Performance of cereal aphids in relation to genetic variation and nitrogen use efficiency of wheat (*Triticum aestivum*)

James Philip Redfern

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Abstract

In this thesis I address the question: does breeding to improve nitrogen use efficiency of winter wheat, *Triticum aestivum,* increase its susceptibility to infestation by cereal aphids? Laboratory and greenhouse experiments were used to monitor population performance of the aphids *Sitobion avenae* and *Metopolophium dirhodum* on double haploid genotypes of a Savannah cross Rialto (SavRia) wheat population. The genotypes varied in nitrogen use efficiency and its two components: nitrogen uptake efficiency and nitrogen utilisation efficiency.

A quantitative trait loci analysis, using 94 genotypes grown under greenhouse and laboratory conditions, identified genetic markers associated with individual performance and population measures of *S. avenae* and *M. dirhodum*. Twelve markers across chromosomes on the SavRia genetic map were statistically associated with nine of the fourteen aphid performance traits measured.

Nine wheat genotypes, representing the range of nitrogen use efficiencies, were used to analyse how aphid development rate, fecundity and intrinsic rate of increase, varied between genotype and whether settling behaviour and preference between genotypes, reflected differences in performance. Significant differences in performance of *S. avenae* between different genotypes were found but performance was not correlated with plant nitrogen uptake.

In a randomised split plot experiment in the field, six SavRia genotypes and both parental varieties were grown under three application rates of nitrogen fertiliser. Significant variation between genotypes and between different nitrogen application rates was observed for eight plant traits, including both nitrogen uptake and utilisation efficiencies. Density of *S. avenae* differed significantly between genotypes with significant genotype x nitrogen application rate interactions. Aphid densities were not significantly correlated to either components of nitrogen use efficiency.

I conclude that this study does not support the hypothesis that increasing nitrogen use efficiency, or either of its primary components, increases the susceptibility of winter wheat to infestation by cereal aphids.

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1 Introduction

In this thesis I investigate how cereal aphid performance is influenced by the genetic variation in a doubled haploid winter wheat population with different nitrogen efficiencies. The aphids *Sitobion avenae* and *Metopolophium dirhodum* are both serious pests on cereal crops due to the losses in yield they can cause (Wratten 1975, Kieckhefer & Gellner 1992). There is a risk that breeding to increase nitrogen use efficiency in wheat could increase the risk of aphid infestation. The overall aim of this thesis is to increase understanding of how aphid performance is influenced by wheat genotypes with different nitrogen use efficiencies.

1.1.1 Human population growth and food security

Modern human societies and animal populations around the world face uncertainty in relation to global environmental change and unsustainable use of natural resources. The human population has continued to increase, and current projections suggest over 9 billion people will be sharing the planet by 2050 (Challinor 2011). The impact of supplying natural resources such as fossil fuels and fresh water for the growing human population is having a profound effect on biodiversity and the environment of the planet (Fields 2004). Not only is the human population growing, but at the same time individuals are increasing their per capita consumption. This combination is putting unprecedented pressure on natural and agricultural systems around the world (Hellin *et al.* 2012, Meyers *et al.* 2015). One solution to this problem is to make more sustainable use of resources.

One of the most important concerns regarding the growing human population is world food security (Meyers *et al.* 2015). The world food summit defined food security as "when all people at all times have access to sufficient, safe, nutritious food to maintain a healthy and active life" (FAO 2003). Current projections for wheat and maize production suggest that food production will not meet the demands of the projected human population (Figure 1.1). This is a serious global concern, not just for developing countries but for all countries and people in the world. Increasing cereal production, in a changing climate with scarcer natural resources, presents a serious global problem that threatens food security (Meyers *et al.* 2015). Historically, food security concerns led to the creation of agriculture which increased food production, fuelling human population growth.

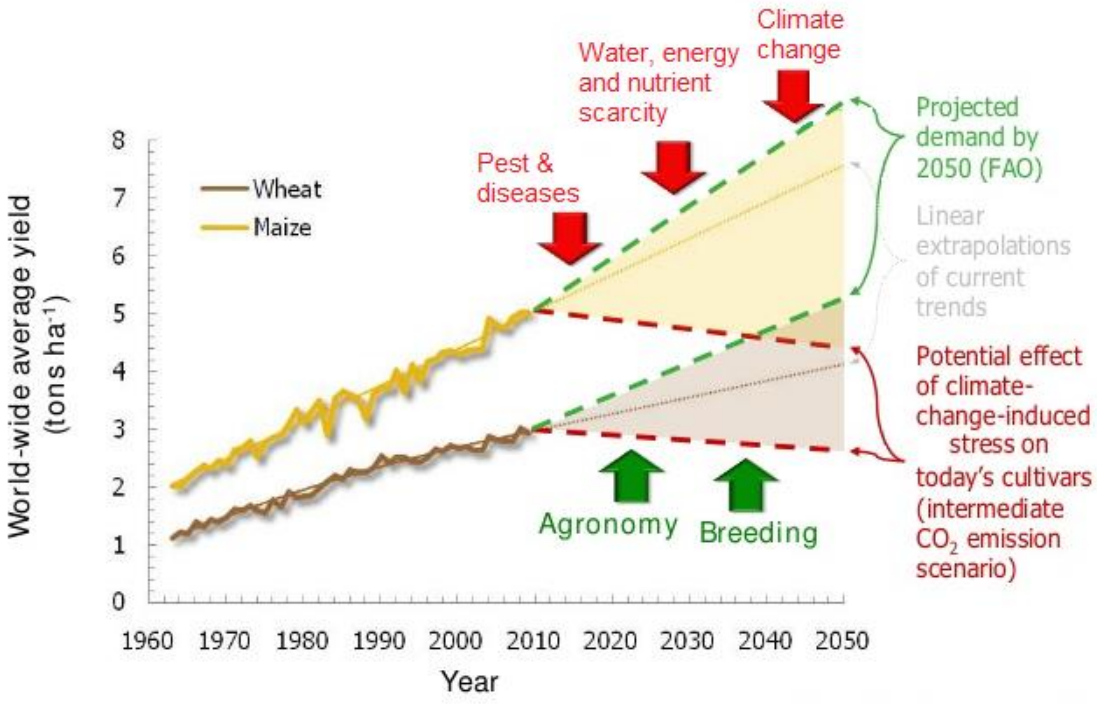

Figure 1.1. The worldwide average yields for wheat and maize (tons ha-1) and the projected yields until 2050. Projected demand (---) for these cereals by 2050 with the potential effects (---) of pest, disease, nutrient scarcity and climate change induced stress on yields. The direction of global trends potentially influencing yield indicated by arrows: ⇧ increase yield, ⇩ decrease yield. Adapted from Hellin *et al.* (2012).

1.1.2 A brief history of agriculture and the green revolution

Agriculture originated ~12000 years ago independently in several regions of the world (Byerlee & Traxler 1995). The earliest sowing and harvesting of food plants that were previously collected in the wild was recorded in the Fertile Crescent stretching from Egypt through western Asia to India (Bonjean & Angus 2001). The Neolithic revolution was when the transition from hunting and gathering of food towards settled agriculture first occurred. During this period wheat was first cultivated (Bonjean & Angus 2001). Since the origin of agriculture and domestication of plants a range of agricultural techniques have been developed, including irrigation, crop rotation and more recently the application of fertilisers.

The green revolution of the 20th century increased agricultural production across the globe through the transfer of knowledge and development of modern management techniques, which included the use of higher yielding varieties (Sylvester-Bradley & Kindred 2009). The green revolution saw the development and distribution of hybridised seeds for high yielding varieties spread across the world. This led to nations that had previously imported cereal grain to supply their demands, being in a position to export excess produce (Byerlee & Traxler 1995). A key aspect of the green revolution was the increased use of synthetic, particularly nitrogen, fertilisers leading to substantial increases in crop yields. The synthesis of ammonium nitrate using the Haber-Bosch process made synthetic fertilisers widely

available for increasing crop production. Modern industrialised processes also led to the creation of synthetic pesticides. The application of synthetic fertilisers and pesticides represented a further major breakthrough, which helped to revolutionise global crop production by removing some of the previous constraints on crop yield.

1.1.3 The importance of wheat for global food security

Amongst the world's most important food crops for both human nutrition and livestock feed are cereals. The three most important cereal crops of the world are maize, rice and wheat. Over 700 million tonnes of wheat were produced in 2013 (FAO 2014). The UK dedicated 1.9 million hectares of land to produce over 16 million tonnes of wheat in 2014 (DEFRA 2014). In the UK, over recent decades wheat yield has increased by around 10% each decade (Evenson & Gollin 2003; Snape *et al.* 2007). Wheat yields in the UK have increased from 3 tons hectare^{-1} in the 1950s to 10 tons hectare^{-1} by 2010 under the most favourable conditions (Ma *et al* 2014). However, wheat yields are not increasing fast enough to supply the increasing global demand and a wheat yield deficit is projected by 2050 (Figure 1.1) (Schmidhuber & Tubiello 2007; Challinor 2011). These projections suggest that wheat yields may not be secure in the near future.

Wheat yields consequently need to be increased. In the past crop productivity has been increased by adding fertiliser and water to increase yields from hybridised varieties and also increasing the area used for production. The problem with applying these approaches in the future is that there is limited available land for crop production, limited supplies of water and of fertilisers. As the human population increases and the climate changes it is imperative to reduce yield loss to drought, disease and crop pests to secure future yields (Schmidhuber & Tubiello 2007). Wheat improvement strategies which include breeding new varieties are, therefore, now key to increasing future food security. Breeding improved varieties of wheat involves developing varieties with higher yields, but which are also responsive to improved agronomic practices that reduce inputs.

1.1.4 The pillars of wheat improvement strategies

Throughout this thesis reference will be made to wheat improvement strategies as having two key pillars, the breeding pillar and the agronomy pillar. The main goal of wheat improvement is to increase yield in a more sustainable way. The drivers negatively impacting wheat yields will be climate change, resource scarcity, diseases and crop pests. Through improving agronomic practice wheat yields can be improved and sustained to some degree. The energy required to produce fertiliser and the associated $C0_2$ production has a detrimental effect on the climate on a global scale (Fuhrer *et al.* 2003). The use of pesticide has negative impacts on the wider environment with local and regional impacts on non-target organisms (Foster *et al. 2014*). Reducing inputs of fertiliser and pesticide for wheat production

would address resource scarcity issues and make production more sustainable, thereby mitigating some of the adverse effects of climate change. Under the breeding pillar, breeding elite wheat varieties with higher nitrogen use efficiencies and increased resistance to pests and disease is a main goal for wheat improvement, fostering more sustainable agronomic practice.

1.1.5 Wheat breeding for increased yields

Traditionally, wheat breeding has focused on identifying phenotypic traits that increase yield and improve crop quality in plot trials and selecting for these traits in breeding populations (Byerlee & Traxler 1995). Traditional wheat breeding methodologies use multiple generations for self-pollinating and backcrossing to create breeding lines to select and fix desirable agronomic traits in new wheat varieties. The biggest increases in yield and quality of bread wheat have been made by the creation of shorter varieties (semi-dwarfs) that are resistant to lodging (falling over), and varieties capable of assimilating large amounts of nitrogen (Berger & Planchon 1990). The creation and introduction of wheat varieties that offer resistance to pathogens or insects was well received by producers that use new varieties from home grown cereals authority (HGCA) recommended growing lists to increase and maintain yields (HGCA 1998, HGCA 2014).

Other advancements in cereal production include the use of varieties with higher nitrogen use efficiencies. Nitrogen use efficiency (NUE) is the total dry matter (DM) grain yield (kg DM grain ha^{-1}) produced per unit of nitrogen (N) absorbed from the soil solution including that applied as fertiliser (kg N ha^{-1}), the units of which are kg DM grain kg^{-1} N supplied (Moll *et al.* 1982, Gaju *et al.* 2011). Complex plant traits such as nitrogen use efficiency are controlled by many different genes, many of which are currently unknown (Obara *et al.* 2004, Karrou & Nachit 2015). Discovering and locating genes that control physiological plant traits influencing nitrogen utilisation efficiency would mean the genes themselves can then be used in modern breeding programs to improve wheat. Growing wheat varieties with higher nitrogen use efficiencies can result in higher yields from a lower application rates of nitrogen fertiliser (Gaju *et al.* 2011).

1.1.6 Wheat production and nitrogen fertiliser application

Currently, global wheat production is heavily reliant on nitrogen fertiliser. Over the past 100 years nitrogen fertiliser production has increased 100 fold (Fields 2004). The annual production of 112 million tonnes of nitrogen fertiliser contributes 1.2% to total anthropogenic greenhouse gas emissions (Fields 2004). Approximately 60% of nitrogen fertiliser produced is applied in the production of maize, rice and wheat (Ladha 2005). Furthermore, to sustain food security it is predicted that a threefold increase in nitrogen production will be required over the next 40 years (Fields 2004).

Although costly, nitrogen inputs dramatically increase wheat quality and yield. Wheat grain used for bread making has the highest market value and is typically required to have ≥12% protein content, equivalent to ~1.9% nitrogen content (Guarda *et al.* 2004). There is a trade-off between wheat yield and grain quality (Berger & Planchon 1990). Breeding to increase wheat yield can have the consequence of reducing grain quality in terms of a reduction in grain nitrogen content (Berger & Planchon 1990). In agricultural systems increasing the ability of plants to take up nitrogen and convert it into nitrogen in the grain will result in a higher yield and better quality grain for bread making (Sylvester-Bradly & Kindred 2009). Therefore plant varieties that have higher nitrogen use efficiencies are advantageous for commercial growers, and can contribute toward global food security.

1.1.7 Nitrogen use efficiency and its components

Nitrogen use efficiency integrates two key components: nitrogen *uptake* efficiency (NupE) and nitrogen *utilisation* efficiency (NutE) (Moll *et al.* 1982). Nitrogen *uptake* efficiency (NupE), can be defined as the above ground nitrogen (AGN) content in the dry plant biomass at harvest per unit nitrogen available in the soil, where nitrogen in the soil may have been increased by the addition of fertiliser (Gaju *et al. 2011*). Nitrogen uptake efficiency is calculated by dividing the above ground nitrogen (AGN) content in the dry plant biomass at harvest by the nitrogen present in the soil and applied as fertiliser. The units of nitrogen uptake efficiency are kg AGN kg^{-1} N available. When plants are grown in substrates with the same amount of nitrogen, the nitrogen content in the plant biomass can be used as a proxy for nitrogen uptake efficiency.

The nitrogen utilisation efficiency of wheat is defined as the amount of nitrogen taken up into above ground plant tissue at harvest that is converted to grain yield (kg grain dry matter kg^{-1} above ground N content in the plant at harvest) (Moll *et al.* 1982, Gaju *et al.* 2011) and can be the component with the greatest contribution to the overall nitrogen use efficiency (Karrou & Nachit 2015). Increasing the nitrogen use efficiency of wheat by means of breeding varieties more efficient at uptake and utilisation of nitrogen, may, however, have the undesirable effect of increasing benefits to phloem feeding insects through increasing the nitrogen concentration in plant phloem (Figure 1.2).

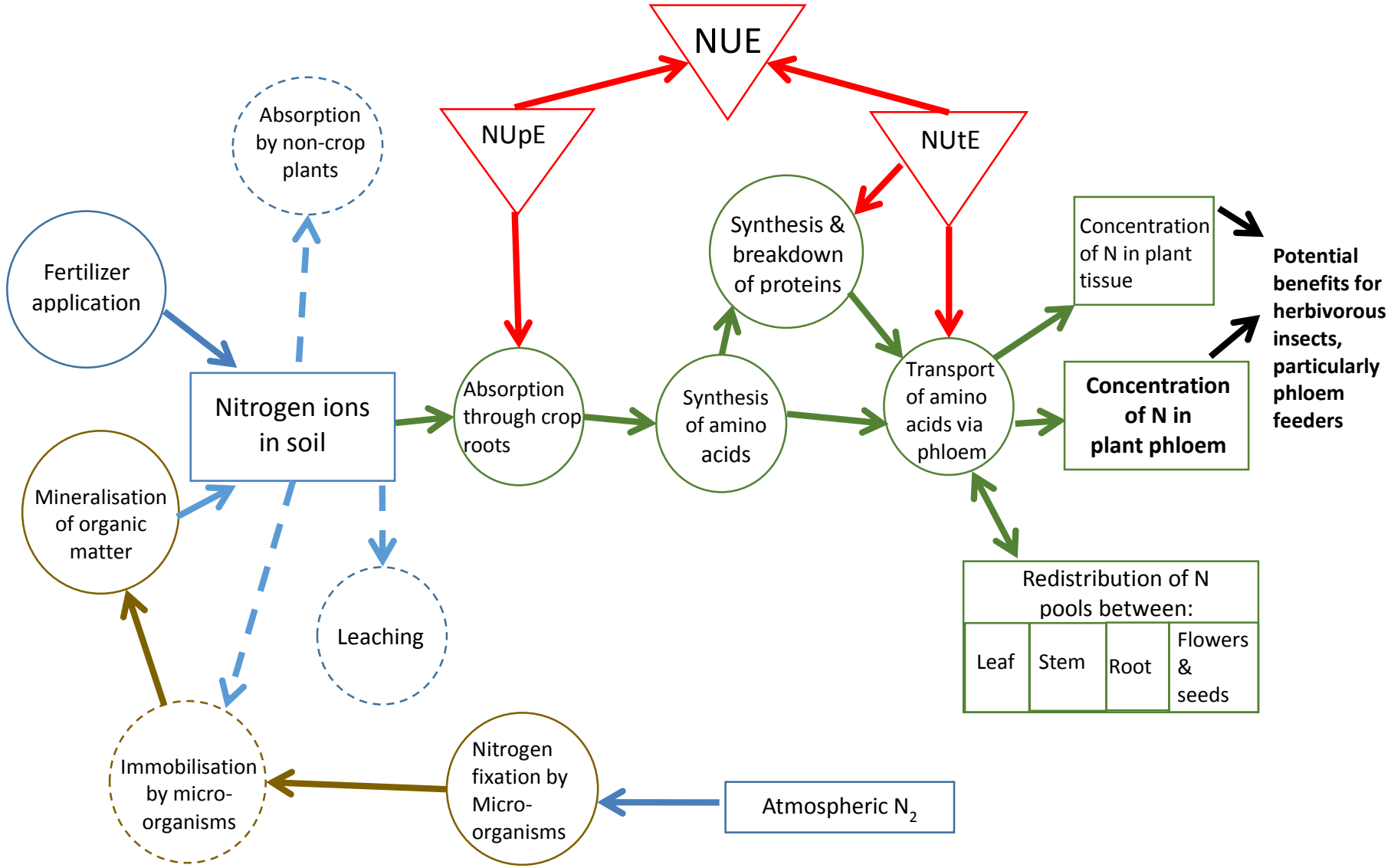

Figure 1.2. A simple summary of soil & plant nitrogen dynamics in relation to nitrogen use efficiency. Circles indicate rates of transfer, rectangles pools or standing crops and triangles genetic influences. Blue nitrogen, red genotypic, brown soil & green plant properties and processes. Dashed lines indicate other losses from soil solution.

1.1.8 The pressure on agricultural crops

The pressure on agricultural systems globally is not limited to increased demand and pressures on the available land area for production. It also includes perturbations from climate change, which can potentially be exacerbated by intensive agricultural practices that depend heavily on fertiliser applications (Fields 2004, Challinor 2011).

As the climate changes the way that pests and disease impact and interact with cereal crops will also change. Insect pests cause substantial yield losses in agricultural systems including wheat production. Phloem feeding insects are serious agricultural pests (Foster *et al. 2014*) that are particularly sensitive to nitrogen application on their host plants (Nowak and Komor 2010, Kuhlmann *et al.* 2013). Phloem feeders mainly classified in the order Hemiptera, cause direct damage to the host plant by removing amino acids from the plant phloem, and indirectly by transmitting plant viruses (Powell *et al* 2006). Phytophagous insects meet their nitrogen requirements from feeding on plants (Mattson 1980). The most important component of amino acids for phloem feeders is nitrogen (Nowak and Komor 2010); plants absorb nitrogen as ammonium or nitrate ions, the nitrogen from which is incorporated into amino acids (Xu *et al.* 2012).

