   0.0321  0.2031  0.2200  0.3450  0.5100

Variable                Q3  Maximum
col0 Rosette weight  0.6475   1.1100
```

Descriptive Statistics: Plant Height of Col0 plants following infestation by *M. persicae*

```
Variable      N  N*    Mean  SE Mean  StDev  Minimum      Q1  Median      Q3
col0 Height  40   0  38.362    0.687  4.345   25.900  36.000  38.050  42.075

Variable     Maximum
col0 Height   47.300
```

Descriptive Statistics: Rosette leaf number of *aap1* plants following infestation by *M. persicae*

```
Variable     N  N*    Mean  SE Mean  StDev  Minimum      Q1  Median      Q3
Rosette No  40   0  15.050    0.340  2.148   10.000  13.250  15.000  16.750

Variable    Maximum
Rosette No   19.000
```

Descriptive Statistics: Rosette Leaf Width of *aap1* plants following infestation by *M. persicae*

```
Variable             N  N*   Mean  SE Mean  StDev  Minimum     Q1  Median
aap1 Rosette width  40   0  8.867    0.253  1.600    6.500  7.600   9.100

Variable               Q3  Maximum
aap1 Rosette width  9.475   13.000
```

Descriptive Statistics: Above soil plant mass of *aap1* plants following infestation by *M. persicae*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
aap1 Rosette weight	40	0	0.4567	0.0344	0.2178	0.1200	0.2925	0.4300	0.6325	0.9600

Descriptive Statistics: Plant height of *aap1* plants following infestation by *M. persicae*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
aap1 Height	40	0	33.913	0.799	5.054	20.000	30.875	34.750	37.000	43.100

Analysis of Plant Characteristics in *aap1, aap2* and Col0 Following Infestation by *B. brassicae*

Descriptive Statistics: Rosette Leaf Number of *aap2* plants following infestation by *B. brassicae*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
AAP2 Rosette No	40	0	13.875	0.308	1.951	10.000	12.250	14.000	15.000	19.000

Descriptive Statistics: Rosette Leaf Width of *aap2* plants following infestation by *B. brassicae*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
AAP2 Rosette width	40	0	7.053	0.265	1.675	3.700	5.850	6.700	8.650	10.100

Descriptive Statistics: Above Soil Plant Mass of *aap2* plants following infestation by *B. brassicae*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median

```
AAP2 Rosette weight  40   0  0.5118   0.0262  0.1657   0.2000  0.3900
0.4750

Variable                Q3  Maximum
AAP2 Rosette weight  0.6100   0.8600
```

Descriptive Statistics: Plant Height of *aap2* plants following infestation by *B. brassicae*

```
Variable      N  N*    Mean  SE Mean  StDev  Minimum      Q1  Median      Q3
AAP2 Height  40   0  32.913    0.741  4.687   20.900  29.750  32.250  37.275

Variable     Maximum
AAP2 Height   42.300
```

Descriptive Statistics: Rosette Leaf Number of Col0 plants following Infestation by *B. brassicae*

```
Variable         N  N*    Mean  SE Mean  StDev  Minimum      Q1  Median
Q3
COL Rosette No  40   0  17.925    0.364  2.303   13.000  16.000  18.000
19.000

Variable        Maximum
COL Rosette No   23.000
```

Descriptive Statistics: Rosette Leaf Width of Col0 plants following infestation by *B. brassicae*

```
Variable            N  N*   Mean  SE Mean  StDev  Minimum     Q1  Median
COL Rosette width  40   0  9.472    0.266  1.682    6.400  8.200   9.600

Variable               Q3  Maximum
COL Rosette width  10.700   12.600
```

Descriptive Statistics: Above Soil Plant Mass of Col0 plants following infestation by *B. brassicae*

```
Variable             N  N*    Mean  SE Mean   StDev  Minimum      Q1  Median
COL Rosette weight  40   0  0.5685   0.0276  0.1748   0.2800  0.4525  0.5500

Variable                Q3  Maximum
COL Rosette weight  0.7475   0.9000
```

Descriptive Statistics: Plant Height of Col0 plants following infestation by *B. brassicae*

```
Variable     N  N*    Mean  SE Mean  StDev  Minimum      Q1  Median      Q3
COL Height  40   0  36.880    0.833  5.267   23.900  33.475  38.050  40.400