1.1.9 Aphids and their host plants in relation to nitrogen

Aphids are very sensitive to changes in nitrogen concentration in plant phloem fluids during different stages of the life cycle of the host plant (Karley *et al.* 2002). Different species of aphids settle on plants that have higher rates of nitrogen fertiliser applied (Nowak and Komor 2010) and plants with higher concentrations of amino acids in the plant phloem (Karley *et al.* 2002, Nowak and Komor 2010). Aphids have been shown to more fecund on host plants with higher plant nitrogen content (Khan & Port 2008). Similarly, aphid performance in terms of longevity, adult weight and fecundity was positively correlated with nitrogen fertiliser application for the grain aphid *Sitobion avenae* and the bird cherry-oat aphid *Rhopalosiphum padi* on different wheat cultivars in the laboratory (Aqueel and Leather 2011).

Aphids infest millions of hectares of wheat each year resulting in substantial reductions in grain yield (Hu *et al.* 2013, Foster *et al. 2014*). In China alone over half a million tons of wheat was lost due to aphid infestation in 2005

(Hu *et al.* 2013). Most aphid species, including *Metopolophium dirhodum* the rose-grain aphid and the grain aphid *Sitobion avenae,* directly damage crops by feeding from amino acids in the plant phloem fluids thereby reducing nitrogen use efficiency (Ladha *et al.* 2005). In addition, they can cause several indirect adverse effects on the plant. Excretion of their honey dew is associated with reduced photosynthesis and the spread of plant pathogens, particularly saprophytic moulds (Rabbinge *et al.* 1981). Moreover, aphids also act as a vector for plant viruses, such as the barley yellow dwarf virus (BYDV) that infects cereal crops (Kurppa *et al.* 1989, Foster *et al. 2014*). Grain yield losses of 30-40% have been attributed to cereal aphid feeding alone, and losses of over 60% have been observed when combined with virus infection (Kieckhefer & Gellner 1992). There is currently no consensus on how increasing nitrogen use efficiency in wheat will influence crop pests. One way to maintain yields globally will be by reducing yield lost to insect crop pests.

Host plant quality is a major determinant of aphid performance (Awmack & Leather 2002, Powell *et al.* 2006). Aphids will adapt their feeding behaviour based on host plant quality. For example, adult *Aphis fabae* fed more often from variegated leaves of *Euonymus japonica aureus* than the green leaves; this was attributed to the increase in mobilised nitrogen to these leaves which compensates for their reduction in photosynthetic capacity (Sadof and Raupp 1991). In a similar way to many other phloem feeders, the performance of the grain aphid *S. avenae* is also heavily influenced by host plant quality (Powell *et al.* 2006), nitrogen fertiliser application (Aqueel & Leather 2011, Gash 2012) and the concentration of nitrogen in the plant phloem (Nowak and Komor 2010). The growth stage of the plant (Leather & Dixon 1981) and the stage at which plant senescence begins strongly affect the distribution of aphids on plant organs and also aphid population parameters such as the intrinsic rate of increase (Watt 1979). Physiological plant traits involved in nitrogen utilisation efficiency, which include senescence dynamics, will be functioning post anthesis, when the grain aphid *Sitobion avenae* can be feeding on wheat ears.

In this thesis population measures, such as development time from nymph to reproduction, the intrinsic rate of increase and population growth rate are all used as indices of aphid performance. Each of these aphid performance components can contribute to aphid population density and abundance, and is, therefore, a good measure of aphid population dynamics (Lamb *et al.* 2009, Gash 2012).

1.2 Thesis aims

The aims of this research are to contribute to an understanding of how wheat production can be improved through breeding and agronomy in relation to the risk of cereal aphid infestations. This broad aim is promoted by identifying and locating genetic markers in the SavRia population of winter wheat that are associated with aphid performance. New information on novel genes associated with a reduction in aphid performance could be used in marker assisted breeding programs to breed wheat populations with increased resistance to aphid infestation. Quantifying the nitrogen use efficiencies of a subset of SavRia genotypes under different nitrogen application rates is key to identifying how aphid populations respond to interactions between increasing nitrogen application rates when feeding on novel genotypes varying in nitrogen use efficiency. A key question that is addressed in this thesis is: are wheat genotypes with higher nitrogen use efficiencies more susceptible to cereal aphid infestations?

1.3 Thesis structure

Chapter 2:

The study system

In chapter 2 the genetic structure and biology of wheat is summarised. The model system of the SavRia doubled haploid winter wheat population is described, with reference to how the components of nitrogen use efficiency can vary between genotypes. The ecology of the rose grain aphid *Metopolophium dirhodum* and the grain aphid *Sitobion avenae*, are introduced as models for phloem feeding pests. The salient factors influencing population performance of these two cereal aphids are also introduced.

Chapter 3:

Quantitative trait loci (QTL) mapping performance measures of the cereal aphids: *Metopolophium dirhodum & Sitobion avenae* on the Savannah x Rialto doubled haploid population

The rationale for this chapter is that, identifying and locating genes which negatively influence aphid performance can potentially be used in the development of new wheat varieties that could contribute to wheat improvement programs. Utilising wheat varieties that negatively influence aphid performance can decrease production costs to growers, reduce environmental degradation associated with pesticide application and maintain future wheat yields. Maintaining future wheat yields is one aspect of wheat improvement that is intended to positively contribute towards food security. The main aim of this chapter is to identify whether genetic variation within the SavRia winter wheat population is associated with the performance of the aphids *Sitobion avenae* and *Metopolophium dirhodum*. The specific objective is to identify and locate genetic markers in the SavRia DH line that are associated with performance measures of *M. dirhodum* and *S. avenae*. These markers and the genes linked to them can potentially be characterised and validated for use in breeding programs as part of wheat improvement strategies.

The questions addressed in this chapter are:

Is there genetic variation in the SavRia wheat population influencing cereal aphid performance?

Are there genetic markers within the SavRia wheat population that are associated with the performance of cereal aphids reared on SavRia plants?

Chapter 4:

Population performance of *Sitobion avenae* on winter wheat genotypes with different nitrogen uptake efficiencies

Breeding plants to increase nitrogen use efficiency is one goal of wheat improvement strategies. Improving nitrogen use efficiency through breeding means that plants can assimilate more nitrogen into the crop from lower applications of nitrogen fertiliser. Growing plants with higher nitrogen use efficiencies also means that the same yield can be achieved from smaller applications of nitrogen fertiliser. There is, however, a need to understand how desirable agronomic traits such as higher nitrogen uptake efficiency will affect aphid fecundity. In this chapter, I investigate in a laboratory study using a high nitrogen substrate, how aphid performance, settling behaviour and preference are influenced by a subset of SavRia genotypes that have different nitrogen uptake efficiencies and therefore different nitrogen content in the plant tissue. The aim is to identify if these aphid traits are influenced by winter wheat genotypes with different nitrogen uptake efficiencies. The specific objective is to identify whether aphid fecundity varies between genotypes with different nitrogen content in the plant tissue. Understanding how aphid performance is affected by increasing nitrogen uptake efficiencies in wheat will identify if this breeding goal will benefit phloem feeding crop pests. This information can inform breeders of detrimental effects associated with improving nitrogen uptake efficiency in winter wheat.

The questions addressed in this chapter:

Does the performance of S. avenae vary between SavRia winter wheat genotypes with different nitrogen content in their somatic plant tissues?

Do the settling behaviour and preference of S. avenae reflect differences in the performance measures of S. avenae on wheat genotypes with different nitrogen uptake efficiencies?

Chapter 5:

Phenotypic components of nitrogen use efficiency in winter wheat genotypes under different nitrogen application rates

A central question addressed in this thesis is "do differences in the nitrogen use efficiency of wheat alter the potential for infestation by aphids?" In order to address this question in chapter 6, it is first necessary to quantify nitrogen use efficiency and its components of nitrogen uptake efficiency and nitrogen utilisation efficiency for the six SavRia genotypes and their parental cultivars 'Savannah' and 'Rialto'. These wheat genotypes and parental cultivars are used as a model system for examining the relationship between genotypic variation in nitrogen use efficiency and cereal aphid densities. The aim of this chapter is to investigate the influence of nitrogen fertiliser application on the components of nitrogen use efficiency and yield characteristics of SavRia genotypes. The specific objective is to quantify the components of nitrogen use efficiency as a basis for investigating how aphid densities respond to wheat genotypes with higher nitrogen use efficiencies in chapter 6.

The questions addressed in this chapter are:

To what extent do different genotypes of the SavRia model system vary in the two components of nitrogen use efficiency?

What are the relative contributions of nitrogen uptake efficiency and nitrogen utilisation efficiency to overall nitrogen use efficiency in SavRia genotypes?

Chapter 6:

Effects on *Sitobion avenae* densities of winter wheat genotypes with different nitrogen use efficiencies under different nitrogen application rates

In this chapter the effects of SavRia genotypes with different nitrogen use efficiencies on aphid densities tiller^{-1} are investigated. Different nitrogen application rates were used in combination with genotypes that have different nitrogen utilisation efficiencies to test the hypothesis that aphid densities will be higher on SavRia genotypes that have higher nitrogen uptake and nitrogen utilisation efficiencies. The overall aim of this chapter is to identify if breeding wheat genotypes with higher nitrogen use efficiency increases the potential for infestation by *Sitobion avenae*.

The questions addressed in this chapter are:

Does the application of different amounts of nitrogen fertiliser affect Sitobion avenae *densities on winter wheat?*

Do components of the nitrogen use efficiency of winter wheat genotypes influence S. avenae density?

Is there an interaction between nitrogen fertiliser application and the nitrogen use efficiency of SavRia genotypes influencing S. avenae density?

Chapter 7.

Discussion

In this chapter the results of the previous four data chapters are synthesised and evaluated particularly in relation to potential applied implications of this study.

2 Study system

The study system which was used to address the question: "are wheat genotypes with higher nitrogen use efficiencies more susceptible to cereal aphid infestations?" is described in this chapter. There is a short section on the history, genetic structure and growth stages of wheat. The SavRia population and how doubled haploid genotypes are produced are introduced and described. Aphids and some salient factors of their interactions with their host plants are introduced. The species of cereal aphids used for these experiments, aspects of their ecology and the timing of their life cycle in relation to the different growth stages of wheat, are presented.

2.1.1 The history and genetic structure of modern bread wheat

Wheat is a monocotyledonous plant in the family Poaceae (Bonjean & Angus 2001). Wheat was first cultivated approximately 10,000 years ago, and domestication has been associated with the spread of agriculture and settled societies (Brenchley *et al.* 2012). Winter wheat is a variant of common wheat, also called bread wheat - *Triticum aestivum* (Linnaeus). Modern bread wheat (*Triticum aestivum*) is a combination of the progenitor species *Triticum urartu* (einkorn wheat), *Aegilops speltoides* and *Aegilops tauschii* which contributed the AA, BB, and DD regions of the bread wheat genome respectively (Brenchley *et al.* 2012). The wheat genome is very large, estimated to be 17GB, which is several times larger than the human genome (Brenchley *et al.* 2012). Wheat is allohexaploid with six sets of chromosomes, two from each of the three progenitor species which contributed to the *AABBDD* genome of modern *Triticum aestivum* (Brenchley *et al.* 2012).

2.1.2 The growth stages of wheat

The developmental growth stages in the lifecycle of cereals can be defined using the Zadoks scale (Figure 2.1) (Zadoks *et al.* 1974). The cereal growth stages follow a specific pattern, which can be influenced by abiotic conditions, such as temperature and rainfall (Karrou & Nachit 2015). Seedling growth begins with the emergence of the first leaf above the soil surface. Several, normally three leaves develop in the seedling stage (GS 00-20). The next growth stage (GS 20-30) is tillering. Tillering is a very important growth stage because tillers contribute importantly to wheat yield due to their

developing grain bearing heads. The following growth stages, stem extension (GS 31-45), booting (GS 45-50), heading - flowering (GS 50-69) and ripening (GS 70- 100) are depicted on the Zadoks scale (Figure 2.1) (Zadoks *et al.* 1974).

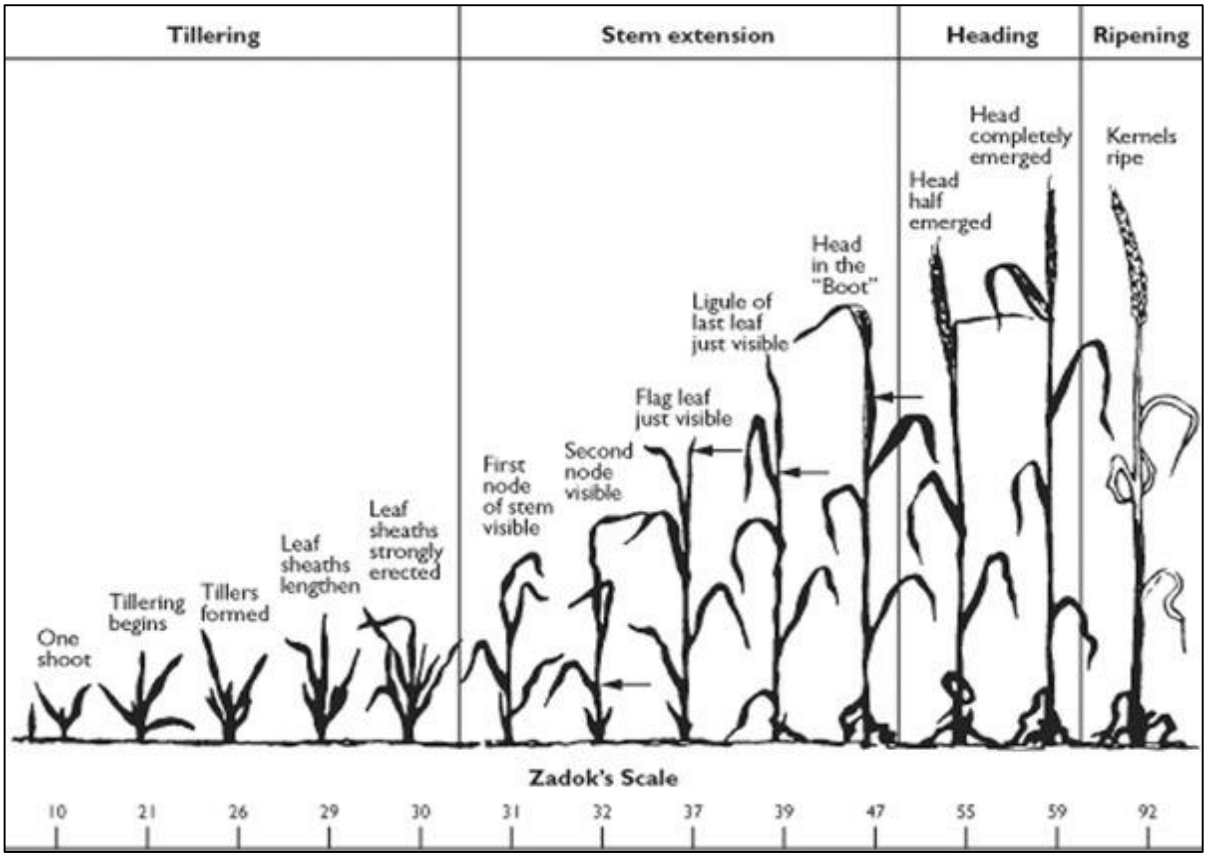

Figure 2.1. Cereal growth stage scale, for winter wheat. The Zadoks scale (Zadoks *et al.* 1974) has been adopted to describe wheat plant growth stages (GS) throughout this thesis.

2.1.3 The SavRia winter wheat population

A doubled haploid population of winter wheat (*Triticum aestivum*) derived from the *F1* cross Savannah x Rialto (SavRia) is used in this study as a model plant population. The SavRia population is comprised of 132 doubled haploid genotypes. The Savannah parent is a semi dwarf UK winter wheat, with potential for high yield that is suitable as a feed wheat. The Rialto parent is also a semi dwarf UK winter wheat, which was commercially successful and can be used in bread making processes (Atkinson *et al.* 2015). The SavRia doubled haploid population was created as a breeding population by the

crop genetic department at the John Innes centre (Germline obtained from Simon Griffiths 2012).

2.1.4 Creating doubled haploid populations

A doubled haploid (DH) is a genotype formed when haploid cells undergo chromosome doubling, taking plants to a homozygous (2n state) without traditional self-pollination techniques (Laurie & Bennett 1988, Suenaga 1994). A population of doubled haploid genotypes is generated from an *F1* cross using the maize wheat technique (Suenaga 1994), whereby maize pollen is used to pollinate a wheat recipient. The wheat plant is then later treated with auxin to maintain the embryo, followed by a colchicine treatment to double the chromosome complement (Laurie & Bennett 1988; Barnabas *et al.* 1999).

Doubled haploids are genetically homozygous inbred plants, which can be produced very quickly compared with inbreeding via back crossing and self-pollination (Suenaga 1994). After up to eight generations of inbreeding the homozygosity of a winter wheat line will be 99.6% (Barkley *et al.* 2012), whereas doubled haploid lines have 100% homozgosity and can be developed relatively quickly (Figure 2.2) (Barkley *et al.* 2012). The 100% homozgosity in a DH line reduces genetic variation allowing associations between insect performance traits and genetic variation in the host plant population to be more precisely investigated. These doubled haploid mapping populations represent replicable and persistent populations that can be used for replicated studies across space and time. This enables breeders to target desirable agronomic traits for wheat improvement strategies.

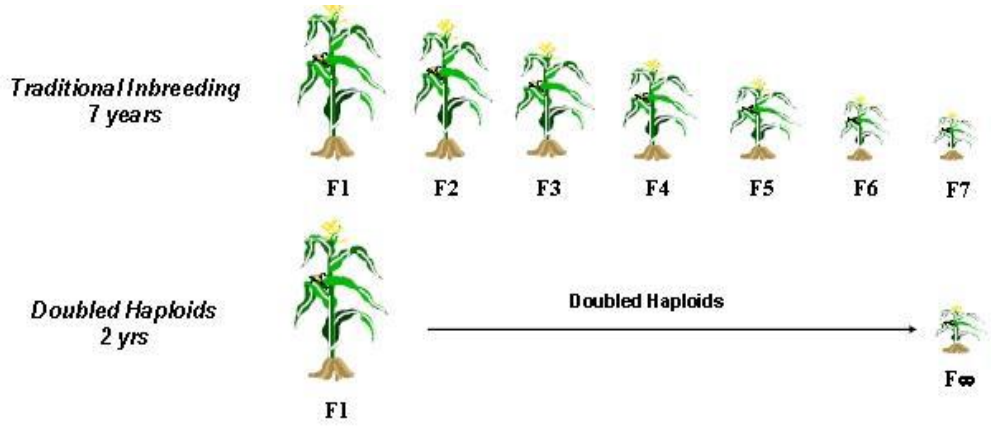

Figure 2.2. A comparison of time investment between traditional inbreeding techniques using single plant selection for backcrossing or self-pollination for 7 generations, compared with producing a doubled haploid line.

2.1.5 The nitrogen use efficiency of the SavRia population

Nitrogen use efficiency is a term used in a variety of ways to describe the ability of a plant to utilise nitrogen resources to increase its productivity. The term nitrogen use efficiency, is used in this thesis as a term integrating its two specific components of nitrogen uptake and nitrogen utilisation efficiency (Moll *et al.* 1982, Gaju *et al.* 2011*)*. The most important variables in determining nitrogen use efficiency in a wheat crop are total nitrogen uptake, nitrogen utilisation efficiency, percentage grain nitrogen, grain yield and nitrogen supplied in the soil and applied as fertiliser (Barraclough *et al.* 2009). The measures of nitrogen efficiency that are used extensively in this thesis are shown in Table 2.1.

Table 2.1. Nitrogen use efficiency and its component traits nitrogen uptake and utilisation efficiency, their abbreviations and units. The term available nitrogen in this context refers to the amount of nitrogen measured in the soil in addition to the amount of nitrogen applied as fertiliser in kg N ha^{-1} (Moll *et al.* 1982, Gaju *et al.* 2011).