Variable    Maximum
COL Height   47.300
```

Descriptive Statistics: Rosette Leaf Number of *aap1* plants following infestation by *B. brassicae*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
AAP1 Rosette No	40	0	13.800	0.298	1.884	10.000	12.000	14.000	15.000	17.000

Descriptive Statistics: Rosette Leaf Width of *aap1* plants following infestation by *B. brassicae*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
AAP1 Rosette width	40	0	8.230	0.193	1.220	5.400	7.250	8.300	9.175	10.100

Descriptive Statistics: Above Soil Plant Mass of *aap1* following infestation by *B. brassicae*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
AAP1 osette weight	40	0	0.4477	0.0303	0.1919	0.2000	0.3025	0.4100	0.5800	0.8700

Descriptive Statistics: Plant Height of *aap1* plants following infestation by *B. brassicae*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
AAP1 Height	40	0	35.128	0.768	4.859	24.100	32.625	35.000	37.550	45.500

General Linear Model: Analysis of Plant Characteristics of *aap1, aap2* and Col0 plants after infestation by *M. persicae* and *B. brassicae*

Factor	Type	Levels	Values
Plant Genotype	fixed	3	1, 2, 3
Aphid Species	fixed	2	1, 2
Characteristic	fixed	4	1, 2, 3, 4

Analysis of Variance for Plant Characteristic after infestation, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Plant Genotype	2	20.182	20.182	20.056	7.81	0.001
Aphid Species	1	1.143	1.143	1.143	0.44	0.510
Characteristic	3	50.231	50.231	24.31	2.14	0.004
Error	232	291.135	291.135			
Total	235	332.471				

S = 1.60247   R-Sq = 11.64%   R-Sq(adj) = 9.46%

Appendix 2.1

Initial Host Preference Analysis

Chi-Square Test: *M. Persicae* on choice tests of *aap1* and Col0

Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

	mp on aap1	mp on col	Total
1	7	5	12
	6.52	5.48	
	0.036	0.043	
2	6	5	11
	5.97	5.03	
	0.000	0.000	
3	8	6	14
	7.60	6.40	
	0.021	0.025	
4	5	4	9
	4.89	4.11	
	0.003	0.003	
5	9	6	15
	8.15	6.85	
	0.090	0.107	
6	6	7	13
	7.06	5.94	
	0.159	0.189	
7	7	8	15
	8.15	6.85	
	0.161	0.191	
8	8	6	14
	7.60	6.40	
	0.021	0.025	
9	8	7	15
	8.15	6.85	
	0.003	0.003	
10	8	5	13
	7.06	5.94	
	0.125	0.149	
11	5	8	13
	7.06	5.94	
	0.601	0.714	
12	6	3	9
	4.89	4.11	
	0.253	0.301	
13	5	7	12
	6.52	5.48	
	0.353	0.419	
14	7	4	11

	5.97	5.03	
	0.177	0.210	
15	9	3	12
	6.52	5.48	
	0.947	1.125	
16	6	5	11
	5.97	5.03	
	0.000	0.000	
17	7	6	13
	7.06	5.94	
	0.000	0.001	
18	5	7	12
	6.52	5.48	
	0.353	0.419	
19	8	5	13
	7.06	5.94	
	0.125	0.149	
20	7	8	15
	8.15	6.85	
	0.161	0.191	
21	3	6	9
	4.89	4.11	
	0.729	0.866	
22	8	7	15
	8.15	6.85	
	0.003	0.003	
23	6	6	12
	6.52	5.48	
	0.041	0.049	
24	7	4	11
	5.97	5.03	
	0.177	0.210	
25	8	5	13
	7.06	5.94	
	0.125	0.149	
26	4	2	6
	3.26	2.74	
	0.169	0.201	
27	8	7	15
	8.15	6.85	
	0.003	0.003	
28	7	5	12
	6.52	5.48	
	0.036	0.043	
29	6	7	13
	7.06	5.94	
	0.159	0.189	
30	8	6	14
	7.60	6.40	
	0.021	0.025	
Total	202	170	372

Chi-Sq = 11.049, DF = 29, P-Value = 0.999
8 cells with expected counts less than 5.

Chi-Square Test: *M. Persicae* on choice tests of *aap2* and Col0

Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

	mp on aap2	mp on col	Total
1	6	5	11
	5.53	5.47	
	0.040	0.040	
2	7	5	12
	6.04	5.96	
	0.154	0.156	
3	6	6	12
	6.04	5.96	
	0.000	0.000	
4	7	4	11
	5.53	5.47	
	0.389	0.394	
5	8	6	14
	7.04	6.96	
	0.131	0.132	
6	6	7	13
	6.54	6.46	
	0.044	0.045	
7	3	8	11
	5.53	5.47	
	1.159	1.173	
8	4	6	10
	5.03	4.97	
	0.211	0.213	
9	5	7	12
	6.04	5.96	
	0.178	0.180	
10	4	5	9
	4.53	4.47	
	0.061	0.062	
11	7	8	15
	7.54	7.46	
	0.039	0.040	
12	6	3	9
	4.53	4.47	
	0.480	0.485	
13	7	7	14
	7.04	6.96	
	0.000	0.000	
14	5	4	9

	4.53	4.47	
	0.050	0.050	
15	8	3	11
	5.53	5.47	
	1.101	1.114	
16	7	5	12
	6.04	5.96	
	0.154	0.156	
17	7	6	13
	6.54	6.46	
	0.033	0.033	
18	6	7	13
	6.54	6.46	
	0.044	0.045	
19	4	5	9
	4.53	4.47	
	0.061	0.062	
20	5	8	13
	6.54	6.46	
	0.362	0.366	
21	4	6	10
	5.03	4.97	
	0.211	0.213	
22	6	7	13
	6.54	6.46	
	0.044	0.045	
23	4	6	10
	5.03	4.97	
	0.211	0.213	
24	6	4	10
	5.03	4.97	
	0.187	0.190	
25	6	5	11
	5.53	5.47	
	0.040	0.040	
26	3	2	5
	2.51	2.49	
	0.094	0.095	
27	7	7	14
	7.04	6.96	
	0.000	0.000	
28	6	5	11
	5.53	5.47	
	0.040	0.040	
29	5	7	12
	6.04	5.96	
	0.178	0.180	
30	7	6	13
	6.54	6.46	
	0.033	0.033	
Total	172	170	342

Chi-Sq = 11.521, DF = 29, P-Value = 0.998
14 cells with expected counts less than 5.

Chi-Square Test: *B. Brassicae* on choice tests of *aap1 and Col0*

Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

	bb on aap1	bb on col	Total
1	7	3	10
	5.70	4.30	
	0.299	0.395	
2	6	4	10
	5.70	4.30	
	0.016	0.022	
3	6	4	10
	5.70	4.30	
	0.016	0.022	
4	5	4	9
	5.13	3.87	
	0.003	0.004	
5	4	6	10
	5.70	4.30	
	0.505	0.668	
6	7	3	10
	5.70	4.30	
	0.299	0.395	
7	7	4	11
	6.26	4.74	
	0.086	0.114	
8	6	6	12
	6.83	5.17	
	0.102	0.135	
9	8	3	11
	6.26	4.74	
	0.481	0.636	
10	7	5	12
	6.83	5.17	
	0.004	0.005	
11	3	6	9
	5.13	3.87	
	0.882	1.166	
12	4	3	7
	3.99	3.01	
	0.000	0.000	
13	5	4	9
	5.13	3.87	
	0.003	0.004	

14	4	4	8
	4.56	3.44	
	0.068	0.090	
15	6	5	11
	6.26	4.74	
	0.011	0.015	
16	8	4	12
	6.83	5.17	
	0.199	0.263	
17	7	4	11
	6.26	4.74	
	0.086	0.114	
18	7	6	13
	7.40	5.60	
	0.022	0.029	
19	6	5	11
	6.26	4.74	
	0.011	0.015	
20	5	6	11
	6.26	4.74	
	0.255	0.338	
21	6	3	9
	5.13	3.87	
	0.149	0.197	
22	3	3	6
	3.42	2.58	
	0.051	0.067	
23	4	6	10
	5.70	4.30	
	0.505	0.668	
24	5	7	12
	6.83	5.17	
	0.492	0.651	
25	7	4	11
	6.26	4.74	
	0.086	0.114	
26	6	5	11
	6.26	4.74	
	0.011	0.015	
27	5	3	8
	4.56	3.44	
	0.043	0.057	
28	3	3	6
	3.42	2.58	
	0.051	0.067	
29	8	4	12
	6.83	5.17	
	0.199	0.263	
30	7	3	10
	5.70	4.30	
	0.299	0.395	

Total	172	130	302

Chi-Sq = 12.160, DF = 29, P-Value = 0.997
29 cells with expected counts less than 5.

Chi-Square Test: *B. Brassicae* on choice tests of *aap2* and Col0

Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

	bb on aap2	bb on col	Total
1	4	3	7
	3.82	3.18	
	0.009	0.010	
2	5	4	9
	4.91	4.09	
	0.002	0.002	
3	6	4	10
	5.45	4.55	
	0.055	0.065	
4	7	4	11
	6.00	5.00	
	0.167	0.200	
5	5	6	11
	6.00	5.00	
	0.167	0.200	
6	5	3	8
	4.36	3.64	
	0.093	0.111	
7	6	4	10
	5.45	4.55	
	0.055	0.065	
8	4	6	10
	5.45	4.55	
	0.388	0.465	
9	3	3	6
	3.27	2.73	
	0.023	0.027	
10	5	5	10
	5.45	4.55	
	0.038	0.045	
11	6	6	12
	6.55	5.45	
	0.045	0.055	
12	4	3	7
	3.82	3.18	
	0.009	0.010	
13	6	4	10
	5.45	4.55	
	0.055	0.065	
14	5	4	9

	4.91	4.09	
	0.002	0.002	
15	5	5	10
	5.45	4.55	
	0.038	0.045	
16	3	4	7
	3.82	3.18	
	0.175	0.210	
17	7	4	11
	6.00	5.00	
	0.167	0.200	
18	5	6	11
	6.00	5.00	
	0.167	0.200	
19	6	5	11
	6.00	5.00	
	0.000	0.000	
20	5	6	11
	6.00	5.00	
	0.167	0.200	
21	5	3	8
	4.36	3.64	
	0.093	0.111	
22	6	3	9
	4.91	4.09	
	0.242	0.291	
23	4	6	10
	5.45	4.55	
	0.388	0.465	
24	8	7	15
	8.18	6.82	
	0.004	0.005	
25	6	4	10
	5.45	4.55	
	0.055	0.065	
26	4	5	9
	4.91	4.09	
	0.168	0.202	
27	5	3	8
	4.36	3.64	
	0.093	0.111	
28	7	3	10
	5.45	4.55	
	0.438	0.525	
29	3	4	7
	3.82	3.18	
	0.175	0.210	
30	6	3	9
	4.91	4.09	
	0.242	0.291	
Total	156	130	286

Chi-Sq = 8.175, DF = 29, P-Value = 1.000
35 cells with expected counts less than 5.

Appendix 2.2

EPG Analysis

Mann-Whitney Test and CI: Analysis of E2 feeding of *M. persicae* on *aap1* and Col0 during hour 1