Term	Abbreviation	Units
Nitrogen uptake efficiency	NupE	kg above ground plant N content kg^{-1} N available
Nitrogen utilisation efficiency	NutE	kg dry matter grain kg^{-1} AGN content in the plant at harvest
Nitrogen use efficiency	NUE	kg dry matter grain kg^{-1} N available

Nitrogen uptake efficiency is the amount of nitrogen that is taken up into the above ground plant tissues excluding grain and flowers. It can be measured at different growth stages including at harvest (Moll *et al.* 1982, Gaju *et al.* 2011). The *nitrogen utilisation efficiency* is defined as the amount of grain dry matter yield per unit nitrogen taken up by above ground (AG) plant tissues at harvest (Moll *et al.* 1982, Gaju *et al.* 2011). *Nitrogen use efficiency* integrates these two components and is defined as the amount of dry matter grain yield per unit nitrogen in the soil and applied as fertiliser (Moll *et al.* 1982, Gaju *et al.* 2011). The nitrogen use efficiency and its components are calculated and expressed in units following Moll *et al.* 1982 & Gaju *et al.* 2011 (Table 2.1).

2.1.6 Calculating nitrogen use efficiency

The equation used to calculate nitrogen uptake efficiency from plant nitrogen content was: total above ground plant nitrogen content at harvest (kg AG N ha^{-1}) ÷ total nitrogen supply (soil nitrogen content + total volume nitrogen applied as fertiliser (kg N ha^{-1}) (Moll *et al.* 1982, Gaju *et al.* 2011).

The nitrogen utilisation efficiencies of the SavRia genotypes were calculated using the equation: Grain yield dry matter (kg ha^{-1}) ÷ plant nitrogen uptake at harvest (kg AG N kg^{-1} N ha^{-1}) (Moll *et al.* 1982, Gaju *et al.* 2011).

The nitrogen use efficiencies of the SavRia genotypes were calculated using the equation: Grain yield (kg ha^{-1}) ÷ total nitrogen supply (soil nitrogen content + total volume nitrogen applied as fertiliser (kg N ha^{-1}) (Moll *et al.* 1982).

A recent study by Gaju *et al.* (2011) quantified the components of nitrogen use efficiency for the parents ('Savannah' and 'Rialto') of the SavRia winter wheat population under different nitrogen treatments at four field sites; a parallel field trial quantified the nitrogen use efficiencies for the entire SavRia population (JIC archives 2011-unpublished data provided by Luzie Wingden & Simon Griffiths). There was significant variation in nitrogen use efficiency and its components of nitrogen uptake and utilisation efficiency between genotypes in the SavRia population. The SavRia genotypes 31 and 46 had the highest and lowest nitrogen uptake efficiencies respectively, while the other seven SavRia genotypes spanned the range in nitrogen uptake efficiency (Table 2.2).

These SavRia genotypes were selected to capture variation in nitrogen uptake, nitrogen utilisation and nitrogen use efficiency, within the SavRia population. SavRia genotypes with similar phenotypes (days till anthesis date) were selected to control for genetic background. Except for when the whole SavRia population was used for QTL analysis, the subset of nine genotypes used for experiments investigating components of nitrogen use efficiency were also selected for high allele similarity, based on Euclidian distance (Data supplied from John Innes archives by Luzie Wingden 2011). This protocol was adopted to control for genetic background, so the component traits of nitrogen use efficiency could be more easily compared with aphid performance.

Table 2.2. Traits of the SavRia genotypes being used for experiments, genotypes ordered by decreasing nitrogen content, data supplied by JIC field trials (2011). Abbreviations; DTAD: days till anthesis date; nitrogen content (gN kg Biomass^{-1}).

SavRia genotype	N-content (gN kg BM^{-1})	Height (cm)	DTAD (d)
46	10.15	81.08	35.41
25	11.05	81.66	38.41
92	11.32	80.81	37.5
SAV	11.51	-	38.25
13	11.53	74.81	35.75
12	11.77	90.53	40.41
RIA	11.89	-	36.08
59	11.92	80.15	39.25
43	11.96	77.84	35.91
90	12.33	87.02	35.16
31	12.47	60.98	35.75

There are many different plant traits that contribute to the component traits of nitrogen uptake and nitrogen utilisation efficiency in wheat (Barraclough *et al.* 2009, Karrou & Nachit 2015). For example, variation in root architecture and root mass can contribute to differences in a plants ability to take up nitrogen into somatic tissues (Xu *et al.* 2012). The nitrogen utilisation efficiency of plants can be influenced by differences in the glutamine synthetase activity; this is an enzyme involved in catalysing the condensation of glutamate and ammonia to glutamine, which is essential for the nitrogen metabolism in wheat (Eisenberg *et al.* 2000). The remobilisation of nitrogen as amino acids post anthesis is a salient factor contributing to the nitrogen utilisation efficiency of wheat (Bernard & Habash 2009). These different traits

are heritable and may have contributed to variation in the components of nitrogen use efficiency in the SavRia population. Furthermore, these traits can interact with nitrogen application rate (Chardon *et al.* 2010).

2.1.7 The SavRia population as a model species

The doubled haploid population of SavRia winter wheat (*Triticum aestivum*) and the genetic data available make SavRia a good model population for identifying and locating genetic markers in winter wheat that are associated with aphid performance (Wilkinson *et al.* 2012). The variation in the components of nitrogen use efficiency identified between genotypes within the SavRia population makes it an ideal model for quantifying how aphid populations respond to novel genotypes varying in nitrogen use efficiencies. The SavRia population and the grain aphid *S. avenae* will be used as model organisms to address the question: are wheat genotypes with higher nitrogen use efficiencies more susceptible to cereal aphid infestations?

2.1.8 Aphid control using insecticides is not a sustainable solution

Insecticide resistance in aphids has been observed for *Myzus persicae,* which has developed knock down resistance to pyrethroids (Foster 2011). The mechanism of resistance is a base pair change which has affected the sodium gated pathway resulting in knock down resistance to pyrethroid insecticides (Foster *et al.* 2011). The common insecticides for aphid control in the UK are pyrethroid based, they are commonly used to control aphids and the spread of associated viruses (Foster *et al.* 2011). The first observation of pyrethroid resistance in *S. avenae* was recorded in 2011 in East Anglia in the UK; this resulted in the spread of BYDV after pyrethroid application (Foster *et al. 2014*). This sort of insecticide resistance developing in insects is a facet of the selection pressure caused by insecticide use in monoculture cropping systems, highlighting the need for more holistic integrated pest management techniques. This will include discovering genes in plant populations that are associated with aphid resistance, which can be used for breeding plants with increased resistance to insect pests.

2.1.9 Aphid host plants in relation to plant resistance and nitrogen

Aphids have a shared evolutionary history with higher plants. Aphids evolved ~280 million years ago in the early Permian, and species radiated with the evolution of Angiosperms in the Cretaceous period, about 135 million years

ago (Peccoud *et al.* 2010). This shared history has led to myriad strategies for aphids to exploit host plants and subsequent plant adaptations for defence against aphids. Host plant resistance to aphids is highly complex; entomologists have identified two broad modes of resistance, antixenosis and antibiosis. Antixenosis affects the behaviour of insects, altering primary infestation by influencing insect preference (Powell *et al.* 2006). Antibiosis influences the fitness and reproduction of insects while feeding on a selected plant (Dogimont *et al.* 2010). In wheat, aphid resistance based on antixenosis can be due to morphological features such as trichomes and surface waxes (Zarpas *et al.* 2006, Wojcicka 2015). Antibiosis based resistance to aphids has also been observed in wheat plants; wheat can produce secondary metabolites, such as benzoxazinoids that reduce aphid population growth rates (Meihls *et al.* 2013). Plants that are resistant to aphids can negatively influence the settling and reproduction of aphids (Powell *et al.* 2006). Plants which are susceptible to aphids do not limit settling, post settling fecundity or population growth rates (Powell *et al.* 2006, Meihls *et al.* 2013). Therefore, nutritional quality of susceptible host plants could play a key role in determining aphid settling and population growth (Karley *et al.* 2002).

The nutritional quality of aphid host plants is another attribute that can determine aphid performance in terms of fecundity and population growth (Karley *et al.* 2002, Awmack & Leather 2002). Aphids are haustellate insects that have specialised mouthparts called stylets, which they insert into plant tissue to locate sieve tubes and feed on the sap, containing sugars and amino acids (Dixon 1973, Hale *et al.* 2003). This mode of feeding makes aphids particularly sensitive to changes in the nitrogen concentration in plant phloem (Nowak & Komor 2010).

Aphids actively track the most readily assimilated nitrogen source on the plant. Flush feeders colonise leaves in the early growth stage of plant phenology to access the highly mobile nitrogen in the new growth. Senescence feeders colonise and feed on leaves that are starting to senesce and are remobilising nitrogen from leaves into other plant tissues (Dixon 1998). Plants can have different concentrations of nitrogen in the phloem due to nitrogen fertiliser application (Nowak & Komor 2010). Plants with different nitrogen utilisation efficiencies can have different concentrations of amino acids in the plant phloem (Eisenberg *et al.* 2000). Aphid fecundity, has been shown to increase in response to higher nitrogen application rates in the laboratory (Aqueel & Leather 2011) and also increases in response to

amino acid concentration in the plant phloem (Karley *et al.* 2002, Nowak & Komor 2010).

2.1.10 The salient factors for measuring aphid performance

The salient factors for measuring performance of aphids include *development time* (***d)*** in days from birth to reproduction, the *fecundity*, the number of progeny produced during a time equal to the development time (***Md*** *)*and a measure of population growth of aphids (Lamb *et al.* 2009). The *intrinsic rate of increase* is an integration of development time and fecundity forms an important factor for assessing aphid performance on plants. The intrinsic rate of increase is a theoretical rate measured empirically and shows how many aphids are added to the population by each parthenogenetic individual per unit of time. It is estimated using the equation $\boldsymbol{r_m}$ = 0.738(ln ***Md/d***). (Birch 1948, Wyatt & White 1977). Aphid populations can grow monotonically and this pattern of population growth lends itself to identifying *maximum abundance*, which is a good measure of the population (Honek, Jarosik and Dixon 2006). Other important factors for monitoring aphid infestation on wheat crops are *the number aphids on each wheat tiller* (Honek 1999), and *the population growth rate* of aphids (Xing *et al.* 2003).

2.1.11 Cereal aphids that were used as model phloem feeders

To identify and locate genetic markers in the SavRia population of winter wheat that are associated with aphid performance the grain aphid *Sitobion avenae* (Fabricius) and the rose-grain aphid *Metopolophium dirhodum* (Walker) were used. Both species are classified in the insect order Hemiptera, the sub-order Sternorrhyncha and the super family Aphidoidea.

The *S. avenae* stock population was sourced from Professor Angharad Gatehouse, in the School of Biology at University of Newcastle on January 2012. The aphid stock population was reared and maintained on oat plants (*Avena sativa* cv. Dula) in a controlled environment (L:D 16:8, 250 μmol m^{-2} s^{-1} , 20 ^{o}C, humidity 48%). The *M. dirhodum* population used for quantative trait loci analysis, was started from wild individuals collected from Barley plants in the John Innes greenhouse (*s*-69) in June 2013.

Both species, *M. dirhodum* and *S. avenae* are considered serious pests on cereals due to the reduction in yields that are caused during their asexual phase in the spring and summer time (Ankersmit & Carter 1981; Maudsley

1996). The cereal aphid *S. avenae* is considered the more serious pest due to the larger reduction in yield it can cause (Ankersmit & Carter 1981, Watt *et al.* 1984). *Sitobion avenae* is the focus of the experiments described in this thesis because it is still considered the more serious pest on wheat (Foster *et al. 2014*). *Sitobion avenae* preferentially feeds on wheat ears but also feeds from the leaves, where it can begin colonisation of the plant; this life history lends itself to experiments at different cereal growth stages (Wratten 1975). The nitrogen utilisation efficiency of wheat, a main component of nitrogen use efficiency is influenced by plant traits that can be functioning during grain fill when *Sitobion avenae* is feeding from the ripening grain (Ankersmit & Carter 1981).

The monophagous *S. avenae* (Figure 2.3) has a cosmopolitan distribution, feeding and reproducing on grasses in the family Poeceae (Helden 2002).There is no host plant alternation in *S. avenae*; the species feeds, reproduces and overwinters on grasses. In the UK *S. avenae* can develop both sexually as well as asexually throughout the year, dependent on winter conditions. As a crop pest *S. avenae* infests commercially cultivated cereals, which include wheat (*Triticum aestivum*) (Wratten 1975, Foster *et al. 2014*).

Figure 2.3. An apterous *Sitobion avenae* adult feeding on a winter wheat leaf.

The rose grain aphid – *M. dirhodum* (Figure 2.4) alternates between host plants, overwintering as eggs on *Rosa* species that hatch at budburst in early spring (Leather 1993). After a small number of generations on *Rosa spp.* alate (winged) *M. dirhodum* migrate to species of grasses and cereals including *Triticum aestivum* (Ankersmit & Carter 1981; Leather 1993). The rose grain

aphid *M. dirhodum* is a leaf feeder, feeding initially on the young green leaves of wheat following colonisation before moving on to higher leaves and eventually feeding on the flag leaf as other leaves senesce (Ankersmit & Carter 1981; Leather 1993). The general life cycle of aphids showing the different aphid forms and their sexual cycle is displayed in Figure 2.5.

Figure 2.4. An apterous adult rose-grain aphid *Metopolophium dirhodum* on a wheat leaf.

2.1.12 The life cycle of cereal aphids in relation to the developmental stages of wheat

The life cycle of *S. avenae* includes a generation of sexual individuals that produce eggs during autumn, which can overwinter on grasses and cereals (Helden 2002). In contrast, the host alternating *M. dirhodum* produces eggs that overwinter on *Rosa* species as part of its winter cycle (Figure 2.5). The approximate timing of the winter wheat lifecycle at key growth stages is shown with reference to the aphid life cycle (Table 2.3).

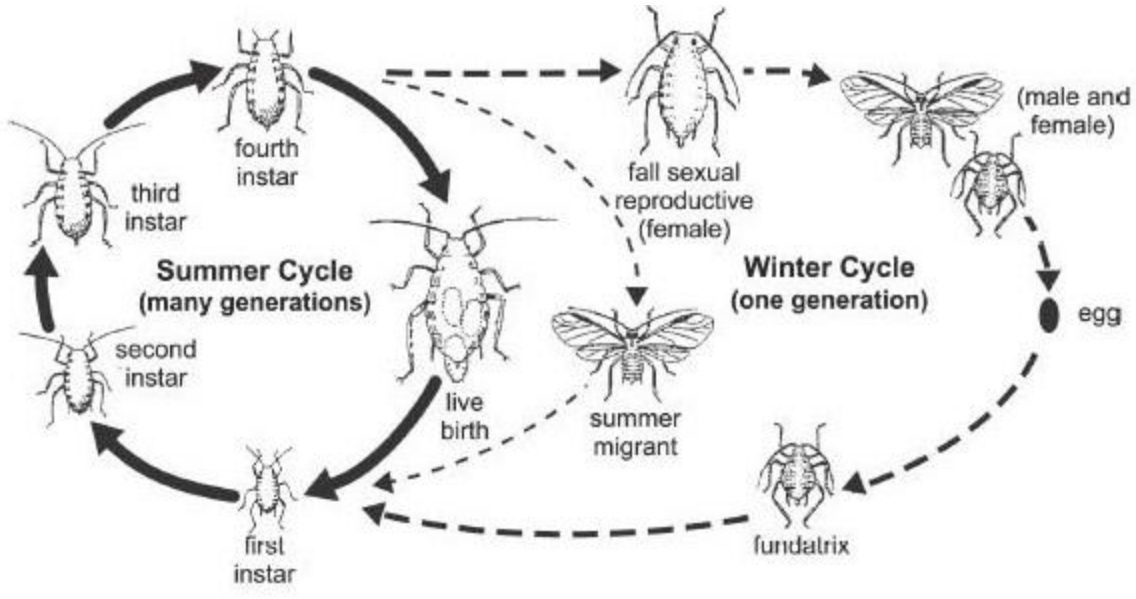

Figure 2.5. General life cycle of aphids. Asexual reproduction occurs during most of the year (summer cycle). The asexual summer cycle for both *S. avenae* and *M. dirhodum* will be on grasses and cereal crops.

Table 2.3. The approximate timing of events in the lifecycle of winter wheat and cereal aphids in the UK, GS is the growth stage on the Zadoks scale (Zadoks *et al.* 1974).

Month	Wheat life cycle	Aphid life cycle
Jan	Tillering GS 21	Eggs & vivipara over winter on grasses
Feb		
Mar	Leaf emergence on main stem continues GS 22-30	Eggs hatch
Apr	Stem elongation and storage GS 31	Asexual reproduction on grasses and cereals
May	Ear formation begins mid may GS 39-50	Infests winter wheat leaves and ears
Jun	Ear emerged above flag leaf GS 59/ flowering GS 61	
July	Grain set GS 61-70, grain filling GS71-80	Infests ears and grain
Aug	Grain ripening GS 79-92	Migration from the crop as sexual forms
Sep		
Oct	seeds sown GS0 - GS10	Eggs & vivipara over winter on grasses
Nov	root growth and leaf production GS10	
Dec	tillering begins	

Increasing our understanding of how genetic variation in winter wheat is associated with aphid performance traits could help identify novel genes that infer a difference in aphid performance. Some genes causing reduced performance could be incorporated into marker assisted breeding with the aim of breeding wheat varieties with increased aphid resistance. Plant resistance to insects is a key part of integrated pest management programs, which can reduce pesticide application contributing to the more sustainable production of wheat. Conversely quantifying changes in aphid performance on plants with different nitrogen use efficiencies can be used to identify if breeding to increase nitrogen use efficiency in wheat increases the potential for infestation by aphids.

3 Quantitative trait loci (QTL) mapping performance measures of the cereal aphids: *Metopolophium dirhodum* & *Sitobion avenae* on the Savannah x Rialto doubled haploid population

3.1 Introduction

Aphids are insect herbivores which feed on plant phloem by inserting their stylet into a plants sieve element to feed from nitrogenous compounds in the plant phloem (Nowak & Komor 2010). Several species of aphid are important crop pests on the cereals of the world (Tagu *et al.* 2008). By feeding on plants cereal aphids can dramatically reduce crop yields due to the drain on nutrients from the plant phloem (Kolbe & Linke 1974), moreover they can also act as a vector for plant viruses, such as Barley yellow dwarf virus (BYDV) that infect cereal crops (Kurppa *et al.* 1989). Cereal aphid infestation combined with virus infection can significantly decrease grain yield (Kieckhefer & Gellner 1992). Pesticide application is commonly used by wheat growers to control aphid outbreaks, growers apply pesticide to reduce the aphid infestation and stop the spread of plant viruses (McKirdy & Jones 1996, Foster *et al.* 2014). However, as insects become more resistant to insecticides (Foster *et al.* 2014), and chemical control continues to have many undesirable effects on the environment, such as killing non-target species; it is important to find novel methods of controlling aphid infestations to incorporate into integrated pest management (IPM) programs (Nyaanga *et al.* 2014). Host plant resistance is a key element of IPM strategies for managing aphid infestations, and can significantly decrease crop production costs for growers (Griffiths 1990, Nyaanga *et al.* 2014).

One approach for incorporating aphid resistance into wheat breeding populations is to determine which genes are influencing aphid performance measures. For this approach to be successful a genetic map of the plant population must be created. There are several different sorts of plant populations commonly created to be used for genetic mapping. The most common types of mapping population are near isogenic lines, recombinant inbred lines, backcrossed populations and doubled haploid (DH). The trait to be mapped and the marker system being used will influence which type of mapping population will be most suitable (Kertesz *et al.* (1991); Jones *et al.* 1997; Mohan *et al.* 1997).

The purpose of creating a doubled haploid line is to capture enough genetic variation from the initial parental *F1* cross to develop new varieties. Doubled haploids are genetically homozygous inbred plants, which can be produced very quickly compared with inbreeding via back crossing and self-pollination (Suenaga 1994). This widely used technique significantly reduces the time to develop new varieties (Kertesz *et al.* 1991; Suenaga 1994). The 100% homozgosity in a DH line makes marker trait association studies and quantitative trait loci analysis much more accurate and efficient at locating genes coding for desirable agronomic traits. These doubled haploid mapping populations represent persistent populations that can be used for replicated studies across space and time. The SavRia DH line used for this study is described in Chapter 2.

Marker assisted selection (MAS) is a technique for selecting phenotypes based on the genotype of a marker (Young 1999, Doerge 2002). For example the genetic markers associated with desirable traits will be used in the creation of new varieties which express these traits, so phenotypes can be predicted based on their genotype (Mohan *et al.* 1997). Gene pyramiding is a technique used to assemble multiple desirable genes that contribute agronomic benefits into a single genotype. Gene pyramiding is now a commonly used technique in MAS breeding programs.

To utilise MAS, molecular markers are needed, which are then used to construct a genetic map (Young 1999). The most common molecular markers used for MAS and quantitative trait loci (QTL) analysis are single sequence repeats (SSR), random assorted polymorphic DNA (RAPD), and single nucleotide polymorphisms (SNP). These and others are described in more detail by Mohan *et al.* 1997. Single nucleotide polymorphic (SNP) microsatellites were used as molecular markers for this study, and are single base pair changes in the DNA sequence. These SNP markers are neutral, meaning they do not code for variation in a phenotype, and will not affect 'fitness' of an individual; they are not subject to the process of natural selection (Mohan *et al.* 1997). The SNP markers are scored using a fluorescent signal that shows one or other (homozygote) or both markers (heterozygote) are present in a genotype. Once the plant population has been genotyped for genetic markers then the recombination frequency between each pair of genetic markers can be estimated using data from successive generations of the plant population. Genetic markers and their recombination frequencies are used to construct a genetic map.

A genetic map is a representation of the order of molecular markers along the chromosomes derived from two different parents (Jones *et al.* 1997). The genetic map is constructed using algorithms that calculate the

most probable order of markers along the chromosome and their relative distances from each other based on recombination frequency (Lorieux 2012). The position of markers along a chromosome and their distance from each other are usually measured in centiMorgans (cM). A centiMorgan is a theoretical unit to measure genetic distance, which are determined by the frequency of recombination between pairs of marker loci; markers are one centiMorgan apart if the probability of recombination between them during crossover events at meiosis equals one percent (Alberts *et al.* 2002).

An analogy for genetic mapping used by Collard *et al.* (2005), and adapted here is to consider the chromosomes of an organism as straight pieces of road with dashed white lines painted on the tarmac. These white lines represent the genes along a chromosome, and these genes code for traits expressed in the phenotype; such as height. For example, we do not know which combination of these white lines are the genes that code for height in the phenotype. Along this imaginary road are sign posts. The road signs are analogous to molecular markers; they do not code for traits expressed in the phenotype. We can imagine the long straight road with many white lines (genes), and every so often there is a road sign (molecular marker), we know how many road signs there are, their most probable order and the distance between them. We do not know how many white lines (genes) are between the road signs, but the more road signs there are and the closer they are together the higher the resolution for detecting marker trait associations.

If a genetic map is constructed using many markers that are close together it is considered to be a dense map, and will be more powerful for QTL detection. With so many marker arrays available modern genetic mapping studies are likely to be population limited (small plant populations) rather than marker limited (fewer markers or far apart along a chromosome) , although plant population sizes of only 50 can be used in preliminary studies (Tanksley 1993, Mohan *et al.* 1997).

Constructing a genetic map from SNP molecular markers has become more accessible to researchers and breeders, due to the availability of molecular marker arrays for wheat (*Triticum aestivum*) that have been made available to the public (Wilkinson *et al.* 2012). A genetic map can be constructed for a mapping population once the SNP markers have been genotyped; by scoring each individual in the population for SNP markers as either A or B (homozygotes) or AB (heterozygotes). To construct a genetic map using SNP molecular markers involves calculating the most probable order of molecular markers and their relative distances from each other along a chromosome. This is done by estimating the

recombination frequency between each pair of marker loci (Jones *et al.* 1997). Estimating recombination frequencies to infer the most probable order of the marker loci and their relative distances along a chromosome is done using an ordering algorithm available in genetic mapping software such as MapDisto (Lorieux 2012). A more comprehensive explanation of the various algorithms available for ordering linkage groups on a genetic map is published by Wu *et al.* (2011).

Recombination frequencies can have a value between 0 (complete linkage) and 0.5 (free recombination) (Jones *et al.* 1997). Complete linkage is when the marker loci are linked, and will have a high probability of being inherited together, because they are close together on the chromosome or in a conserved gene complex that will not be broken apart during meiosis when chiasma occurs during crossover of the chromosomes. Free recombination is observed when marker loci are not linked and are more likely to recombine randomly during crossover; this is indicative of the marker loci being further apart along the chromosome and therefore less likely to be inherited together (Jones *et al.* 1997). This is because the probability of chiasma between the two marker loci along the chromosome is proportional to the distance between these loci (Jones *et al.* 1997).

Genetic mapping software calculates recombination frequencies between each pair of marker loci, to allow the order of the molecular markers along a chromosome and their relative genetic distance in centiMorgans to be inferred (Lorieux 2012). Once a genetic linkage map has been constructed for a mapping population and the individuals within the population have been phenotyped for the trait, quantitative trait loci (QTL) analysis can be used to identify putative genes that are coding for this trait (Zeng 1994).

Many desirable agronomic traits such as yield (Tzarfati *et al.* 2014), ear emergence (Griffiths *et al.* 2009) and disease resistance are controlled by many genes. This is true for more complex traits such as disease resistance and insect performance (Hickey *et al.* 2012; Kloth *et al.* 2012). These genes will segregate in the progeny showing a continuous expression of a trait. For example the trait height is expressed in the phenotype and can be measured on a quantitative scale rather than just short or tall as one would expect for single gene inheritance following classical Mendelian segregation (Jones *et al.* 1997). These traits are known as *quantitative traits*, and the genomic region that codes for these traits are known as quantitative trait loci (QTL). QTL are detected using a statistical approach to identify variance in the molecular markers on a

genetic map that are associated with variation in the phenotypic data evaluated for the mapping population (Zeng 1994). The position of the molecular markers along a chromosome mapped on to a genetic map are used to locate QTL (Tanksley 1993; Jones *et al.* 1997). The premise is that variance in the marker loci that are associated with variation in the measured phenotypic trait are identified using a statistical model, with significant marker loci and trait associations above a predetermined significance threshold indicating the QTL position along a chromosome (Zeng 1994).

The commonly used statistical models for QTL mapping are single marker analyses using ANOVA, one of the simpler statistical models. The single marker ANOVA detects trait value differences between different marker classes and is a robust model for locating QTL (Rebai *et al.* 1995). Although single marker analysis is robust for locating QTL it is more appropriate as a first quick scan of all the chromosomes to check the data file and locate marker trait associations. It is then appropriate to supplement it with a more powerful statistical approach such as interval mapping (IM) or composite interval mapping (CIM). The interval mapping approach by Landere and Botstein (1989) is an extension of single marker analysis but more precise; this approach analyses each marker interval at a time to construct a putative QTL based on likelihood ratio tests. A drawback with the IM approach is having to consider one marker interval at a time. Estimating QTL location is biased if multiple QTL are located on a chromosome leading to an underestimate of effects and IM may not determine all QTL positions (Zeng 1994).

The IM method was extended by Hadley and Knott (1992) by replacing likelihood ratio tests with a regression model simplifying the IM model and improving precision for QTL location. The biased estimation remains however if more than one QTL is located along the chromosome. To overcome this drawback Zeng (1994) developed the composite interval mapping (CIM) approach. Composite interval mapping (CIM) combines IM with a multiple regression model which is much more powerful and precise for detecting QTL, and determining the most probable position. When testing an interval for QTL CIM reduces residual variance caused by other markers by including them as co-variants in the model, by fitting partial regression coefficients to background markers to account for variance caused by non-target QTL. The CIM approach therefore removes biased estimates of IM when detecting multiple QTL along a chromosome (Silva *et al.* 2012; Zeng 1994). The QTL model will also describe the percentage of variation in a trait that is explained by the genetic variance for each QTL position identified, as well as the direction of effect; i.e.

whether the QTL is contributing positively or negatively to the phenotypic trait (Tanksley 1993; Zeng 1994). The statistical threshold value for locating QTL is known as logarithm of odds (LOD) values.

In the past LOD values of 2 - 3 were arbitrarily used as a significance threshold, and marker trait associations that exceeded the LOD threshold score of 3 were considered significant for determining QTL positions (Churchill & Doerge 1994). More recently the LOD threshold for significant QTL positions can be calculated for each trait in the analysis using permutations (Churchill & Doerge 1994). This technique is now widely accepted, and studies for publishing QTL locations routinely use 500-1000 permutations with a significance threshold of $P<0.05$ (Churchill & Doerge 1994; Meihls *et al.* 2013). The LOD profile for each trait is plotted on the Y-axis against the molecular marker position along the chromosome on the X-axis. The peak of the LOD profile, if exceeding the predetermined statistical threshold indicates the most probable location of a QTL associated with changes in the phenotypic trait. The percentage of variation in the trait that is explained by the QTL is reported as r^2 in the model. An r^2 value of >10% represents a QTL has major effects, and r^2 values of <10% are considered to represent minor QTL (Lander & Kruglyak 1995). The QTL that are described as having minor effects may be environmentally sensitive or be components of complex traits that are controlled by several QTL, such as those associated with disease and insect resistance (Hickey *et al.* 2012; Kloth *et al.* 2012).

There have been many QTL studies on desirable agronomic traits in cereals, with yield components receiving a lot of attention (Tzarfati *et al.* 2014*;* Azadi *et al.* 2015). The physiological characteristics of wheat that influence yield (Sukumaran *et al.* 2015), crop quality (Deng *et al.* 2015), ear emergence (Griffiths *et al.* 2009), environmental adaptation and resilience have also been widely studied (Snape *et al.* 2007; Bai *et al.* 2013; Azadi *et al.* 2015). Wheat is such an economically important crop that published genetic maps available for several wheat varietal crosses have been made publicly available, making QTL studies accessible to research laboratories without the need to create large genetic marker databases themselves (Wilkinson *et al.* 2012). As serious crop pests of cereals several QTL and genetic mapping studies have been carried out for numerous species of aphids on cereals (Migui & Lamb 2003; Li & Peng 2014; Betsiashvili 2015). Meihls *et al.* 2013 located QTL associated with benzoxazinoids production in maize controlling *Rhopalosiphum maidis* resistance. A QTL associated with *Rhopalosiphum padi* resistance in barley was mapped to chromosome 3H by Cheung *et al.* (2010). Several QTL associated with resistance of *R. padi and Schizaphis graminum* on wheat were mapped by

Crespo-Herrera *et al.* (2014). The complexity and time investment has made locating QTL associated with aphid performance and aphid resistance problematic, because it can compromise investigations for other important agronomic traits. QTL studies for *Sitobion avenae* performance measures on wheat cultivars has received less attention than for other aphid species on different cereal crops (Liu *et al* 2012).

A key aspect of wheat improvement is breeding and developing new varieties with increased resistance to aphids. It is clear that locating genes in wheat that confer a reduction in aphid performance measures can be used for gene pyramiding in MAS breeding programs for wheat improvement (Joshi & Nayak 2010).

Identifying and locating genes that confer a reduction in aphid performance is the rationale for this chapter, these genes can be used in the development of new wheat varieties and can contribute to wheat improvement strategies. Utilising genes in wheat varieties that are associated with reduced aphid performance can decrease production costs to growers, reduce environmental degradation associated with pesticide application and maintain future wheat yields. Maintaining wheat yields, in terms of reducing yield lost to insect pests; is one aspect of wheat improvement intended to positively contribute towards future food security (Meyers *et al.* 2015). The main aim is to identify genetic variation within the SavRia winter wheat population associated with *S. avenae* and *Metopolophium dirhodum* performance measures. The objective of this chapter is to identify and locate genetic markers in the SavRia DH line that are associated with a reduction in the performance measures of *M. dirhodum* and *S. avenae*. These markers and the genes linked to them can be characterised and validated for use in breeding programs as part of wheat improvement strategies.

3.2 Methods and materials

The cultivation techniques for the plants used in each experiment, the aphid performance measures, genetic mapping, and the analysis with preparation of data for the QTL analysis are all described. The DH lines of the Savannah X Rialto (SavRia) wheat population will be described in brief; a more concise description can be found in chapter 2.

There were three experiments conducted to gather data for QTL analysis. They were a laboratory experiment measuring the development time, fecundity and the intrinsic rate of increase for *Sitobion avenae* individuals (LabIND). A laboratory experiment measuring the population performance of *S. avenae* (LabPOP), and a greenhouse experiment (GH) monitoring the population growth of a natural infestation of both *S. avenae and M. dirhodum* (Table 3.1). The aphid traits were all recorded on a population of 94 DH lines that were derived from a cross between wheat cultivars Savannah and Rialto

The laboratory experiment monitoring individual performance of *S. avenae* (LabIND) on 56 SavRia genotypes was a random assortment design. The SavRia genotypes were not replicated in the **LabIND** experiment (Table 3.1). The genes within the SavRia population may however be replicated many times within this subset of plants. The small plant population was of ample size to collect data on individual aphid performance traits cultivated on the plants, which were used for QTL analysis (Mohan *et al.* 1997).

The laboratory experiment that monitored aphid population performance traits (LabPOP) was a random assortment design. There were 94 SavRia genotypes that were replicated three times and randomly assorted into 10 trays (52cm x 42cm x 9cm) of 30 genotypes (Table 3.1). This random assortment design was used for the **labPOP** experiment to control for confounding variables such as the effects of plant positioning within the trays.

The greenhouse (**GH**) experiment was a random assortment design. There were 94 SavRia genotypes that were replicated three times for the greenhouse (GH) experiment (Table 3.1). The greenhouse (**GH**) plant population were arranged in one randomly assorted plot for aphid infestation. Plants were placed in a randomly assorted plot as close together as the pots would allow, to simulate a field plot.

Table 3.1. The three experiments with their abbreviations, environmental conditions, soil type, number of genotypes, number of replicates, the growth stage of the plants and the experimental design. Day length is denoted as number of hours of light (L), and the number of hours of dark (D).

Experiment	Abbreviation	Environment	Substrate	N.O of GTs	Reps	Growth stage	Design
Laboratory: Intrinsic rate of increase	**LabIND**	22°C, 48% humidity L16:D8	John Innes N.O 2 compost	56	1	25-30	Random assortment
Laboratory: population performance	**LabPOP**	22°C, 48% humidity L16:D8	John Innes N.O 2 compost	94	3	25-30	Random assortment
Greenhouse: population growth	**GH**	25°C, 55% humidity L18:D6	John Innes N.O 2 compost	94	3	31-40	Random assortment

3.2.1 Doubled Haploid SavRia population

The SavRia doubled haploid (DH) population was established using the wheat-maize technique (Laurie & Bennett 1988). The SavRia population of 132DH lines was created from the F_1 between Savannah and Rialto cultivars (Snape *et al.* 2007). The Savannah parent is a semi dwarf UK winter wheat, with potential for high yield used as a feed wheat; the Rialto parent is also a semi dwarf UK winter wheat, which may be used for certain bread making processes and the resulting DH population is suitable for QTL analysis of phenotypic traits (Atkinson *et al.* 2015). Of 132DH lines only 94DH lines were used in these experiments, the number of genotypes in each experiment is displayed in Table 3.1 and described under the genetic mapping heading in this method section.

3.2.2 Plants

The seeds for all three experiments were sown 15 mm deep into John Innes N.o.2 (JI N.o.2) compost (Khan & Port 2008), fresh bags were used each time to avoid contamination with pesticides or fertilizer. The seeds for both of the laboratory experiments were sown in to 60 well trays (Dimensions: 52 cm x 29 cm, Cells: 4 cm^2 x 5 cm^2), commercially known as P60 trays. After sowing the plant trays were placed in a controlled environment (Day length 18 h light: 6 h dark, light intensity 290 μmol m^{-2} s^{-1}, Temp 26 °C, humidity 50% ±5%) and grown for 14 days. All the plants grown for all experiments were watered from below as required every 24-72 h. The plant genotypes used in the Laboratory experiments were

vernalised (day length 18 h light: 6 h dark, light intensity 120 μmol m^{-2} s^{-1}, temperature 5 °C ±1 °C , humidity 55 % ±5 %) for 56 days (Brooking 1996).

Following vernalisation the plants for the laboratory experiments were potted in 7*7*8 cm^3 pots of JI N.o.2 compost, and grown in a controlled environment for a further 14days (Day length 18 h light: 6 h dark, light intensity 290 μmol m^{-2} s^{-1}, Temp 26 °C, humidity 50% ±5%), at which time the plant growth stage was 25 – GS25 following the cereal development scale proposed by Zadoks (1974). These plants were transferred to a controlled environment room (Day length 18 h light: 6 h dark, light intensity 250 μmol m^{-2} s^{-1},temperature 22 °C, humidity 48 %) for 7 days to acclimatise before the aphid inoculations.

The plants for the LabIND experiment were placed in 2 randomly assorted trays (52 cm x 42 cm x 9 cm) in two insect enclosures (dimensions: 10 mm thick Perspex, 550 mm high X 530 mm wide, two access windows 210 mm X 210 mm in the front and a 390 mm x 110 mm vent in each side guarded by 50 µm mesh, a 70 mm deep watering tray is housed in the bottom of the enclose) for these 7 days and the subsequent time for measuring individual aphid performance measures.

The plants for the LabPOP experiment were randomly assorted into plant trays (52 cm x 42 cm x 9 cm), of which there were 10, each containing 30 plants. Each of the plants was then put into micro-perforated polypropylene bags (SM570 – 305 mm x 460 mm), which were used to contain the aphids on the plants. Growing wheat plants in these bags reduces air flow around the plant and slightly increases humidity in the plants micro-environment (*personal observation*). These factors combined with the lower light levels in the insectary can lead to an increase in powdery mildew, which can be present in the wider environment potentially infecting plants before bagging them. There is a relationship between the fungus that causes powdery mildew (*Blumeria graminis tritici*) and cereal aphids (Von Burg *et al.* 2012). Therefore, the level of powdery mildew (PM) infection on the plants was recorded as a categorical variable, ranked 0-10; with zero representing no PM present, and 10 representing a severe level of PM infection symptoms.

The plants for the greenhouse (GH) experiment were germinated and grown for 14 days in a controlled environment (Day length 18 h light: 6 h dark, light intensity 290 μmol m^{-2} s^{-1}, Temp 26 °C, humidity 50 % ±5 %), before 56 days vernalisation in a cold room (day length 18 h light: 6 h

dark, light intensity 120 μmol m^{-2} s^{-1}, temperature 5 ^{o}C ±1 ^{o}C, humidity 55 % ±5 %) (Brooking 1996).

Following vernalisation the plants were potted on into 7*7*8 cm^3 pots of cereal mix (40 % peat, 40% sterilised soil, 20% washed horticultural grit 1-5 mm, 1.3 kg m^{-3} base fertiliser -14:16:18 + trace elements (TE), 1kg m^{-3} Osmocote mini 3-4 month – 16:8:11 2MgO + TE, wetting agent, 3 kg m^{-3} Maglime, and 300 g m^{3-1} Exemptor) and grown in a controlled greenhouse for <24 h (natural light, maximum temperature 25 ^{o}C, humidity 55% ±10%). Exemptor is a systemic insecticide mixed into the cereal mix. Plants that were potted into cereal mix containing Exemptor was a mistake for culturing insects on these plants. These plants contaminated with Exemptor were consequently re-potted into 3 litre pots of John Innes N.o.2 within 24 h (Dimensions: Diameter 179 mm^3 across the top. 140 mm^3 at the base. 155 mm^3 high). After 48h in the JI-N.o.2 compost a subset of 5 random plants were bagged in micro-perforated polypropylene bags (SM570 305 mm x 460 mm) and transferred to the insectary controlled environment (L:D 16:8, 250 μmol m^{-2} s^{-1} 22 ^{o}C, humidity 48%), for aphid bioassays to monitor mortality and reproduction.