```
                     N  Median
Mp on aap1 hour 1   13     0.0
Mp on col0 hour 1   13  1389.0


Point estimate for ETA1-ETA2 is -728.0
95.4 Percent CI for ETA1-ETA2 is (-1741.9,-0.1)
W = 130.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0210
The test is significant at 0.0175 (adjusted for ties)
```

Mann-Whitney Test and CI: Analysis of E2 feeding of B. brassice on *aap1* and Col0 during hour 1

```
                     N  Median
Bb on aap1 hour 1   13     0.0
Bb on col0 hour 1   13  1278.0


Point estimate for ETA1-ETA2 is -627.0
95.4 Percent CI for ETA1-ETA2 is (-1643.1,-49.0)
W = 127.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0138
The test is significant at 0.0119 (adjusted for ties)
```

Mann-Whitney Test and CI: Analysis of E2 feeding of *B. brassicae* on *aap2* and Col0 during hour 1

```
                     N  Median
Bb on aap2 hour 1   13     0.0
Bb on col0 hour 1   13  1006.0


Point estimate for ETA1-ETA2 is -741.0
95.4 Percent CI for ETA1-ETA2 is (-1574.9,-15.1)
W = 122.5
```

```
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0071
The test is significant at 0.0063 (adjusted for ties)
```

Mann-Whitney Test and CI: Analysis of E2 feeding of *M. persicae* on *aap*2 and Col0 during hour 1

```
                     N  Median
Mp on aap2 hour 1  13    10.0
Mp on col0 hour 1   13  1009.0


Point estimate for ETA1-ETA2 is -700.0
95.4 Percent CI for ETA1-ETA2 is (-2095.1,-19.9)
W = 126.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0120
The test is significant at 0.0108 (adjusted for ties)
```

Appendix 2.3

Honeydew Production Analysis

General Linear Model: Honeydew Production Analysis of *B. brassicae* on *aap2* and Col0

```
Factor    Type   Levels  Values
Genotype  fixed       2  1, 2
hour      fixed      10  3, 4, 5, 6, 7, 8, 9, 10, 11, 12


Analysis of Variance for drop number, using Adjusted SS for Tests

Source     DF    Seq SS    Adj SS   Adj MS      F      P
Genotype    1   29.0417   31.3307  31.3307  53.40  0.000
hour        9   36.1956   36.1956   4.0217   6.85  0.000
Error     213  124.9770  124.9770   0.5867
Total     223  190.2143


S = 0.765994   R-Sq = 34.30%   R-Sq(adj) = 31.21%
```

General Linear Model: Honeydew Production Analysis of *B. brassicae* on *aap1* and Col0

```
Factor    Type   Levels  Values
Genotype  fixed       2  1, 2
hour      fixed      10  3, 4, 5, 6, 7, 8, 9, 10, 11, 12


Analysis of Variance for drop number, using Adjusted SS for Tests

Source     DF    Seq SS    Adj SS   Adj MS      F      P
Genotype    1   16.1998   18.3573  18.3573  32.80  0.000
hour        9   34.2642   34.2642   3.8071   6.80  0.000
Error     195  109.1525  109.1525   0.5598
Total     205  159.6165


S = 0.748169   R-Sq = 31.62%   R-Sq(adj) = 28.11%
```

General Linear Model: Honeydew Production Analysis of *M. persicae* on *aap1* and Col0

```
Factor    Type   Levels  Values
Genotype  fixed       2  1, 2
hour      fixed      10  3, 4, 5, 6, 7, 8, 9, 10, 11, 12


Analysis of Variance for drop number, using Adjusted SS for Tests

Source     DF    Seq SS    Adj SS   Adj MS      F      P
Genotype    1   31.5950   28.3282  28.3282  32.91  0.000
hour        9   33.3100   33.3100   3.7011   4.30  0.000
Error     222  191.1035  191.1035   0.8608
```

```
Total     232  256.0086

S = 0.927807   R-Sq = 25.35%   R-Sq(adj) = 21.99%
```

General Linear Model: Honeydew Production Analysis of *M. persicae* on *aap2* and Col0

```
Factor    Type   Levels  Values
Genotype  fixed       2  1, 2
hour      fixed      10  3, 4, 5, 6, 7, 8, 9, 10, 11, 12


Analysis of Variance for drop number, using Adjusted SS for Tests

Source     DF    Seq SS    Adj SS   Adj MS      F      P
Genotype    1   22.3097   25.4532  25.4532  37.70  0.000
hour        9   44.2642   44.2642   4.9182   7.28  0.000
Error     223  150.5757  150.5757   0.6752
Total     233  217.1496


S = 0.821722   R-Sq = 30.66%   R-Sq(adj) = 27.55%
```

Appendix 2.4

Honeydew Volume Analysis

One-way ANOVA: Honeydew Volume Analysis of *M. persicae* on *aap1*, *aap2* and Col0

Source	DF	SS	MS	F	P
Plant Genotype	2	0.509	0.254	1.90	0.156
Error	87	11.675	0.134		
Total	89	12.184			

S = 0.3663 R-Sq = 4.17% R-Sq(adj) = 1.97%

One-way ANOVA: Honeydew Volume Analysis of *B. brassicae* on *aap1, aap2* and Col0.

Source	DF	SS	MS	F	P
Plant Genotype1	2	1.248	0.624	5.49	0.006
Error	87	9.881	0.114		
Total	89	11.129			

S = 0.3370 R-Sq = 11.21% R-Sq(adj) = 9.17%

One-way ANOVA: Honeydew Volume Analysis of *M. persicae* on *aap1* and Col0

Source	DF	SS	MS	F	P
PLANT GENOTYPE 5	1	0.267	0.267	2.13	0.150
Error	58	7.257	0.125		
Total	59	7.523			

S = 0.3537 R-Sq = 3.54% R-Sq(adj) = 1.88%

One-way ANOVA: Honeydew Volume Analysis of *M. persicae* on *aap2* and Col0

Source	DF	SS	MS	F	P
PLANT GENOTYPE 5	1	0.468	0.468	3.20	0.079
Error	58	8.480	0.146		
Total	59	8.948			

S = 0.3824 R-Sq = 5.23% R-Sq(adj) = 3.60%

One-way ANOVA: Honeydew Volume Analysis of *B. brassicae* on *aap1* and Col0

Source	DF	SS	MS	F	P
Plant Genotype 4	1	0.600	0.600	5.24	0.026
Error	58	6.647	0.115		

```
Total             59  7.247

S = 0.3385   R-Sq = 8.28%   R-Sq(adj) = 6.70%
```

One-way ANOVA: Honeydew Volume Analysis of *B. brassicae* on *aap2* and Col0

```
Source            DF     SS     MS     F      P
Plant Genotype 4   1  0.096  0.096  0.86  0.357
Error             58  6.467  0.112
Total             59  6.563

S = 0.3339   R-Sq = 1.46%   R-Sq(adj) = 0.00%
```

General Linear Model: Honeydew Volume Analysis of *M. persicae* and *B. brassicae* on *aap1*, *aap2* and Col0

```
Factor          Type   Levels  Values
Plant Genotype  fixed       3  1, 2, 3
Aphid Genotype  fixed       2  1, 2


Analysis of Variance for Honeydew Volume, using Adjusted SS for Tests

Source           DF   Seq SS   Adj SS  Adj MS      F      P
Plant Genotype    2   0.8823   0.8823  0.4412   3.46  0.034
Aphid Genotype    1   3.4445   3.4445  3.4445  27.03  0.000
Error           176  22.4307  22.4307  0.1274
Total           179  26.7575


S = 0.356997   R-Sq = 16.17%   R-Sq(adj) = 14.74%
```

Descriptive Statistics: *B. brassicae* on Col0

```
Variable     N  N*    Mean  SE Mean   StDev  Minimum    Q1  Median    Q3
Bb on col0  30   0  9.8433  0.0610  0.3339   9.2000  9.6000  9.7500  10.2000

Variable    Maximum
Bb on col0  10.6000
```

Descriptive Statistics: *B. brassicae* on *aap1*

```
Variable     N  N*    Mean  SE Mean   StDev  Minimum    Q1  Median    Q3
Bb on aap1  30   0  9.6433  0.0626  0.3431   9.1000  9.3750  9.6000  10.0000

Variable    Maximum
Bb on aap1  10.2000
```

Descriptive Statistics: *B. brassicae* on *aap2*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Bb on aap2	30	0	9.9233	0.0610	0.3339	9.3000	9.6750	9.9000	10.1250	10.6000

Descriptive Statistics: *M. persicae* on Col0

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Mp on col0	30	0	10.183	0.0683	0.374	9.500	9.875	10.200	10.425	10.900

Descriptive Statistics: *M. persicae* on *aap1*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Mp on aap1	30	0	10.050	0.0606	0.332	9.400	9.775	10.100	10.300	10.600

Descriptive Statistics: *M. persicae* on *aap2*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Mp on aap2	30	0	10.007	0.0713	0.390	9.300	9.675	10.100	10.300	10.700

Appendix 2.5

Aphid Feeding Rate Analysis

General Linear Model: Feeding Rate Analysis – *M. persicae* and *B. brassicae* on *aap1*, *aap2* and Col0

```
Factor          Type   Levels  Values
Plant Genotype  fixed       3  1, 2, 3
Aphid Genotype  fixed       2  1, 2
```

```
Analysis of Variance for Feeding Rate, using Adjusted SS for Tests

Source           DF   Seq SS   Adj SS  Adj MS      F      P
Plant Genotype    2  1079.85  1063.17  531.59  99.69  0.000
Aphid Genotype    1     7.66     7.66    7.66   1.44  0.234
Error            97   517.26   517.26    5.33
Total           100  1604.77


S = 2.30924   R-Sq = 67.77%   R-Sq(adj) = 66.77%
```

One-way ANOVA: Feeding Rate of *B. brassicae* on *aap1* and Col0

```
Source          DF      SS      MS      F      P
Plant Genotype   1  450.72  450.72  75.26  0.000
Error           33  197.62    5.99
Total           34  648.35

S = 2.447   R-Sq = 69.52%   R-Sq(adj) = 68.60%
```

One-way ANOVA: Feeding Rate of *B. brassicae* on *aap2* and Col0

```
Source          DF      SS      MS      F      P
Plant Genotype   1  353.48  353.48  56.23  0.000
Error           33  207.43    6.29
Total           34  560.92

S = 2.507   R-Sq = 63.02%   R-Sq(adj) = 61.90%
```

One-way ANOVA: Feeding Rate of *M. persicae* on *aap1* and Col0

```
Source          DF      SS      MS      F      P
Plant Genotype   1  287.35  287.35  47.88  0.000
Error           40  240.08    6.00
Total           41  527.43