The plants contaminated with Exemptor were allowed to grow for 56 days in the controlled greenhouse, during which time a random batch of 5 of these contaminated plants were moved into the insectary every week to monitor aphid morality and reproduction. This continued for the 56 days, at which time it was observed that nymphs which had developed into adults with <10% mortality, reproduced, leaving fresh nymphs that could feed and survive on the plants previously contaminated with Exemptor. Therefore, this plant population was deemed suitable to be used in the greenhouse experiment for QTL analysis. It is worth noting at this point that after 56 days in the greenhouse the SavRia population for the GH experiment had been naturally infested with wild populations of two species of cereal aphid, *M. dirhodum* and *S. avenae,* the life cycle and ecology of both species are described in chapter 2.

3.2.3 Aphids

There were three populations of aphids used for the experiments. Both of the laboratory experiments used an indoor reared population of the cereal aphid *S. avenae,* which was used as a model phloem feeder. Aphid stock was sourced from Angharad Gatehouse, Professor of Invertebrate Molecular Biology in the School of Biology at University of Newcastle on

January 2012. The Aphid stock population was reared and maintained on oat plants (*Avena sativa* –cv. Dula) to avoid confounding variables associated with aphids previously feeding on wheat cultivars.

The stock aphid population was housed in Perspex enclosures (dimensions: 10 mm thick Perspex, 550 mm high X 530 mm wide, two access windows 210 mm X 210 mm in the front and a 390 mm x 110 mm vent in each side guarded by 50 µm mesh, a 70 mm deep watering tray is housed in the bottom of the enclosure) in a controlled environment (L:D 16:8, 250 μmol m^{-2} s^{-1} , 20 ^{o}C, humidity 48%). The greenhouse experiment was naturally infested from wild populations of *M. dirhodum* and *S. avenae* that had been feeding on barley plants (*Hordeum sp*) in a controlled greenhouse environment (natural light, maximum temperature 25 ^{o}C, humidity 55% ±10%).

3.2.4 Aphid performance measures

The Laboratory individual performance (**LabIND**) experiment had 56 SavRia genotypes (Table 3.1). Each SavRia genotype had one clip cage (25 mm diameter x 15 mm deep) placed two thirds of the way along a wheat leaf (MacGillivray & Anderson 1957) (Figure 3.1). Three *S. avenae* adults were enclosed in each clip cage. The cages were checked every 24 h until reproduction had begun, at which time each adult was then removed from the clip cages leaving one freshly produced nymph of the same age class (produced within 24 h) in each cage. The aphid performance measures recorded (Table 3.2) were **development time** from nymph to reproduction, the **fecundity** 7 d^{-1}; which was the number of nymphs produced in a seven day period following development time, and the **intrinsic rate of increase** (r_M). The intrinsic rate of increase is a theoretical measure of population growth per capita with no density dependence effects (Wyatt & White 1977). The equation used to calculate intrinsic rate of increase was: $r_m = 0.738(\ln M_d/d)$, where d is the development time in days and M_d is the number of progeny produced in a period equal to d (Wyatt & White 1977).

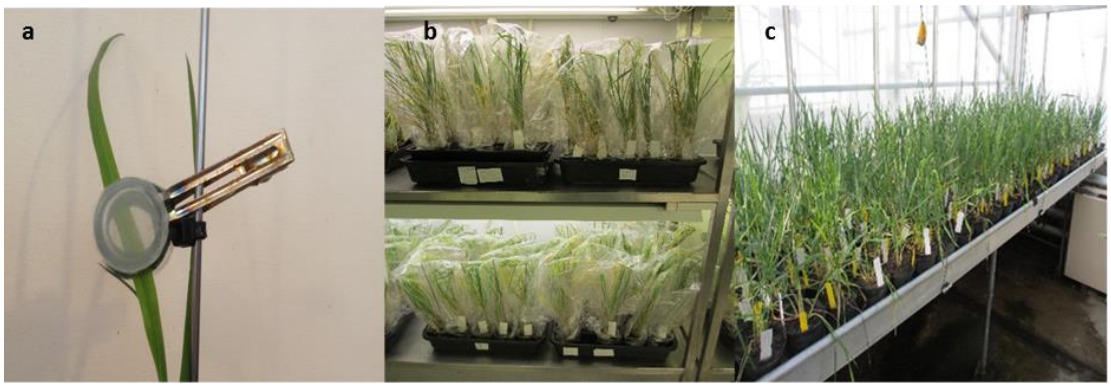

Figure 3.1. **a**). A clip cage (macgilivray & Anderson 1957) used to contain aphids on a wheat leaf for the laboratory experiment measuring individual performance (LabIND), **b**). Plants contained in micro-perforated bags for the laboratory population experiment (LabPOP), **c**). The greenhouse plant population plot.

Table 3.2. The aphid performance measures (traits) from each experiment used in the QTL analysis. The abbreviations for each trait used throughout the results section.

	Aphid performance measures	Abbreviation
Laboratory Intrinsic rate of increase	Development time of *S. avenae*	LIDevtime
	Fecundity $7d^{-1}$	LIFecundity
	Intrinsic rate of increase	LIrM
Laboratory population parameters	Starting population of *Sitobion avenae*	startPOP
	Development time of *S. avenae*	Devtime
	Peak population of *Sitobion avenae*	peakPOP
	Population growth rate	popGR
	Population growth rate unit $time^{-1}$	popGR/T
	Aphid $tiller^{-1}$	aphid/tiller
Greenhouse population growth	Staring population of aphids	GHstartPOP
	Peak population of *Metopolophium dirhodum*	GHpeakPOPMD
	Peak population of *Sitobion avenae*	GHpeakPOPSA
	Peak population *M. dirhodum* & *S. avenae*	GHpeakPOPALL
	Population growth rate	GHpopGR

A population of 300 *S. avenae* adults were established on oat plants (*Avena sativa* –cv. Dula) to produce a new apterous adult population that was used to inoculate the SavRia genotypes used in the laboratory population (**LabPOP**) experiment. After 48h the 300 adults were removed leaving >900 nymphs of the same age class (Produced within 48h). After nine days this nymph population had developed into adults and were beginning reproduction; they will be referred to here as *'A1'* adults. These

S. avenae A1 adults of the same age class were used to inoculate the SavRia genotypes for this laboratory population experiment.

Each SavRia genotype was inoculated with 3 apterous *Sitobion avenae A1* adults. The aphids were contained on SavRia plants in micro-perforated polypropylene bags (SM570 – 305 mm x 460 mm) following Meihls *et al.* (2013). The *A1* adults were removed from the SavRia plants after 72h when all the nymphs produced were counted and left on the plant; this was the starting population and will be referred to as *'N1'* nymphs. This technique for establishing a starting population of nymphs of the same age class reproduced by adults of the same age class on the SavRia genotypes meant that the variable 'starting population' was influenced by the SavRia genotypes rather than maternal effects from other host plants (Dahlin & Ninkovic 2013) or confounded by progeny being produced by founders of a different age class (He *et al.* 2013).

The aphid population performance traits for the laboratory population (**LabPOP**) experiment were; aphid '**starting population**', '**development time**' from nymph to reproduction, '**peak population**', **aphids tiller^{-1}** and **population growth rate** (Table 3.2). Development time for the *'N1'* nymph population was monitored every 24 H and the first nymph produced on each plant was recorded as development time for the aphid population on that genotype. The peak population was a count of all aphid morphs on the plant. The number of aphids tiller^{-1} were calculated by dividing the peak population of *S. avenae* on each plant by the number of tillers on that plant. The population growth rate was calculated using the equation: $\ln(Nt/N0) = rt$; where Nt is the peak population of aphids, N0 is the starting population of aphids, and t is time in days (Odum and Barrett 2005). These were the aphid performance measures in the LabPOP experiment that were the traits used in the QTL analysis (Table 3.2).

The greenhouse plant population was naturally infested with *M. dirhodum* and *S. avenae* from source populations 10 m away from the experimental plant population, which allowed natural infestation by these aphids that had free choice between SavRia genotypes (Moharramipour *et al.* 1997). The plants were infested at GS 31. After the natural infestation of the SavRia population by *M. dirhodum* and *S. avenae* the plants were potted into their final 3ltr pot size (Dimensions: Diameter 179 mm across the top, 140 mm at the base, 155 mm high). The naturally infested plant population was put into a randomly assorted plot in the controlled insectary greenhouse (L:D 18:6, maximum temp: 25 ^{o}C, humidity: 55% ± 10%).

The aphid performance measures were observed and recorded in the insectary greenhouse. The starting population of aphids was counted 7 days after transferring them to the insectary greenhouse at GS31 on the Zadoks scale, and the peak population count was made 10 days later when the plants were at GS40 on the Zadoks scale (Zadoks 1974). The **starting population** was the infestation level of all morphs of both aphid species at the first count, and the **peak population** of aphids was the last count of aphids on the plants. There is a peak population measure for the adults and nymphs of both species of aphids. The **population growth rate** was calculated from these data using the density-independent population growth equation: $\ln(Nt/N0) = rt$; where *Nt* is the peak population of aphids, *N0* is the starting population of aphids, and *t* is time in days (Odum and Barrett 2005). Population growth rate was calculated for all forms and both species of aphid together. These were the aphid performance measures in the greenhouse experiment that were the traits used in the QTL analysis (Table 3.2).

3.2.5 Genetic mapping

The SavRia genetic map was constructed using the software MapDisto (Lorieux 2012) with data downloaded from CerealsDB website (accessed 12/12/14). The SNP database that is published on CerealsDB is publicly and freely accessible, requiring no registration and with no restrictions on use (Wilkinson *et al.* 2012). The SavRia public linkage map was uploaded by the Biological Sciences department at Bristol University and the markers are described and characterised by Allen *et al.* (2012). The 94DH lines used to construct the genetic map are subset of the 132DH lines in the SavRia population; they are not the same 94DH lines that were used in the three experiments. Consequently, there are 23 genotypes with aphid population data that could not be included in the QTL analysis. The reason for selecting 94 lines from the SavRia population was that these lines had been characterised for nitrogen efficiency which was also investigated in this PhD.

The SavRia genetic linkage map used in the QTL analysis was constructed using the Kosambi mapping function in MapDisto version 1.7 (Lorieux 2012). The SavRia genetic map is comprised of 1923 single nucleotide polymorphic (SNP) marker Loci, across 94 doubled haploid Savanah cross Rialto winter wheat genotypes. The marker loci were put into linkage groups and assigned along the 21 chromosomes in the wheat genome (Mayer *et al.* 2014). There were 36 linkage groups, 32 of which were assigned to the correct chromosomes using the Basic local alignment search tool (BLAST). The marker sequences were assigned to correct

chromosomes using the FASTA sequence format through a central repository established by URGI (2015), on behalf of international wheat genome sequencing consortium (IWGSC).

3.2.6 Data analysis

The software package SPSS was used for descriptive statistics and preliminary statistical analysis. A one-way ANOVA was used as a preliminary to test for differences in the mean aphid performance measures between genotypes. A two tailed t-test was used to compare means between each SavRia parent for the aphid performance measures. The correlation matrix was created using Pearson product moment correlation coefficients; the Pearson correlation tested the extent of a linear relationship between traits from all three experiments (IBM SPSS Version 22.0 2013). The R-package V3.02 was used for creating and displaying the histograms for the aphid performance measures used in the QTL analysis (R core team 2014).

A single marker ANOVA was used in MapDisto to identify significant variation in the SNP markers on the SavRia genetic map that were associated with aphid performance traits (Rebai *et al.* 1995). Aphid performance measures were dissected into their component traits which contribute to aphid performance. These component traits were each used in the QTL analysis using composite interval mapping (CIM) to find any correlated gene region within the SavRia plant population that is associated with aphid performance measures (Zeng 1994).

Complex traits such as insect resistance or performance measures are dissected into their component traits because mapping these component traits will reduce false positive results and increase the statistical significance (Kloth *et al.* 2012).The genetic map and aphid performance measures data were exported into the program Windows QTL Cartographer version 2.5 that was used for QTL analysis (Silva *et al.* 2012). The LOD threshold values were set using 500 permutations for each trait with a significance level of 0.05 following Meihls *et al.* (2013). The composite interval mapping (CIM) model was used in Windows QTL cartographer (Silva *et al.* 2012). The program settings were: CIM Model 6: Standard Model, walking speed = 2 centiMorgans, control marker numbers = 5, window size = 10 centiMorgans, regression method = backward regression method (Li *et al.* 2012 & Meihls *et al.* 2013).

3.3 Results

The results are presented in three sections.

- *Section 1:* results from monitoring aphid performance measures in three experiments.

- *Section 2:* the genetic linkage map and single marker ANOVA associations.

- *Section 3:* the results of QTL analysis using composite interval mapping.

3.3.1 Section 1: Aphid performance measures

The aphid performance measures were the traits measured for QTL analysis; there were 14 traits in total from the three experiments (**LabIND**- laboratory experiment used to measure the performance of individual aphids, **LabPOP**- laboratory experiment used to measure aphid population performance traits, and **GH**- greenhouse experiment used to measure aphid population traits in greenhouse conditions -Table 3.2). The number of genotypes, and the number of replicates used in each experiment are given in Table 3.3. The descriptive statistics for each of the traits recorded during each experiment are displayed in (Table 3.3-3.7). Abbreviations used for each of the three experiments, and each aphid performance trait are given in Table 3.2 and in the Glossary section.

Table 3.3. The number of replicates of each genotype in each of the three experiments used to monitor aphid performance measures.

	Number of SavRia genotypes included in each experiment		
Replicate	LabIND	LabPOP	GH
3	0	64	63
2	0	18	20
1	56	10	6
Total number of genotypes	56	92	89

The labIND experiment measured population performance per capita. The traits measured were development time, fecundity 7 d^{-1}, and the intrinsic

rate of increase of *S. avenae*. The SavRia genotypes were not replicated in this experiment (Table 3.3). The SavRia genotypes used in the experiment are aggregated for the descriptive statistics, and the observed values for the Savannah parent are included (Table 3.4).

Table 3.4. Descriptive statistics for the individual performance traits of the aphids measured during the LabInd experiment for the whole SavRia population, and the values for the Savannah parent (n=1); Rialto was not included in the population subset.

	LI-Devtime	LI-Fecundity	LI-(r_M)
Savannah parent			
Values for Savannah (n=1)	8	28	0.31
Lab-IND population			
n	50	44	44
Mean	9.36	26.36	0.262
S.D	1.61	9.67	0.0624
S.E	0.23	1.46	0.0094
Min	7	1	0
Max	16	44	0.35

The 94 wheat genotypes in the LabPOP experiment included both parental cultivars. The remaining 92 SavRia genotypes used in the experiment were replicated 3 times, resulting in 1-3 replicates for aphid performance measures (Table 3.3, 3.5). The LabPOP experiment monitored aphid performance measures as the traits for QTL analysis, both parents Rialto and Savannah were included and replicated 10 times per parent, which resulted in between 7-9 replicates for each trait measured (Table 3.5). The population growth rate per day was significantly different between the parental cultivars Savannah and Rialto (Table 3.5).

There was a significant difference in each aphid performance measure between SavRia genotypes for each trait used in the QTL analysis (Table 3.6).

Table 3.5. Descriptive statistics for the whole SavRia population for all aphid performance traits included in the QTL analysis. The categorical variable: powdery mildew score is displayed but was not included in QTL analysis. The descriptive statistics for each parental cultivar- Rialto and Savannah. The t-test results comparing parental means is included, significant differences between parental means in italics (P<0.05). Data from the LabPOP experiment.

	StartPOP	Devtime	peakPOP	PopGR	popGR/T	Aphid tiller[1]	PMscore
SavRia Population							
n	90	89	92	90	89	92	94
Mean	8.22	7.58	82.31	2.15	0.23	16.43	2.88
S.D	6.63	1.27	69.68	0.77	0.07	14.11	1.18
S.E	0.42	0.08	4.36	0.05	0	0.89	0.07
Min	1	5	1	-1.61	-0.05	0.25	0.5
Max	41	12	319	4.13	0.41	65.5	5
Rialto parent							
n	9	9	7	7	7	7	9
Mean	11.11	7.11	57.43	1.46	0.15	15.13	7.29
S.D	7.72	1.17	48	0.62	0.06	12.9	1.25
S.E	2.57	0.39	18.14	0.24	0.02	4.88	0.47
Min	3	5	12	0.18	0.03	3.4	5
Max	28	9	147	2.08	0.22	36.75	9
Savannah parent							
n	8	7	8	8	7	8	8
Mean	5.75	6.86	65.75	2.15	0.26	19.44	6.63
S.D	4.53	0.38	45.1	1.25	0.05	19.18	1.3
S.E	1.6	0.14	15.94	0.44	0.02	6.78	0.46
Min	2	6	1	-0.69	0.02	0.25	5
Max	15	7	142	2.86	0.34	51	9
t-test SavRia Parents							
d.f	15	14	13	13	12	13	-
t-value	1.77	0.61	-0.34	-1.36	-3.38	-0.51	-
Sig	0.1	0.553	0.736	0.201	0.006	0.615	-

Table 3.6. Preliminary one-way ANOVA results comparing aphid performance traits measured in the LabPOP experiment, and in the greenhouse experiment. Traits preceded by GH denote traits measured in the greenhouse experiment. Significant differences between SavRia genotypes in italics ($P<0.05$) and bold italics ($P\leq0.01$).

Aphid performance trait	sum of squares	mean sum of squares	F	d.f	*P*-value
startPOP	3423.66	60.06	1.573	57,127	*0.05*
Devtime	121.84	2.34	1.992	52,116	***0.01***
peakPOP	609132.16	9824.71	3.379	62,135	***0.001***
popGR	50.13	0.93	2.364	54,119	***0.001***
popGRT	0.41	0.01	2.493	48,98	***0.001***
aphidTILLER	20478.39	330.3	2.266	62,134	***0.001***
Pmscore	751.19	10.58	2.079	71,155	***0.001***
GHpeakPOPMD	2460540.46	40336.73	1.734	61,126	***0.01***
GHpeakPOPSA	43241.8	708.88	1.8	61,126	***0.01***
GHpeakPOPall	2768434.46	45384.17	1.766	61,126	***0.01***

The descriptive statistics for aphid population traits measured in the greenhouse experiment, the peak population of *M. dirhodum* was a magnitude larger than that of *S. avenae* (Table 3.7).

Table 3.7. Descriptive statistics for aphid population traits measured on the whole SavRia population in the greenhouse experiment (GH). Suffix: SA *Sitobion avenae,* MD *Metopolophium dirhodum*.

	GH-startPOP	GH-peakPOPSA	GH-peakPOPMD	GH-peakPOPall	GH-popGR
SavRia population GH					
n	89	89	89	89	86
Mean	43.74	27.97	374.93	402.90	2.39
S.D	30.27	22.14	171.41	180.06	0.83
S.E	1.96	1.43	11.33	11.92	0.054
Min	2	1	66	73	0.51
Max	165	148	1104	1169	4.89

The powdery mildew score (PMscore) was significantly associated with the peak population of *S. avenae*. The powdery mildew score explained 9% of the variation in the peak population of *S. avenae* (n=71, $mean$=2.88, $S.D$=1.18, r^2 =0.09, $P<0.05$) in the LabPOP experiment (Figure 3.2).

Figure 3.2. The powdery mildew score (PMscore) against the peak population of *S. avenae* plant^{-1} (X-axis). These data were from the LabPOP experiment (r^2 =0.09, P<0.05).

The aphid performance measures used as traits in the QTL analysis deviated from normal distribution (Shaprio-wilk normality test *p<0.05*); with the exception of *S. avenae* fecundity in the LabIND experiment (*W = 0.96, p-value = 0.14*), and population growth rate (GHpopGR) in the greenhouse experiment (*W = 0.99, p-value = 0.07)*. The histograms showing data distribution are displayed in Figure 3.3.