S = 2.450   R-Sq = 54.48%   R-Sq(adj) = 53.34%
```

One-way ANOVA: Feeding Rate Analysis of *M. persicae* on *aap2* and Col0

Source	DF	SS	MS	F	P
Plant Genotype	1	322.79	322.79	55.42	0.000
Error	40	232.98	5.82		
Total	41	555.77			

S = 2.413 R-Sq = 58.08% R-Sq(adj) = 57.03%

One-way ANOVA: Feeding Rate Analysis of *M. persicae* on *aap1* and *aap2*

Source	DF	SS	MS	F	P
Plant Genotype	1	0.72	0.72	0.23	0.640
Error	22	70.43	3.20		
Total	23	71.15			

S = 1.789 R-Sq = 1.01% R-Sq(adj) = 0.00%

One-way ANOVA: Feeding Rate Analysis of *B. brassicae* on *aap1* and *aap2*

Source	DF	SS	MS	F	P
Plant Genotype	1	4.49	4.49	1.64	0.214
Error	22	60.23	2.74		
Total	23	64.72			

S = 1.655 R-Sq = 6.94% R-Sq(adj) = 2.71%

Descriptive Statistics: Feeding Rate Analysis - *B. brassicae* on *aap1*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
bb aap1	12	0	16.415	0.437	1.514	14.700	15.680	15.680	17.395	19.600

Descriptive Statistics: Feeding Rate Analysis - *B. brassicae* on Col0

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
bb col	23	0	23.975	0.584	2.799	17.820	21.780	23.760	26.730	28.710

Descriptive Statistics: Feeding Rate Analysis - *B. brassicae* on *aap2*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3
bb aap2	12	0	17.280	0.515	1.784	14.400	16.320	17.280	18.960

Variable	Maximum
bb aap2	20.160

Descriptive Statistics: Feeding Rate Analysis - *M. persicae* on *aap1*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3
mp aap1	12	0	18.180	0.542	1.877	15.150	17.170	18.180	19.947

Variable	Maximum
mp aap1	21.210

Descriptive Statistics: Feeding Rate Analysis - *M. persicae* on Col0

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3
mp col	30	0	23.970	0.481	2.635	17.340	22.440	23.970	25.500

Variable	Maximum
mp col	28.560

Descriptive Statistics: Feeding Rate Analysis - *M. persicae* on *aap2*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3
mp aap2	12	0	17.833	0.490	1.697	16.000	17.000	17.000	18.750

Variable	Maximum
mp aap2	21.000

Appendix 3.1

Aphid Growth Analysis – Weight Analysis of *M. persicae* and *B. brassicae* on *aap1, aap2* and control plants

Descriptive Statistics: *M. persicae* on *aap1* measured at 24hrs

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
MP aap1 24HRS	21	0	38.333	0.590	2.704	33.200	36.400	38.300	40.300	43.400

Descriptive Statistics: *M. persicae* on *aap2* measured at 24hrs

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
MP aap2 24HRS	20	0	35.725	0.691	3.091	29.800	33.000	36.600	38.300	39.400

Descriptive Statistics: *M. persicae* on col0 measured at 24hrs

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
MP col0 24HRS	25	0	38.168	0.605	3.027	32.100	36.000	38.400	40.200	43.600

Descriptive Statistics: *M. persicae* on *aap1* measured at 72hrs

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
MP aap1 72HRS	21	0	97.614	0.851	3.898	86.700	95.900	97.900	99.950	104.100

Descriptive Statistics: *M. persicae* on *aap2* measured at 72hrs

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
MP aap2 72HRS	20	0	100.10	0.596	2.66	95.10	98.48	99.95	102.25	104.50

Descriptive Statistics: *M. persicae* on *col0* measured at 72hrs

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
MP col0 72HRS	25	0	112.49	1.36	6.78	98.60	110.45	114.20	118.05	119.60

***Descriptive Statistics: M*. persicae on *aap1* measured at 120hrs**

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
MP aap1 120HRS	21	0	204.44	0.940	4.31	197.50	200.45	205.60	208.10	210.60

Descriptive Statistics: *M. persicae* on *aap2* measured at 120hrs

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
MP aap2 120HRS	20	0	213.53	2.25	10.04	197.40	206.78	212.60	220.77	230.50

Descriptive Statistics: *M. persicae* on col0 measured at 120hrs

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
MP col0 120HRS	25	0	197.52	1.16	5.81	187.70	192.45	199.30	202.10	210.40

Descriptive Statistics: *B. brassicae* on *aap1* measured at 24hrs

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
BB aap1 24HRS	21	0	31.671	0.617	2.825	25.600	29.900	31.100	33.400	37.700

Descriptive Statistics: *B. brassicae* on *aap2* measured at 24hrs

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
BB aap2 24HRS	20	0	30.440	0.511	2.284	27.300	28.700	29.950	32.325	35.100

Descriptive Statistics: *B. brassicae* on col0 measured at 24hrs

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
BB col0 24HRS	25	0	32.160	0.648	3.239	25.700	29.750	32.800	34.850	37.100

Descriptive Statistics: *B. brassicae* on *aap1* measured at 72hrs

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
BB aap1 72HRS	21	0	94.805	0.812	3.723	89.700	91.500	94.500	97.250	102.400

Descriptive Statistics: *B. brassicae* on *aap2* measured at 72hrs

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
BB aap2 72HRS	20	0	98.765	0.675	3.018	92.800	96.750	99.150	101.250	103.800

Descriptive Statistics: *B. brassicae* on col0 measured at 72hrs

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
BB col0 72HRS	25	0	100.16	0.772	3.86	94.90	97.55	99.50	101.25	110.20

Descriptive Statistics: *B. brassicae* on *aap1* measured at 120hrs

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
BB aap1 120HRS	21	0	189.98	1.45	6.66	179.40	185.10	187.90	196.85	202.40

Descriptive Statistics: *B. brassicae* on *aap2* measured at 120hrs

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
BB aap2 120 HRS	20	0	210.08	0.948	4.24	199.70	207.50	210.75	213.23	217.40

Descriptive Statistics: *B. brassicae* on col0 measured at 120hrs

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
BB col0 120HRS	25	0	203.80	0.842	4.21	196.60	199.60	206.10	206.85	209.70

Tabulated statistics: Plant Genotype, Timepoint

Rows: Plant Genotype Columns: Timepoint

	1	2	3	All
1	21	21	21	63
2	20	20	20	60
3	25	25	25	75
All	66	66	66	198

Cell Contents: Count

General Linear Model: Analysis of Mean Aphid Weight of *M. persicae* on *aap1, aap2* and Col0 at 24hr, 72hr and 120hr

Factor	Type	Levels	Values
Plant Genotype	fixed	3	1, 2, 3
Timepoint	fixed	3	1, 2, 3

Analysis of Variance for Mean Aphid Weight, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Plant Genotype	2	335	335	167	3.01	0.052
Timepoint	2	934145	934145	467073	8384.63	0.000
Error	193	10751	10751	56		
Total	197	945231				

S = 7.46363 R-Sq = 98.86% R-Sq(adj) = 98.84%

General Linear Model: Analysis of Mean Aphid Weight of *B. brassicae* on *aap1, aap2* and Col0 Plants at 24hr, 72hr and 120 hr

Factor	Type	Levels	Values
Plant Genotype 1	fixed	3	1, 2, 3
Timepoint 1	fixed	3	1, 2, 3

Analysis of Variance for Mean Aphid Weight 1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Plant Genotype 1	2	2156	2156	1078	37.29	0.000
Timepoint 1	2	966530	966530	483265	16712.43	0.000
Error	193	5581	5581	29		
Total	197	974268				

S = 5.37741 R-Sq = 99.43% R-Sq(adj) = 99.42%

General Linear Model: Analysis of Mean Aphid Weight of *M. persicae* and *B. brassicae* on *aap1, aap2* and Col0 plants at 24hr, 72hr and 120hr.

Factor	Type	Levels	Values
Aphid Species	fixed	2	1, 2
Plant Genotype	fixed	3	1, 2, 3
Timepoint	fixed	3	1, 2, 3

Analysis of Variance for Mean Aphid Weight, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Aphid Species	1	2554	2554	2554	58.98	0.000
Plant Genotype	2	2095	2095	1048	24.19	0.000
Timepoint	2	1900514	1900514	950257	21942.17	0.000
Error	390	16890	16890	43		
Total	395	1922053				

S = 6.58083 R-Sq = 99.12% R-Sq(adj) = 99.11%

One-way ANOVA: Weight analysis of *B. brassicae* on *aap1* and col0 at 24hrs

Source	DF	SS	MS	F	P
Plant Genotype 2	1	2.72	2.72	0.29	0.592
Error	44	411.40	9.35		
Total	45	414.13			

S = 3.058 R-Sq = 0.66% R-Sq(adj) = 0.00%

One-way ANOVA: Weight analysis of *B. brassicae* on *aap2* and Col0 at 24hrs

Source	DF	SS	MS	F	P
Plant Genotype 2	2	33.16	16.58	2.21	0.120
Error	50	374.75	7.49		
Total	52	407.90			

S = 2.738 R-Sq = 8.13% R-Sq(adj) = 4.45%

One-way ANOVA: Weight analysis of *B. brassicae* on *aap1* and Col0 at 72hrs

Source	DF	SS	MS	F	P
Plant Genotype 2	1	326.8	326.8	22.67	0.000
Error	44	634.3	14.4		
Total	45	961.2			

S = 3.797 R-Sq = 34.00% R-Sq(adj) = 32.50%

One-way ANOVA: Weight analysis of *B. brassicae* on *aap2* and Col0 at 72 hrs

Source	DF	SS	MS	F	P
Plant Genotype 2	1	21.5	21.5	1.74	0.194
Error	43	530.2	12.3		
Total	44	551.7			

S = 3.511 R-Sq = 3.90% R-Sq(adj) = 1.66%

One-way ANOVA: Weight analysis of *B. brassicae* on *aap1* and Col0 at 120 hrs

Source	DF	SS	MS	F	P
Plant Genotype 2	1	2181.0	2181.0	73.20	0.000
Error	44	1311.0	29.8		
Total	45	3492.0			

S = 5.458 R-Sq = 62.46% R-Sq(adj) = 61.60%

One-way ANOVA: Weight analysis of *B. brassicae* on *aap2* and Col0 at 120 hrs

Source	DF	SS	MS	F	P
Plant Genotype 2	1	438.2	438.2	24.59	0.000
Error	43	766.3	17.8		
Total	44	1204.5			

S = 4.222 R-Sq = 36.38% R-Sq(adj) = 34.90%

Appendix 3.2

Aphid Body Dimension Analysis

General Linear Model: Aphid Bodily Dimension Analysis (Measurement 1) – *M. persicae* and *B. brassicae* on *aap1, aap2* and Col0 plants at 24, 72 and 120 hrs

```
Factor          Type   Levels  Values
plant genotype  fixed       3  1, 2, 3
aphid species   fixed       2  1, 2
hour            fixed       3  1, 2, 3


Analysis of Variance for measurement, using Adjusted SS for Tests

Source           DF   Seq SS   Adj SS  Adj MS       F      P
plant genotype    2   0.0805   0.0297  0.0149    0.95  0.388
aphid species     1   0.0120   0.0120  0.0080    0.00  0.943
hour              2  19.6932  19.6932  9.8466  629.80  0.000
Error           336   5.2532   5.2532  0.0156
Total           341  25.0269


S = 0.125038   R-Sq = 79.01%   R-Sq(adj) = 78.70%
```

General Linear Model: Aphid Bodily Dimension Analysis (Measurement 2) – *M. persicae* and *B. brassicae* on *aap1, aap2* and Col0 plants at 24, 72 and 120 hrs

```
Factor          Type   Levels  Values
plant genotype  fixed       3  1, 2, 3
aphid species   fixed       2  1, 2
hour            fixed       3  1, 2, 3


Analysis of Variance for measurement, using Adjusted SS for Tests

Source           DF   Seq SS   Adj SS  Adj MS       F      P
plant genotype    2     3.45     1.74    0.87    1.54  0.215
aphid species     1     0.02     0.01    0.00    0.00  0.903
hour              2  1025.24  1025.24  512.62  910.13  0.000
Error           336   189.25   189.25    0.56
Total           341  1217.94


S = 0.750491   R-Sq = 84.46%   R-Sq(adj) = 84.23%
```

General Linear Model: Aphid Bodily Dimension Analysis (Measurement 3) – *M. persicae* and *B. brassicae* on *aap1, aap2* and Col0 plants at 24, 72 and 120 hrs

```
Factor          Type   Levels  Values
plant genotype  fixed       3  1, 2, 3
aphid species   fixed       2  1, 2
hour            fixed       3  1, 2, 3


Analysis of Variance for measurement, using Adjusted SS for Tests

Source           DF   Seq SS   Adj SS   Adj MS       F      P
plant genotype    2    0.819    1.364    0.682    1.67  0.189
aphid species     1    0.001    0.001    0.001    0.01  0.943
hour              2  259.779  259.779  129.889  994.50  0.000
Error           336   43.884   43.884    0.131
Total           341  304.482