Each of the aphid performance measures in each experiment were tested for correlations before being used in the QTL analysis. There were significant correlations between several aphid performance traits (Table 3.8). The intrinsic rate of increase for *Sitobion avenae* was significantly correlated with their development time (r=-0.75, n=50, $P \leq 0.01$) and fecundity (r=0.67, n=44, $P \leq 0.01$) in the LabIND experiment (Table 3.8). The peak population of *S. avenae* was significantly correlated with both population growth rate (r=0.48, $P \leq 0.01$) and aphid tiller $^{-1}$ (r=0.95, $P \leq 0.01$) in the LabPOP experiment (Table 3.8). In the greenhouse experiment there was a significant correlation between the starting population of aphids and the population growth rate of aphids (r=-0.74, $P \leq 0.01$) (Table 3.8). There was a significant correlation between the aggregated peak population of aphids and the peak population of *M. dirhodum* (r=0.99, n=89, $P \leq 0.01$), and the peak population of *S. avenae* (r=0.47, n=89, $P \leq 0.01$) (Table 3.8). The peak population of *S. avenae was* significantly correlated with the peak population of *M. dirhodum* (r=0.36, n=89, $P \leq 0.01$) (Table 3.8).

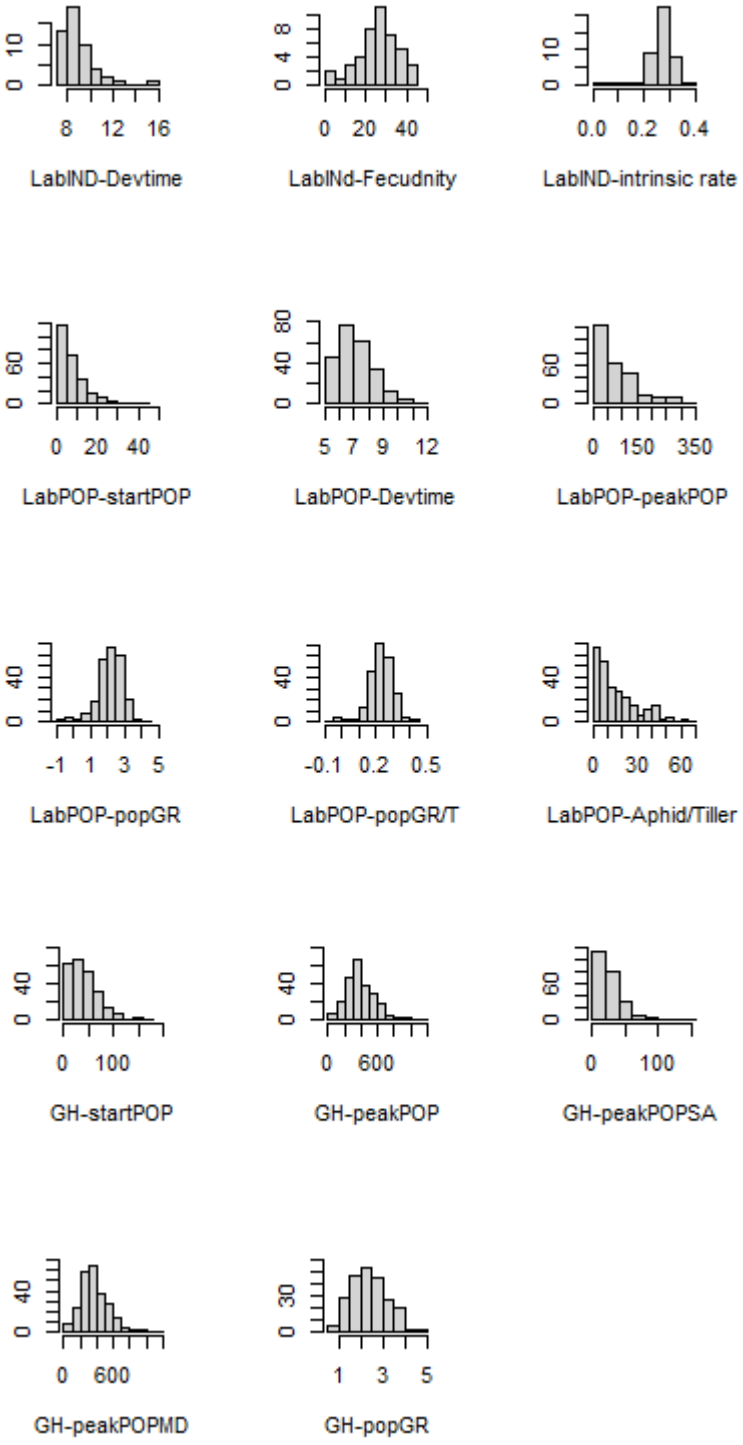

Figure 3.3. Histograms of all traits used in the QTL analysis from each of the experiments. The 'frequency of DH lines' (Y-axis) against the aphid population trait values on the X-axis. The sample sizes, means and standard deviation for each trait are displayed (Tables 3.3-3.7).

Table 3.8. A correlation matrix for all traits from all experiments. The values are the Pearson correlation coefficient (r). Correlation coefficients (r) significant at $P \leq 0.05$ bold, significant at $P \leq 0.01$ bold italics.

	LabIND experiment			LabPOP experiment						Greenhouse experiment			
Trait	*LIDevtime*	*LI Fecundity*	*LIrM*	*startPOP*	*Devtime*	*peakPOP*	*popGR*	*popGR/T*	*aphidsTILLER*	*GHstartPOP*	*GHpeakPOPSA*	*GHpeakPOPMD*	*GHpeakPOP*
LI Fecundity	-0.10	1											
LIrM	***-0.75***	***0.67***	1										
startPOP	0.01	0.00	-0.04	1									
Devtime	0.07	0.10	0.04	-0.10	1								
peakPOP	0.15	0.07	-0.07	***0.3***	***-0.18***	1							
popGR	0.20	-0.02	-0.08	***-0.23***	*-0.16*	***0.48***	1						
popGR/T	*0.30*	0.10	-0.07	***-0.29***	0.03	***0.30***	***0.92***	1					
aphidsTILLER	0.10	-0.01	-0.07	***0.29***	***-0.19***	***0.95***	***0.46***	***0.27***	1				
GHstartPOP	-0.04	-0.11	0.01	-0.02	-0.04	0.05	-0.11	-0.02	0.09	1			
GHpeakPOPSA	0.04	*0.33*	0.10	0.00	-0.12	0.08	0.14	0.09	0.03	0.03	1		
GHpeakPOPMD	0.10	0.04	0.00	0.01	-0.05	0.06	0.02	0.09	0.07	0.12	***0.36***	1	
GHpeakPOP	0.11	0.09	0.01	0.01	-0.06	0.07	0.03	0.10	0.07	0.12	***0.47***	***0.99***	1
GHpopGR	0.11	0.11	0.03	0.02	-0.01	-0.02	0.14	0.10	-0.03	***-0.74***	*0.16*	***0.37***	***0.38***

3.3.2 Section 2: Genetic linkage map and single marker ANOVA

The map was created from a population of 94 doubled haploid genotypes, with 1923 SNP markers (Appendix I). There were 37 Linkage groups (LG) on the SavRia genetic map, spanning a total length of 1925.05cM, therefore, on average of one marker for every <1cM. Five of the 37LG represented failed to assign to any chromosome, but the remaining 32LG could be assigned across the 21 chromosomes in wheat (Appendix I). This linkage map (Appendix I) was used for composite interval mapping to locate potential quantative trait loci (QTL).

Aphid performance traits were correlated to markers on several chromosomes (Table 3.9). More than 500 marker sites on the SavRia genetic map were associated with aphid performance traits. Marker trait associations that were highly significant ($P \leq 0.0001 - 0.01$ and $R^2 > 0.1$) are displayed (Table 3.9).

Table 3.9. Single marker ANOVA output. The abbreviations: cM is centi-Morgan distances, chromo are the chromosomes the markers are found on. Each aphid trait that were statistically associated ($P \geq 0.001$ <0.01) with markers on the SavRia genetic linkage map. Aphids were reared on SavRia wheat genotypes in a controlled environment.

Marker	Chromo	cM	hmzA	hmzB	*n*	m(hmzA)	m(hmzB)	*R2*	*A*	*F*	*p*	*trait*
BS00153038	1D	59.21	29	36	65	439.98	359.12	0.11	-40.43	7.55	0.01	GHpeakPOPALL
BS00089310	2A	99.58	21	43	64	1.79	2.34	0.16	0.28	11.76	0.00	LppopGR
BS00022265	2A	99.58	23	40	63	1.83	2.37	0.16	0.27	11.57	0.00	LppopGR
BS00030165	2A	104.04	23	42	65	0.21	0.24	0.10	0.02	7.15	0.01	LPpopGR/T
BS00023974	2A	105.23	22	39	61	0.20	0.24	0.12	0.02	7.86	0.01	LPpopGR/T
BS00029696	3A	118.15	39	26	65	7.35	7.92	0.10	0.28	7.23	0.01	LPDevtime
BS00022395	4A1	15.02	13	21	34	10.38	8.95	0.30	-0.72	13.86	0.00	LI devtime
BS00022956	5B1	28.31	35	30	65	51.44	34.37	0.16	-8.53	11.90	0.00	GHstartPOP
BS00085610	5D	79.76	36	27	63	50.22	32.62	0.16	-8.80	11.99	0.00	GHstartPOP
BS00022373	5D	23.34	41	26	67	2.16	2.74	0.18	0.29	14.37	0.00	GHpopGR
BS00162083	5D	79.76	22	17	39	0.16	0.26	0.20	0.05	9.16	0.00	LIrM
BS00029317	5D	79.76	22	18	40	16.41	27.39	0.16	5.49	7.48	0.01	LI Fecundity
BS00022373	5D	23.34	40	28	68	13.77	20.75	0.11	3.49	8.26	0.01	aphid/tiller
BS00009514	6D	0.00	16	21	37	28.00	16.33	0.19	-5.83	8.11	0.01	LI Fecundity
BS00003753	6D	73.36	33	35	68	13.14	19.95	0.11	3.40	8.07	0.01	aphid/tiller
BS00003753	6D1	73.36	33	35	68	66.54	101.41	0.11	17.44	8.09	0.01	LPpeakPOP
BS00023172	7A	27.08	43	24	67	2.58	1.99	0.20	-0.29	15.75	0.00	GHpopGR
BS00069242	7A	29.49	38	23	61	2.59	1.98	0.24	-0.30	18.16	0.00	GHpopGR
BS00078359	7A	30.67	39	28	67	2.62	2.09	0.16	-0.27	12.22	0.00	GHpopGR
BS00004136	7A	33.00	39	24	63	2.55	2.01	0.17	-0.27	12.86	0.00	GHpopGR
BS00023055	7A	35.32	41	23	64	2.59	1.96	0.22	-0.32	17.29	0.00	GHpopGR
BS00012317	7A	39.92	44	20	64	2.56	1.97	0.18	-0.29	13.39	0.00	GHpopGR
BS00009404	7A	2.38	32	33	65	65.67	98.79	0.10	16.56	7.38	0.01	LPpeakPOP
BS00009404	7A	2.38	32	33	65	12.95	19.70	0.11	3.37	7.77	0.01	aphid/tiller
BS00150370	7D	11.70	34	29	63	23.88	33.74	0.08	4.93	5.43	0.02	GHpeakPOPSA
BS00139213	7D	11.70	33	31	64	0.24	0.21	0.06	-0.01	4.05	0.05	LPpopGR/T
BS00181652	7D	0.00	20	20	40	26.35	16.00	0.15	-5.18	6.66	0.01	LI Fecundity
BS00181652	7D	0.00	20	20	40	0.25	0.16	0.15	-0.05	6.86	0.01	LIrM

3.3.3 Section 3: Composite interval mapping to locate potential QTL

These results are from the QTL analysis using composite interval mapping (CIM). The QTL analysis identified genomic regions associated with individual aphid performance and aphid population performance traits. The LOD significance threshold values for significant QTL locations, were determined by *500* permutations at significance level *P=0.05* for each trait included in the CIM analysis. There were 12 markers along chromosomes on the SavRia genetic map that were statistically associated with 9 out of the 14 aphid traits measured and analysed (Figure 3.4, Table 3.10). A visual display of all potential QTL locations (Figure 3.4) and each chromosome with significant QTL are displayed (Figure 3.5-3.11).

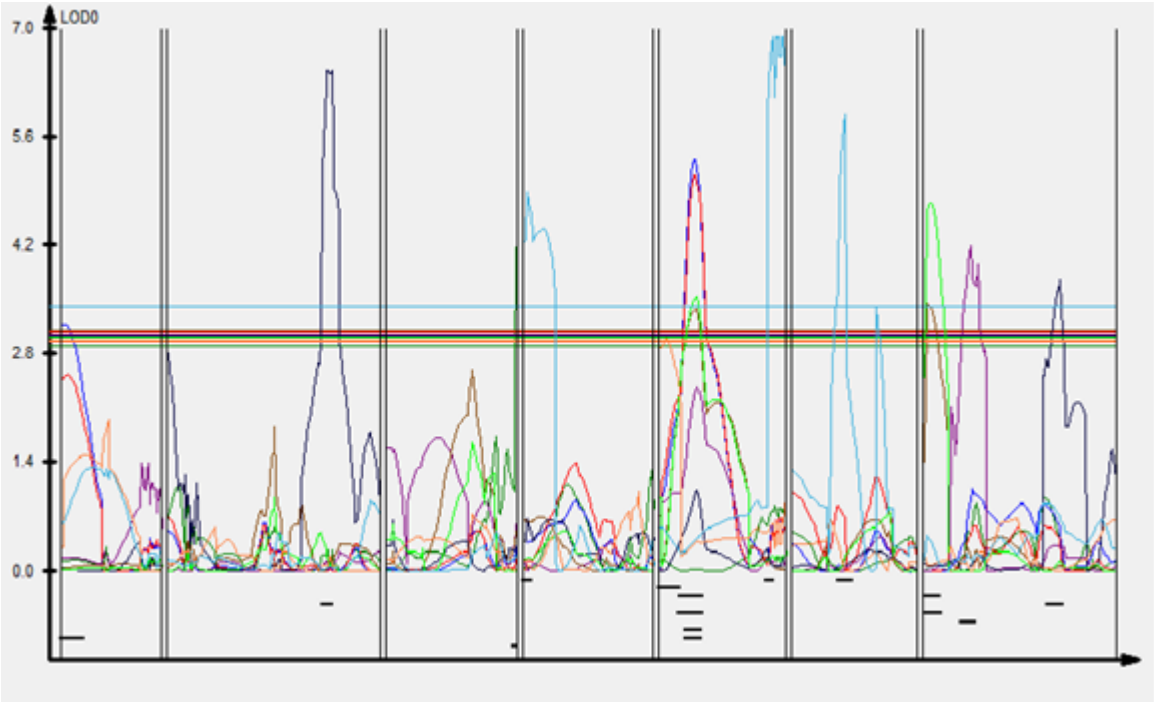

Figure 3.4. The putative QTL locations significantly associated with aphid performance traits on the SavRia genetic linkage map. The LOD value (Y-axis). The LOD significance thresholds are colour coded for each trait and form the horizontal lines out from the Y-axis. Putative QTL are located where the peak of the colour coded lines exceed their threshold. The traits are colour coded as: LI-DevTime –, LI-*rM* –, LP-aphid/tiller –, LP-peakPOP –, LP-popGR/T –,GH-peak POPMD –,GH-peakPOPSA –, GH-peakPOPALL –, and GH-popGR –. The double black vertical lines separate the chromosomes, 1D, 2A, 2B, 3B, 5D, 6D, and 7A from left to right respectively. The horizontal black dashes above the x-axis below the 0 LOD value identify the putative QTL locations.

There was a significant association between marker BS00003823 on chromosome 1D at 2.1cM with the Peak population of *Metopolophium dirhodum* in the greenhouse experiment (Figure 3.5). The marker BS00003823 was associated with an increase in the peak population of *Metopolophium dirhodum* and explained 13% of the variation in this trait (Table 3.10).

Table 3.10. The nine aphid performance traits that were statistically associated with markers along chromosome arms on the SavRia genetic map, s: short arm, L: long arm. Position of the marker in cM. Effect; shows the direction (+/-) of the effect of the A-allele from the Savannah parent on the trait, the R^2 value, LOD score for each trait in the analysis (CIM results).

Trait	Chromosome	marker	Position	Effect	R^2	LOD score
GHpeakPOPMD	1Ds	BS00003823	2.1	46.95	0.13	3.2
LPpopGR/T	2A$_L$	BS00089308	99.6	-0.03	0.27	6.5
GHpeakPOPSA	2Bs	BS00004262	81.3	-16.17	0.26	4.2
LIr$_M$	3B	BS00075879	2.3	0.06	0.22	4.9
GHpeakPOPMD	5D$_L$	BS00150192	21.4	-70.20	0.24	5.3
GHpeakPOPall	5D$_L$	BS00150192	21.4	-75.30	0.25	5.1
LPpeakPOP	5D$_L$	BS00022373	23.4	-20.67	0.14	3.4
Aphid/Tiller	5D$_L$	BS00022373	23.4	-4.03	0.14	3.5
LIDevTime	5D$_L$	BS00074877	4.1	0.62	0.19	3.0
LIr$_M$	5D$_L$	BS00013077	70.9	-0.07	0.32	6.9
LIr$_M$	6D$_L$	BS00138278	33.2	0.08	0.28	5.9
GHpopGR	7As	BS00069242	29.5	0.28	0.15	4.2
LPpeakPOP	7As	BS00012880	2.4	-20.13	0.14	3.5
LPpopGR/T	7A$_L$	BS00094959	86.0	0.02	0.14	3.8
Aphid/Tiller	7As	BS00012880	4.4	-4.97	0.22	4.8

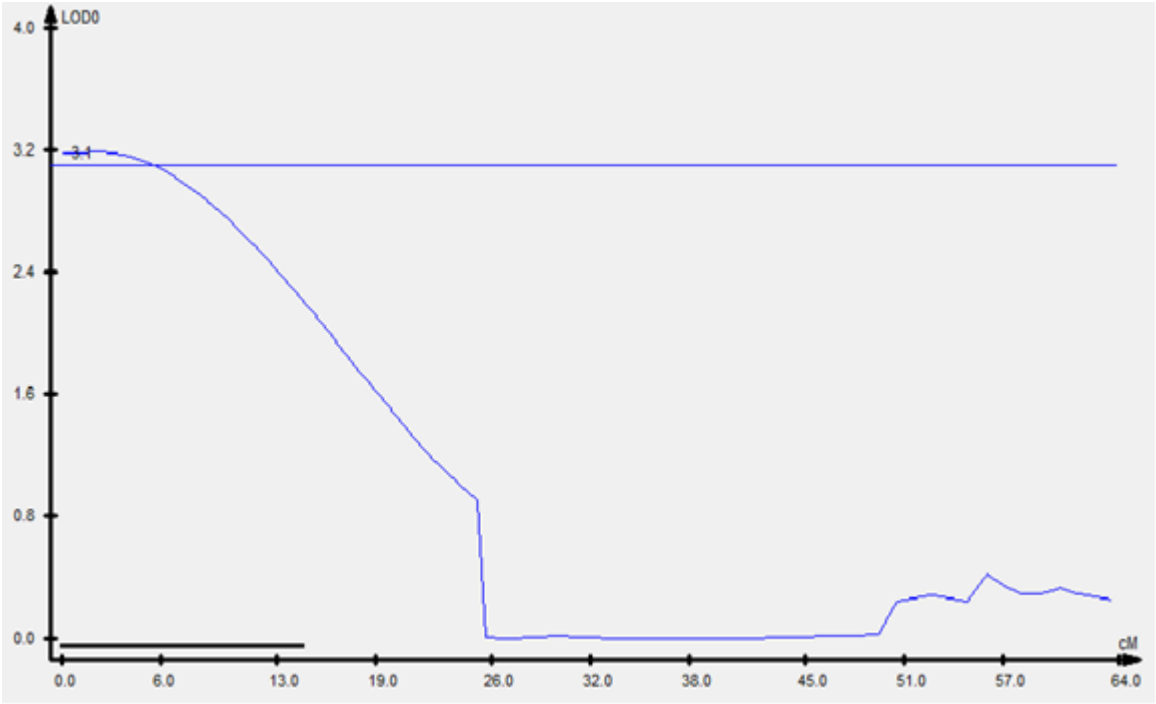

Figure 3.5. CIM analysis of chromosome 1D with a significant QTL location at 2.1cM for the peak population of *Metopolophium dirhodum* in the greenhouse experiment. LOD value (Y-axis) against centiMorgan distance along chromosome 1D (X-axis). LOD significance threshold for GHpeakPOPMD (horizontal line–).