S = 0.361397   R-Sq = 85.59%   R-Sq(adj) = 85.37%
```

General Linear Model: Aphid Bodily Dimension Analysis (Measurement 4) – *M. persicae* and *B. brassicae* on *aap1, aap2* and Col0 plants at 24, 72 and 120 hrs

```
Factor          Type   Levels  Values
plant genotype  fixed       3  1, 2, 3
aphid species   fixed       2  1, 2
hour            fixed       3  1, 2, 3


Analysis of Variance for measurement, using Adjusted SS for Tests

Source           DF   Seq SS   Adj SS   Adj MS       F      P
plant genotype    2   0.2799   0.1298   0.0649    2.86  0.069
aphid species     1   0.0012   0.0012   0.0012    0.05  0.816
hour              2  20.6272  20.6272  10.3136  454.65  0.000
Error           336   7.6221   7.6221   0.0227
Total           341  28.5304


S = 0.150615   R-Sq = 73.28%   R-Sq(adj) = 72.89%
```

General Linear Model: Aphid Bodily Dimension Analysis (Measurement 5) – *M. persicae* and *B. brassicae* on *aap1, aap2* and Col0 plants at 24, 72 and 120 hrs

Factor	Type	Levels	Values
plant genotype	fixed	3	1, 2, 3
aphid species	fixed	2	1, 2
hour	fixed	3	1, 2, 3

Analysis of Variance for measurement, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
plant genotype	2	1.81	3.09	1.54	3.54	0.070
aphid species	1	0.01	0.01	0.01	0.01	0.915
hour	2	995.89	995.89	497.94	1142.43	0.000
Error	336	146.45	146.45	0.44		
Total	341	1144.15				

S = 0.660199 R-Sq = 87.20% R-Sq(adj) = 87.01%

Appendix 3.3

Cumulative Nymph Production

Mann-Whitney Test and CI: Cumulative Nymph Production of *M. persicae* on *aap1* and Col0

```
           N  Median
mp aap1   11   17.00
mp col 1  11   17.92


Point estimate for ETA1-ETA2 is -0.48
95.1 Percent CI for ETA1-ETA2 is (-9.58,7.76)
W = 122.5

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.8182
The test is significant at 0.8182 (adjusted for ties)
```

Mann-Whitney Test and CI: Cumulative Nymph Production of *M. persicae* on *aap2* and Col0

```
          N  Median
mp aap2  13   17.00
mp col   13   15.00


Point estimate for ETA1-ETA2 is -1.00
95.4 Percent CI for ETA1-ETA2 is (-9.00,8.10)
W = 170.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.7976
The test is significant at 0.7975 (adjusted for ties)
```

Mann-Whitney Test and CI: Cumulative Nymph Production of *B. brassicae* on *aap2* and Col0

```
           N  Median
bb aap2   12   14.35
bb Col_1  12   16.30


Point estimate for ETA1-ETA2 is -1.35
95.4 Percent CI for ETA1-ETA2 is (-10.60,7.30)
W = 140.5

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.6033
The test is significant at 0.6032 (adjusted for ties)
```

Mann-Whitney Test and CI: Cumulative Nymph Production of *B. brassicae* on *aap1* and Col0

```
          N  Median
bb aap1  11   16.40
bb col   11   18.10


Point estimate for ETA1-ETA2 is -1.50
95.1 Percent CI for ETA1-ETA2 is (-10.30,7.70)
W = 118.0

Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.5994
The test is significant at 0.5993 (adjusted for ties)
```

Appendix 3.4

Aphid Establishment

General Linear Model: Aphid Establishment of *M. persicae* and *B. brassicae* on *aap1*, *aap2* and wildtype plants

```
Factor            Type   Levels  Values
Plant Genotype    fixed       3  1, 2, 3
Aphid Species     fixed       2  1, 2


Analysis of Variance for Establishment 1, using Adjusted SS for Tests

Source             DF   Seq SS   Adj SS  Adj MS     F      P
Plant Genotype      2   40.111   40.111  20.056  7.81  0.001
Aphid Species       1    1.143    1.143   1.143  0.45  0.506
Error             122  313.286  313.286   2.568
Total             125  354.540


S = 1.60247   R-Sq = 11.64%   R-Sq(adj) = 9.46%
```

One-way ANOVA: Analysis of Establishment of *B. brassicae* on *aap1* and Col0

```
Source            DF      SS     MS     F      P
Plant Genotype 2   1   13.71  13.71  5.68  0.022
Error             40   96.57   2.41
Total             41  110.29

S = 1.554   R-Sq = 12.44%   R-Sq(adj) = 10.25%
```

One-way ANOVA: Analysis of Establishment of *B. brassicae* on *aap2* and Col0

```
Source            DF      SS     MS     F      P
Plant Genotype 2   1   12.60  12.60  4.44  0.041
Error             40  113.52   2.84
Total             41  126.12

S = 1.685   R-Sq = 9.99%   R-Sq(adj) = 7.74%
```

One-way ANOVA: Analysis of Establishment of *M. persicae* on *aap1* and Col0

```
Source            DF      SS     MS     F      P
Plant Genotype 1   1   12.60  12.60  5.73  0.021
Error             40   87.90   2.20
Total             41  100.50
```

S = 1.482   R-Sq = 12.53%   R-Sq(adj) = 10.35%

One-way ANOVA: Analysis of Establishment of *M. persicae* on *aap2* and col0

Source	DF	SS	MS	F	P
Plant Genotype 1	1	21.43	21.43	9.98	0.003
Error	40	85.90	2.15		
Total	41	107.33			

S = 1.465   R-Sq = 19.96%   R-Sq(adj) = 17.96%

Appendix 3.5

Whole Population Analysis

General Linear Model: Whole population analysis of *M. persicae* and *B. brassicae* on *aap1*, *aap2* and wildtype plants

```
Factor          Type   Levels  Values
Plant Genotype  fixed       3  1, 2, 3
Aphid Species   fixed       2  1, 2
```

```
Analysis of Variance for Weight, using Adjusted SS for Tests

Source           DF     Seq SS     Adj SS     Adj MS     F      P
Plant genotype    2  0.0000559  0.0000579  0.0000290  0.26  0.768
Aphid Species     1  0.0000112  0.0000112  0.0000112  0.10  0.749
Error           118  0.0129026  0.0129026  0.0001093
Total           121  0.0129698


S = 0.0104568   R-Sq = 0.52%   R-Sq(adj) = 0.00%
```

One-way ANOVA: Whole Aphid Population Analysis of *M. persicae* on *aap1* and Col0 plants