There was a significant association between marker BS00089308 on chromosome 2A at 99.6cM with the aphid population growth rate in the labPOP experiment (Figure 3.6). The marker BS00089308 was associated with a decrease in the population growth rate of *S. avenae* and explained 27% of the variation in this trait in the laboratory population experiment (Table 3.10).

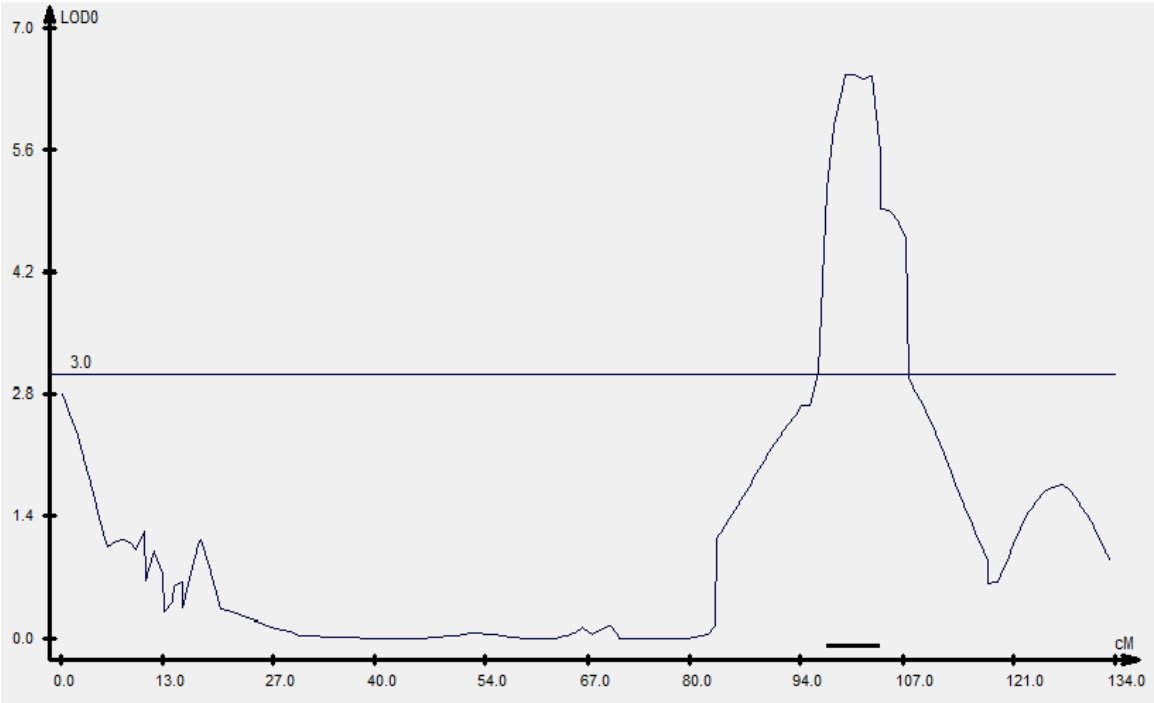

Figure 3.6. A significant QTL location on chromosome 2A at 99.6cM associated with *S. avenae* population growth rate in the laboratory population experiment. LOD value (Y-axis). Genetic distance (centiMorgan) along chromosome 2A (X-axis). The LOD significance threshold for LPpopGR/T (horizontal line -).

A significant association was found between marker BS00004262 on chromosome 2B at 81.3cM with the peak population of *S. avenae* in the

greenhouse experiment (Figure 3.7). The marker BS00004262 was associated with a decrease in *S. avenae* peak population in the greenhouse experiment and explained 26% of the variation in this trait (Table 3.10).

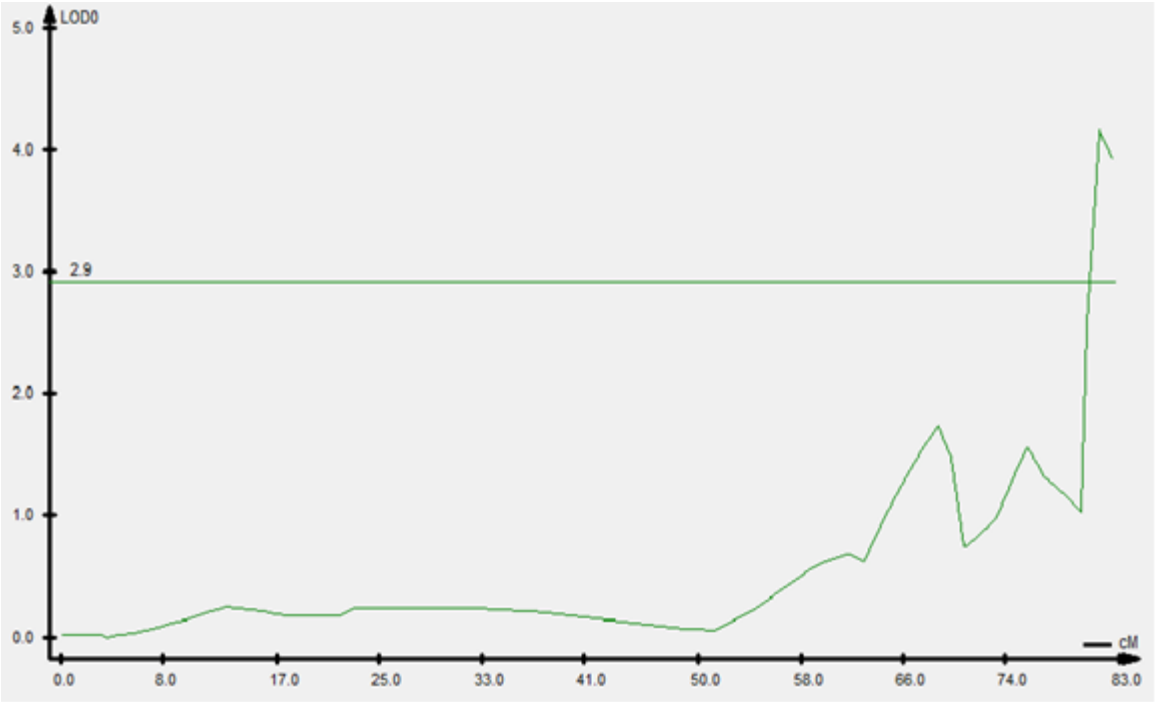

Figure 3.7. A significant QTL location on chromosome 2B at 81.3cM associated with the peak population of *S. avenae* in the greenhouse experiment. LOD value (Y-axis). Genetic distance (centiMorgan) along chromosome 2B (X axis). The LOD significance threshold for GHpeakPOPSA (horizontal line –).

There was a significant association between marker BS00075879 on chromosome 3B at 2.3cM with the intrinsic rate of increase of *S. avenae* in the laboratory IND experiment (Figure 3.8). Marker BS00075879 was associated with an increase in the intrinsic rate of increase of *S. avenae*. The marker BS00075879 explained 22% of the variation in this trait during the laboratory IND experiment (Table 3.10).

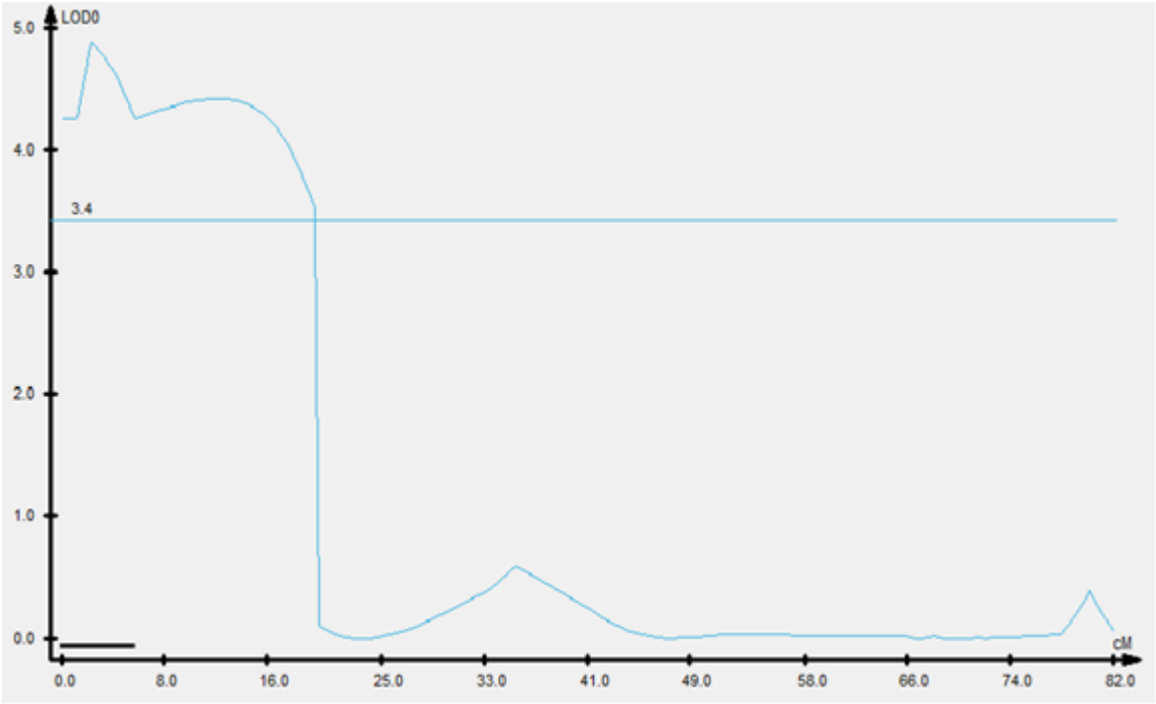

Figure 3.8. A significant marker on chromosome 3B at 2.3cM associated with the intrinsic rate of increase (r_M) for *S. avenae* in the laboratory IND experiment. LOD significance threshold for the trait LabINDr_M (horizontal line -).

There were four markers associated with six aphid traits on the long arm of chromosome 5D. There were significant associations between the marker BS00150192 at 21.4cM with the peak population of *Metopolophium dirhodum,* and the peak population of both aphid species in the greenhouse experiment; the marker explained 24% and 25% of the variation in these traits respectively (Figure 3.9). The A-allele contributed by the Savannah parent was associated with a decrease in the peak population of aphids. The marker BS00022373 at 23.4cM on 5D$_L$ explained 14% of the variation in both aphid tiller^{-1} and the peak population of *S. avenae* in the laboratory population experiment (Figure 3.9). The marker BS00022373 was associated with a decrease in aphid tiller^{-1} and peak population of *S. avenae* (Table 3.10). The marker BS00074877 at 4.1cM on 5DL was associated with an increase in development time of *S. avenae* in the laboratory IND experiment, and explained 19% of the variation in this trait (Table 3.10). There was a significant association between marker BS00013077 at 70.0cM on chromosome 5D$_L$ with the intrinsic rate of increase of *S. avenae* in the laboratory IND experiment (Figure 3.9), the marker was associated with a decrease in the intrinsic rate of increase of *S. avenae* and explained 32% of the variation in this trait (Table 3.10).

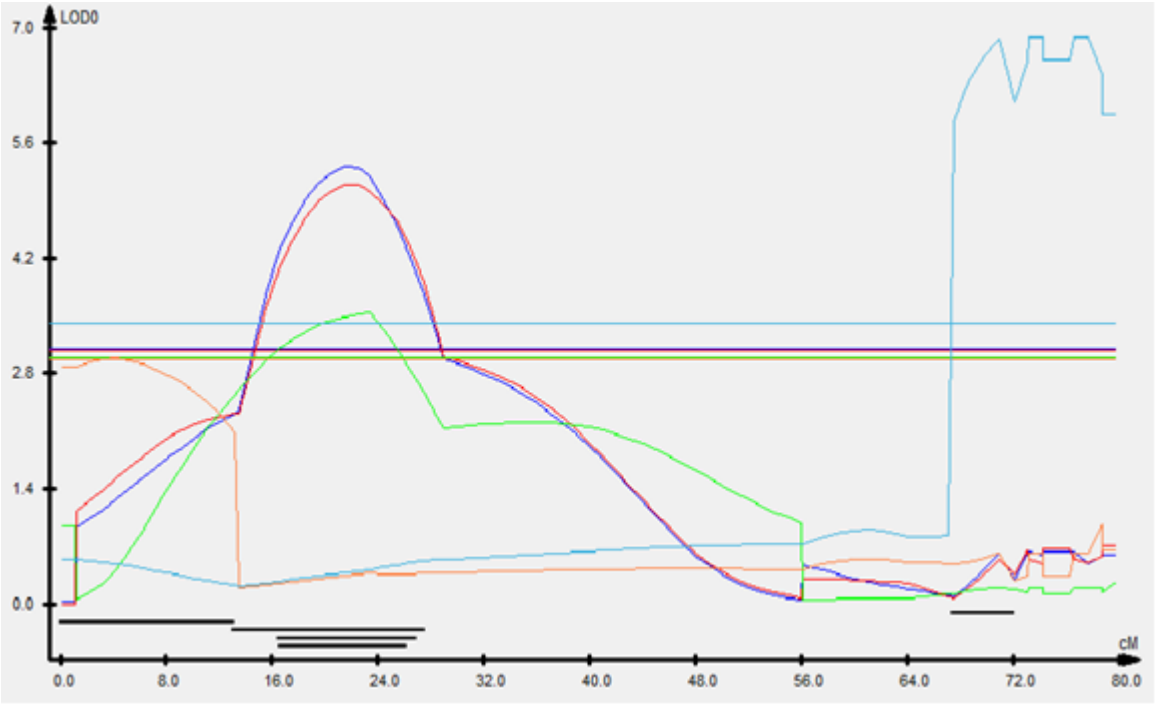

Figure 3.9. Chromosome 5D (CIM output). The marker BS00074877 at 4.1cM was associated with LI-DevTime –, marker BS00150192 at 21.4cM is associated with both GH-peakPOPMD –, and GH-peakPOPALL –. Marker BS00022373 at 23.4cM was associated with both LP-aphid/tiller –, and LP-peakPOP –. Marker BS00013077 at 70.9cM was associated with LIrM –. LOD value (Y-axis). The LOD threshold values for each trait (horizontal lines-colour coded the same as the traits). Genetic distance (cM) along chromosome 5D (X-axis). The putative QTL locations (horizontal black dashes above x-axis below the 0 LOD value).

Marker BS00138278 at 33.2cM on the long arm of chromosome 6D was associated with the intrinsic rate of increase of *S. avenae* in the laboratory IND experiment; the marker was associated with an increase in *S. avenae* intrinsic rate of increase, and explained 28% of the variation in this trait (Figure 3.10).

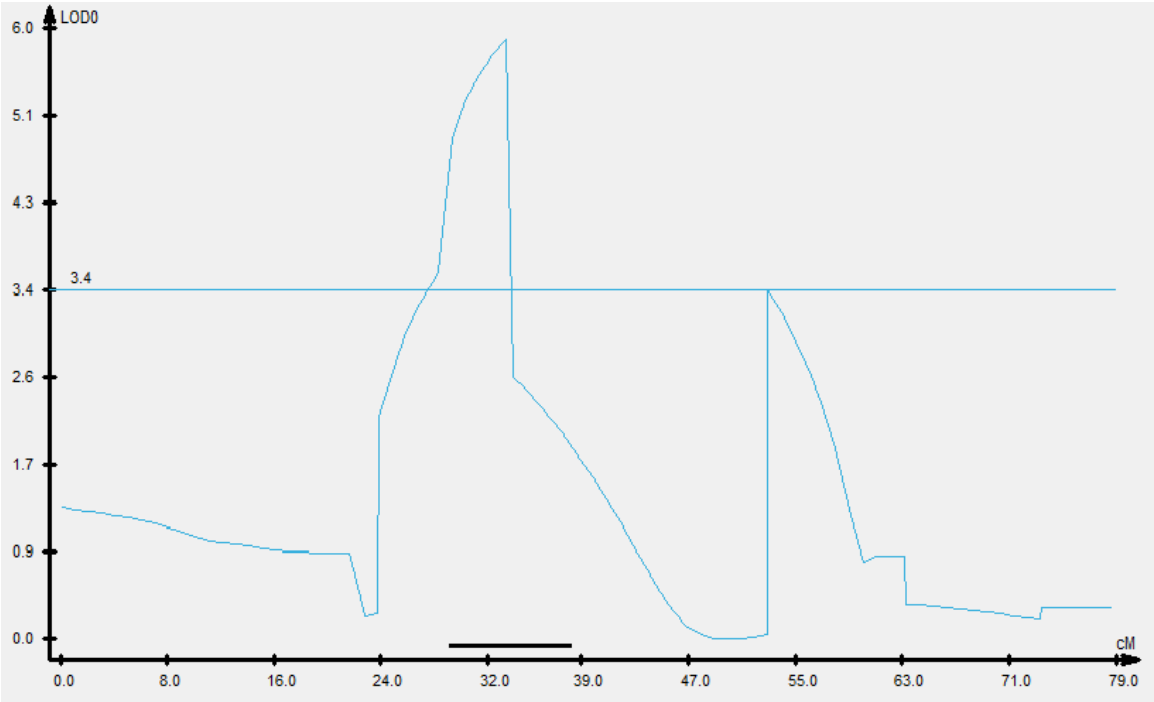

Figure 3.10. Chromosome 6D (CIM –output). Marker BS00138278 at 33.2cM significantly associated with the intrinsic rate of increase (r_M) of *S. avenae* in the laboratory IND experiment. LOD significance threshold for LabINDr_M (horizontal line -).

There were three markers associated with four aphid traits on chromosome 7A. The marker on the short arm of chromosome 7A at 2.4cM was significantly associated with aphids tiller^{-1}, and the peak population of *S. avenae;* in the laboratory POP experiment (Figure 3.11). Marker BS00012880 explained 22% and 14% of the variation in these traits respectively and was associated with a decrease in aphids tiller^{-1} and the peak population of *S. avenae* (Table 3.10). The marker BS00069242 at 29.5cM on the short arm of 7A explained 15% of the variation in population growth rate of aphids in the greenhouse experiment (Figure 3.11). The marker BS00069242 was associated with an increase in the population growth rate of aphids in the greenhouse experiment (Table 3.10). The marker BS00094959 at 86cM on the long arm of chromosome 7A (Figure 3.11) was associated with an increase in population growth rate unit time^{-1} in the laboratory POP experiment, and explained 19% of the variation in this trait (Table 3.10).