```
Source            DF        SS        MS     F      P
Plant Genotype 1   1  0.000008  0.000008  0.05  0.829
Error             38  0.006647  0.000175
Total             39  0.006656

S = 0.01323   R-Sq = 0.12%   R-Sq(adj) = 0.00%
```

One-way ANOVA: Whole Population Analysis of *M. persicae* and on *aap2* and col0

```
Source            DF        SS        MS     F      P
Plant Genotype 1   1  0.000011  0.000011  0.09  0.767
Error             44  0.005578  0.000127
Total             45  0.005590

S = 0.01126   R-Sq = 0.20%   R-Sq(adj) = 0.00%
```

One-way ANOVA: Whole Aphid Population Analysis of *B. brassicae* on *aap1* and Col0

Source	DF	SS	MS	F	P
Plant Genotype 1	1	0.000028	0.000028	0.19	0.666
Error	35	0.005184	0.000148		
Total	36	0.005212			

S = 0.01217 R-Sq = 0.54% R-Sq(adj) = 0.00%

One-way ANOVA: Whole Aphid Population Analysis of *B. brassicae* on *aap2* and Col0

Source	DF	SS	MS	F	P
Plant Genotype 1	1	0.000048	0.000048	0.47	0.499
Error	39	0.004006	0.000103		
Total	40	0.004054			

S = 0.01014 R-Sq = 1.18% R-Sq(adj) = 0.00%

Descriptive Statistics: Mean Total Aphid Population Weight (g) *M. persicae* on *aap1*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
aap1	17	0	0.00996	0.00248	0.01024	0.00070	0.00407	0.00872	0.01035	0.04676

Descriptive Statistics: Total Aphid Population Weight (g) *M. persicae* on Col0

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
col	23	0	0.01088	0.00313	0.01503	0.00078	0.00151	0.00512	0.01064	0.05260

Descriptive Statistics: Total Aphid Population Weight (g) *M. persicae* on *aap2*

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3

```
aap2      23   0  0.00989  0.00110  0.00526  0.00218  0.00664  0.00874  0.01240

Variable  Maximum
aap2      0.01998
```

Descriptive Statistics: Total Aphid Population Weight (g) *B. brassicae* on *aap1*

```
Variable   N  N*     Mean  SE Mean    StDev  Minimum       Q1   Median       Q3
bbaap1    18   0  0.01041  0.00231  0.00980  0.00070  0.00623  0.00900  0.01074

Variable  Maximum
bbaap1    0.04676
```

Descriptive Statistics: Total Aphid Population Weight (g) *B. brassicae* on *aap2*

```
Variable   N  N*      Mean   SE Mean     StDev   Minimum        Q1    Median
bb aap2   22   0  0.009991  0.000993  0.004659  0.003330  0.006613  0.008455

Variable        Q3   Maximum
bb aap2   0.013160  0.019450
```

Descriptive Statistics: Aphid Population Weight (g) *B. brassicae* on Col0

```
Variable   N  N*     Mean  SE Mean    StDev  Minimum       Q1   Median       Q3
bb col    19   0  0.01216  0.00322  0.01404  0.00093  0.00229  0.00750  0.01275

Variable  Maximum
bb col    0.05206
```

Appendix 4.1

Aposymbiotic Aphid Performance on *aap1* and *aap2* Plants

Two-way ANOVA: Performance of aposymbiotic and symbiotic *B.brassicae* on *aap2* and wildtype plants

Source	DF	SS	MS	F	P
Genotype	1	0.0000005	0.0000005	15.42	0.004
Treatment	1	0.0000028	0.0000028	82.74	0.000
Interaction	1	0.0000001	0.0000001	2.44	0.157
Error	8	0.0000003	0.0000000		
Total	11	0.0000037			

S = 0.0001840 R-Sq = 92.63% R-Sq(adj) = 89.87%

Two-way ANOVA: Performance of aposymbiotic and symbiotic *B. brassicae* on *aap1* and wildtype plants

Source	DF	SS	MS	F	P
Genotype	1	0.0000002	0.0000002	5.85	0.042
Treatment	1	0.0000036	0.0000036	90.07	0.000
Interaction	1	0.0000002	0.0000002	5.85	0.042
Error	8	0.0000003	0.0000000		
Total	11	0.0000044			

S = 0.0001995 R-Sq = 92.71% R-Sq(adj) = 89.98%

Two-way ANOVA: Performance of aposymbiotic and symbiotic *M. persicae* on *aap1* and wildtype plants

Source	DF	SS	MS	F	P
Genotype	1	0.0000011	0.0000011	104.14	0.000
Treatment	1	0.0000019	0.0000019	185.52	0.000
Interaction	1	0.0000002	0.0000002	18.25	0.003
Error	8	0.0000001	0.0000000		
Total	11	0.0000032			

S = 0.0001014 R-Sq = 97.47% R-Sq(adj) = 96.52%

Two-way ANOVA: Performance of aposymbiotic and symbiotic *M. persicae* on *aap2* and wildtype plants

Source	DF	SS	MS	F	P
Genotype	1	0.0000012	0.0000012	281.71	0.000
Treatment	1	0.0000020	0.0000020	461.97	0.000
Interaction	1	0.0000003	0.0000003	58.80	0.000
Error	8	0.0000000	0.0000000		
Total	11	0.0000035			

S = 0.00006591 R-Sq = 99.01% R-Sq(adj) = 98.64%

Appendix 5.1 - Table of EPG Parameters

Mean Duration (sec) of Activity Over 8 Hour Experimental Period	**Wildtype (n=13)**		***aap1*** **(n=14)**		***aap2*** **(n=15)**	
	M. persicae	B. brassicae	*M. persicae*	*B. brassicae*	M. persicae	B. brassicae
Non-probing activities (out)	199 (±56.1)	112 (±49.6)	561 (±129.2)	492 (±136.9)	505 (±225.7)	378 (±197.6)
Pathway Activities (pathway)	2879 (±967.5)	4830 (±1056.2)	5780 (±1784.1)	6325 (±1662.3)	7192 (2106.4)	8434 (±2345.1)
E1	436 (±114.3)	111 (±69.8)	457 (±99.6)	529 (±138.7)	435 (±142.5)	620 (±161.1)
E2 (> 10 mins)	25128 (±764.2)	23747 (±884.9)	21637 (±663.2)	20143 (±771.4)	20685 (±669.8)	19368 (±602.1)

Table 3.11b – Summary of EPG parameter results for *M.persicae* and *B. brassicae*. Values shown are means ± s.e. Asterisk indicate significant differences ($p < 0.05$). Sample sizes are indicated in brackets at the top of each genotype column.