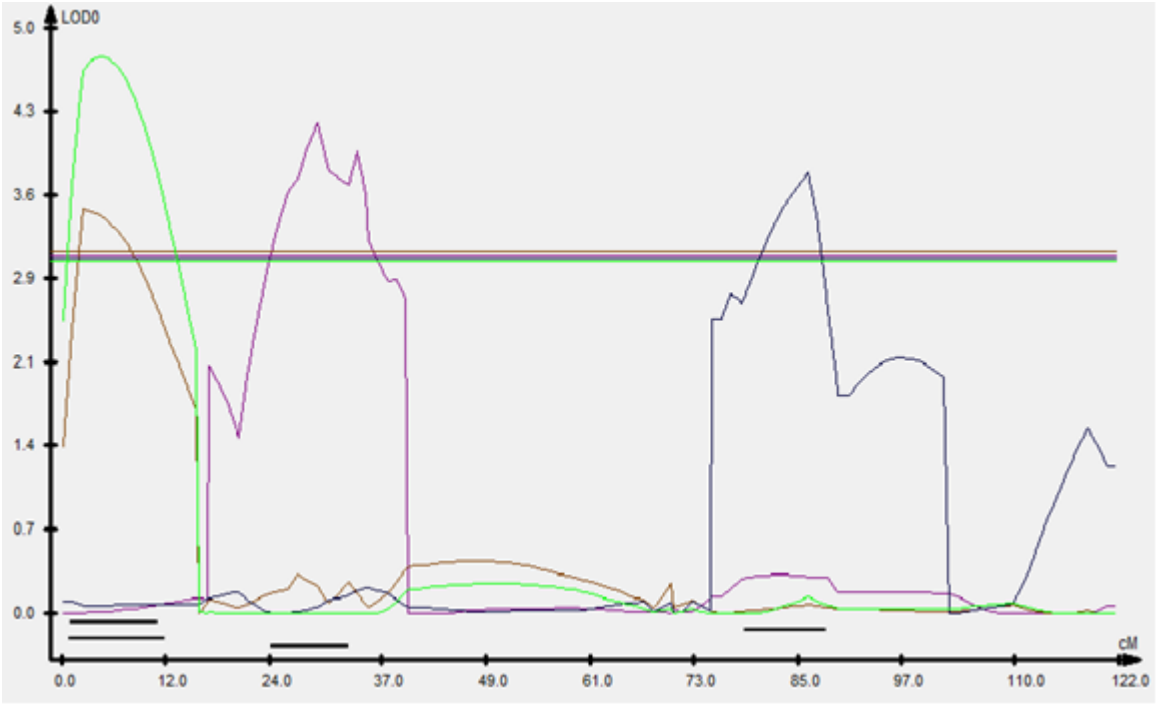

Figure 3.11**.** Chromosome 7A (CIM-output). Marker BS00012880 at 2.4cM was associated with aphids tiller^{-1} -, and the peak population of *Sitobion avenae* – in the laboratory POP experiment. Marker BS00069242 at 29.5cM is associated with the population growth rate of aphids – in the greenhouse experiment. Marker BS00094959 at 86cM was associated with aphid population growth rate - in the laboratory POP experiment.

3.4 Discussion

Three experiments are reported in this chapter that aimed to investigate the relationship between genetic variation in the SavRia winter wheat population and the performance measures of the aphid species *Metopolophium dirhodum* and *S. avenae*. The models used for quantitative trait loci (QTL) analysis were then shown to be suitable for identifying genomic regions associated with aphid performance traits (Zeng 1994; Cresspo-Herrera *et al* 2014). It is clear that locating novel genes in wheat that are associated with a negative influence on aphid performance can be applied in MAS programs under the breeding pillar for wheat improvement strategies.

The main aim of this chapter was to identify if genetic variation within the SavRia winter wheat population is associated with performance measures of the aphids *S. avenae* and *Metopolophium dirhodum*. The aphid performance measures, which have been previously identified as particularly salient (Honek *et al.* 2006, Lamb *et al.* 2009), were significantly different between genotypes (Table 3.6) and were then used in the QTL analysis. The 94 genotypes from the SavRia DH population were chosen to capture genetic variation in a winter wheat population. The SavRia DH line has SNP molecular marker arrays available for genetic mapping (Allen *et al.* 2012, Wilkinson *et al.* 2012). Identifying which wheat genotypes have a negative influence on aphid performance infers these genotypes have genes that are associated with aphid performance or code for traits that infer a negative influence on aphid performance.

Identifying and locating genetic markers that are associated with negative aphid performance is the first step in discovering and characterising these genes for use in wheat breeding programs. A Single marker ANOVA was a preliminary test used to identify if genetic variation in the SavRia population was associated with aphid performance traits. Genetic markers were significantly associated with all aphid performance measures, with the exception of the starting population of *S. avenae* in the laboratory population experiment. Over 500 significant marker trait associations with aphid performance measures were identified using single marker ANOVA and a subset of 29 that were highly significant are displayed in Table 3.9. The genetic marker BS00022373 on chromosome 5D had a highly significant association with aphids tiller^{-1} from the laboratory population experiment, and the growth rate of *S. avenae* and *M. dirhodum* in the greenhouse experiment. The genetic marker BS00022373 explained 11% and 18% respectively of the variation in these traits. An association

between a genetic marker with different aphid performance traits from two different species under different environments suggest this marker flanks genes that may have a major influence on aphid performance (Table 3.9). There were several other markers on chromosome 5D that had highly significant associations with aphid performance measures (Table 3.9).

The single marker ANOVA identified markers that were significantly associated with aphid performance, the amount of variation in a trait that is explained by the genetic marker and which chromosomes these markers are located on (Table 3.9). However, although a robust technique for identifying marker trait associations, the single marker ANOVA can be flawed by the many false positive marker/trait associations that are identified (Rebai *et al.* 1995). These results are ample justification for using a higher resolution technique such as QTL analysis to elucidate more precisely the location of genetic markers significantly associated with aphid performance traits.

The composite interval mapping model used for QTL analysis is more robust than a single marker ANOVA with a higher resolution for locating genetic markers that have significant associations with aphid performance measures (Zeng 1994). The composite interval mapping model (CIM) identified 11 genetic markers associated with nine aphid performance traits along seven different chromosomes (Table 3.10). The influence of the genes that are flanked by these genetic markers can be associated with an increase or decrease in aphid performance traits. There were seven markers that were associated with a reduction in aphid performance measures. There were five markers (BS00003823, BS00075879, BS00138278, BS00069242, and BS00094959) that were associated with an increase in aphid performance.

The marker BS00094959 at 86cM on the long arm of chromosome 7A was associated with an increase in the population growth rate of *S. avenae,* explaining 14% of the variation in population growth rate of *S. avenae* (Figure 3.11*).* These markers may be associated with plant traits that infer benefits for phloem feeding insects. Powdery mildew growth has been shown to be negatively influenced by genes on chromosome 7A (Sears and Briggle 1969), and a reduction in powdery mildew may benefit the population growth rate of *S. avenae*. The peak population of *S. avenae* was negatively correlated to powdery mildew infection (Figure 3.2). This interaction between factors offers one potential explanation for the association between markers on chromosome 7A with an increase in the population growth rate of *S. avenae*.

Genes that are flanked by markers associated with positive aphid performance should be characterised, so a deeper understanding of intrinsic plant factors influencing phloem feeding insects is attained. However, markers that are associated with an increase in aphid performance may infer benefits to phloem feeding insects, and therefore should be selected against in breeding programs, assuming the genes that flank these markers do not confer desirable agronomic traits.

The marker BS00089308 at 99.6cM on the long arm of chromosome 2A is associated with a reduction in the population growth rate of *S. avenae,* and explains 27% of the variation in this trait (Figure 3.6). This result with a LOD score of 6.5 and R^2 of 0.27 suggests this marker flanks major QTL that were associated with a reduction in aphid growth rate. A study by Tan *et al.* (2013) used CIM with the WincartQTL program to identify QTL on chromosome 2A conferring wheat resistance to the Hessian fly. The results of Tan *et al.* (2013) provide compelling evidence that insect resistance genes are present along chromosome 2A in hexaploid wheat; these genes may also be associated with a negative influence on the population growth rate of aphids.

This study identified a genetic marker (BS00004262) at 81.3cM on chromosome 2Bs that was associated with a reduction in the peak population of *S. avenae*. A recent study by Li and Peng (2013) identified genes on wheat chromosome 2Bs that were associated with tolerance in wheat to *S. avenae*. Genes on chromosome 2Bs that are associated with a reduction in aphid performance may also increase aspects of wheat tolerance to aphid infestation. There can be interactions between genes that confer tolerance or resistance to plant pathogens and the performance of insect pests (Von Burg *et al.* 2012, Drakulic *et al.* 2015). Tolmay *et al.* (2016) combined genes identified on chromosome 2B and 4A that inferred resistance to *Diuraphis noxia* and also resistance to stem, leaf and stipe rust in five new wheat varieties. Characterising the function of putative genes linked to markers significantly associated with aphid performance measures is imperative and validating their effects in different environments could yield novel genes suitable for pyramiding into elite wheat varieties using MAS breeding techniques (Joshi & Nayak 2010).

Modern bread wheat (*Triticum aestivum*) is a synthetic hexaploid, with 7 chromosomes each with A, B and D copies (Brenchley *et al.* 2012). The biology and genetic structure of wheat are introduced in chapter 2. The progenitor species *Triticum urartu*, *Aegilops speltoides* and *Aegilops*

tauschii that contributed the A, B, and D parts of the wheat genome respectively donated their specific characteristics (Brenchley *et al.* 2012), which have in some cases remained conserved in their genetic region of contribution (Peterson *et al.* 2015). The D region of the wheat genome is often linked to sources of disease resistance (Liu *et al.* 2000). Genomic regions on chromosome 5D have been shown to code for traits conferring resistance to *Blumeria graminis* and other fungal pathogens (Miranda *et al.* 2006). These regions coding for disease resistance can also infer resistance to insect pests (Von Burg *et al.* 2012, Tolmay *et al.* 2016). For example, a stem rust resistance gene on chromosome 7D has been shown to be associated with Russian wheat aphid (*D. noxia*) resistance genes in wheat (Marais *et al.* 1998).

There were four markers along the long arm of chromosome 5D associated with six aphid performance traits (Table 3.10, Figure 3.9). The four markers (BS00150192, BS00022373, BS00074877, and BS00013077) were all associated with a reduction in aphid performance. Marker BS00074877 was associated with an increase in the development time of *S. avenae*. Therefore, this marker was associated with a negative influence on aphid performance by increasing the development time of *S. avenae* nymphs to reach their reproductive stage. The marker BS00022373 at 23.4cM that was associated with the peak population of *S. avenae* in the laboratory experiment is only 2cM away from the marker BS00150192 at 21.4cM on chromosome 5D that is associated with the peak population of *M. dirhodum* in the greenhouse experiment. Two markers so close together associated with negative aphid performance for two different species in different environments is an important finding. This finding suggests that targeting genomic regions on chromosome 5D associated with a reduction in aphid performance are worth further investigation, which should include discovery and characterising putative genes.

Marker BS00022373 at 23.4cM on $5D_L$ was associated with both the peak population of *S. avenae* and the number of *S. avenae* tiller^{-1} in the laboratory population experiment (Table 3.10 & Figure 3.11). These aphid performance traits were significantly correlated (Table 3.8). The number of aphids tiller^{-1} was calculated by dividing the peak population of aphids by the number of tillers on the plant. This equation, therefore, offers a potential explanation for the correlation between these performance traits. The association between marker BS00022373 and both aphid traits may be an artefact of this correlation. The association between marker BS00150192 and both the peak population of *M. dirhodum* and the peak population of all aphid species in the greenhouse experiment (Figure 3.9)

may also be an artefact of the correlation between these aphid traits (Table 3.8).

The marker BS00012880 on chromosome 7A was associated with a reduction in aphids tiller^{-1} and peak population of *S. avenae* in the laboratory population experiment (Figure 3.11). Again, this significant association may be an artefact of correlated aphid performance traits (Table 3.8). However, genes on chromosome 7A have been associated with resistance to *Schizaphis graminum* (Boyko *et al.* 2004). Marker associations with reduced aphid performance on chromosome 7A may, therefore, warrant validation with other aphid species. The results of this study identified and located genetic markers that are associated with performance measures of *S. avenae* and *M. dirhodum*.

The limitations of this study were the small population of genotypes used for QTL analysis. However, a population size of 50 is ample for QTL detection (Mohan *et al.* 1997). The lack of replication of SavRia genotypes in the Laboratory experiment on aphid performance per capita is justifiable, due to dense molecular marker coverage (Mohan *et al.* 1997, Wilkinson *et al.* 2012). Furthermore, the genes and the genetic markers can be replicated many times within the plant population (Allen *et al.* 2013). There was no significant difference in aphid performance measures between the Savannah and Rialto parents (Table 3.5). This may be due to the genetic background of each parent not influencing the aphid performance traits significantly. However, transgressive segregation, which is the creation of extreme phenotypes compared with either parental phenotype implies that progeny can be transgressive for traits that may be associated with aphid performance. Many heritable traits can be transgressive, especially traits segregating in domesticated hybrid plant populations (Rieseburg 1999). These limitations do not flaw the results of this study but warrant discussion.

Quantitative genetics and concomitant marker assisted selection (MAS) breeding programs have advanced considerably over recent decades (Joshi & Nayak 2010, Tolmay *et al.* 2016, Zhang *et al.* 2016). Gene pyramiding as part of MAS is now a commonly used technique intended to assemble multiple desirable genes into a single genotype (Forte *et al.* 2014, Tolmay *et al.* 2016). Modern quantitative genetics and molecular plant breeding techniques have increased the applicability of gene pyramiding for durable resistance to crop pests by incorporating a suite of techniques (Tolmay *et al.* 2016), which include powerful statistical models capable of identifying associations between insect performance traits with neutral markers linked to functional genes (QTL) (Wang *et al.*

2013). For gene pyramiding to be successful, plant populations must be phenotyped, and the relative genomic location of neutral markers linked to functional genes that are associated with the desirable agronomic traits must be identified (Peterson *et al.* 2015). Marker assisted selection programs can reduce phenotyping investment and breeding duration by pyramiding genes with similar effects into elite varieties (Forte *et al.* 2014). Creating more durable resistance in wheat to insect pests will come from pyramiding many genes that negatively influence insect performance traits, or code for aspects of resistance such as secondary plant metabolites (Meihls *et al.* 2015).

Resistance genes in plants often confer resistance to one species, race, or biotype of insect pest. Pyramiding genes with similar effects can, therefore, broaden plant resistance considerably (Singh *et al.* 2000, Joshi & Kumar 2010). Plant resistance to insect pests can be overcome quickly if the resistance conferred is for one biotype only, for example if different biotypes of crop pest introgress then the plant resistance may breakdown (Singh *et al.* 2000). As the breakdown of resistance to pathogens or insect pests in wheat could threaten future food security (Meyers *et al.* 2015, Singh *et al.* 2015) it is essential we combat resistance breakdown and create more durable resistance in wheat to aphid pests by expanding the database of markers linked to genes that negatively influence aphid performance on wheat genotypes (Singh *et al.* 2000, Joshi & Kumar 2010). Genetic markers associated with a reduction in aphid performance and the relative genomic location of these markers were identified during this study. Genes linked to these markers need characterising and validating for utilisation in MAS programs that could contribute to wheat improvement strategies under the breeding pillar (Tolmay *et al.* 2016).

In summary the aims and objectives of this chapter were realised in expanding the current data base of genetic markers in winter wheat associated with a reduction in aphid performance. This new information on the associations between genetic variation in winter wheat with aphid performance measures can contribute to wheat improvement. There are also implications for improving agronomy, in terms of identifying novel genes that can be used to increase aphid resistance in wheat as part of IPM strategies (Nyaanga *et al.* 2014). The benefits of increasing host plant resistance using genes that confer a reduction in aphid performance are reduced insecticide use, which can reduce production costs, and reduced environmental degradation associated with insecticide application (Foster *et al.* 2014).

Seven genetic markers and their relative genomic locations, which were associated with a reduction in the performance of *S. avenae* and *M. dirhodum,* were identified during this study. This new information can be used to locate the putative genes linked to these markers. These genes will need to be characterised and validated in different environments at different growth stages of wheat before they have the potential for incorporation into MAS breeding programs (Joshi & Kumar 2010), which are aiming to increase resistance in wheat to aphid infestation (Tolmay *et al.* 2016). Marker assisted selection programs that develop wheat varieties with increased resistance to aphids have the potential to contribute to wheat improvement strategies under both the breeding and agronomy pillars (Meyers *et al.* 2015).

4 Population performance of *Sitobion avenae* on winter wheat genotypes with different nitrogen uptake efficiencies

4.1 Introduction

Earth's atmosphere is approximately 80% nitrogen but it is unavailable to most organisms as atmospheric gas. While it is widely accepted that nitrogen can be a limiting resource for plants and animals (White 2003; Nowak & Komor 2010; Paine & Hanlow 2010), nitrogen limitation is a case of relative shortage rather than absolute shortage, as organisms have developed numerous adaptations to acquire it (White 2003). Nitrogen is a key component of all proteins and is essential for all growth and cellular function. Increasing the availability of nitrogen can, therefore, lead to higher growth rates and increased metabolic function in both plants and animals (Garnier *et al.* 1999, Fields 2004). It has been shown for various animal groups that nitrogen availability drives animal behaviour in such a way to optimise their access to nitrogen through consuming plants or other animals (White 2003).

Plants around the world are reliant on nitrogen for growth, but are unable to utilise atmospheric nitrogen (N_2) directly. In most cases plants acquire their nitrogen from the soil solution where nitrogen fixation is performed by microorganisms called diazotrophs, which fix inorganic nitrogen into organic forms available to plants. Nitrogen fixation is the process of fixing atmospheric nitrogen (N_2) into ammonia (NH_3) through breaking the triple bonded N_2 (Xu, Fan & Miller 2012). Ammonification of ammonia (NH_3) into ammonium (NH_4^+) is facilitated by anaerobic and aerobic bacteria and fungi in soil. Ammonium is then converted to nitrite (NO_2^-) and nitrate (NO_3^-) by nitrifying bacteria (Xu, Fan & Miller 2012). Following fixation through ammonification and nitrification provided by microorganisms in the soil, atmospheric nitrogen is then available as nitrite (NO_2^-) and nitrate (NO_3) and ammonium (NH_4^+) for assimilation by plants (Xu, Fan & Miller 2012).

The assimilation of nitrogen by plants describes the process of cycling inorganic nitrogen to organic compounds that are absorbed by plants and incorporated into cells and tissues. Plants absorb nitrogen in the form of nitrite (NO_2^-) and nitrate (NO_3^-) through their roots; absorbed nitrate is then reduced to nitrite ions and ammonium which is assimilated into organic form as glutamine and glutamate (Xu, Fan & Miller 2012). These amino acids act as nitrogen donors for biosynthesis into all other amino acids, nucleic acids and chlorophyll (Distelfeld *et al.* 2014). A positive correlation has been documented in wheat between increasing nitrogen application and amino

acid concentration in the plant phloem (Caputo & Barneix 1997), and chlorophyll concentration in plant leaves (Bojović & Marković 2009). The biological processes of fixation, nitrification and ammonification of inorganic nitrogen by soil microorganisms for absorption and assimilation by plants is fundamental to global agriculture.

For agricultural cropping systems, in addition to the biological conversion of atmospheric nitrogen, the supply of nitrogen to the crop is usually increased with chemical nitrogen fertiliser, e.g. in the form of ammonium nitrate (NH_4NO_3) (Fields 2004, Sylvester-Bradley & Kindred 2009). Nitrogen application increases crop production, providing that light, water and temperature are all favourable. Most non-leguminous plants require 20-50 g of nitrogen taken up by their roots to produce 1 kg of dry biomass (Robertson & vitousek 2009). Crop yield is, therefore, nitrogen limited in most agricultural cropping systems.

Crop nitrogen use efficiency (NUE) is composed of uptake efficiency, which is the nitrogen captured by the roots and assimilated into plant tissues, together with utilisation efficiency, which is the mobilisation of nitrogen to the grain from the stem, leaves and flowers (Ladha *et al.* 2005). The components of nitrogen use efficiency are described in detail in chapter 1. On average in the UK winter wheat has a NUE of 25 kg DM kg^{-1} N available (Sylvester-Bradley & Kindred 2009). There are various physiological plant traits that can lead to higher nitrogen use efficiency in wheat plants. For example, plants that have greater root mass may uptake nitrogen more efficiently (Ladha *et al.* 2005, Xu, Fan & Miller 2012). Plants that remobilise more nitrogen to the grain during senescence may also utilise nitrogen more efficiently (Hörtensteiner 2009, Distelfeld *et al.* 2014). Therefore nitrogen use efficiency is a complex polygenic trait influenced by different physiological plant factors that vary between individual genotypes within a population (Ladha *et al.* 2005, Karrou & Nachit 2015). Breeding plants to improve components of nitrogen use efficiency, such as nitrogen uptake, can increase yields and decrease production costs.

Increasing nitrogen uptake efficiency in wheat has commercial as well as environmental benefits. Wheat varieties with higher nitrogen uptake efficiencies can have increased yield from the same volume of nitrogen inputs with less nitrogen lost to the wider environment, as more nitrogen can be absorbed by the plants (Barraclough *et al.* 2009). Improving the productivity from wheat without increasing nitrogen application could potentially improve food security, and clearly contributes to wheat improvement under the agronomy pillar. However, increasing nitrogen

uptake efficiency in wheat may have the undesirable effect of increasing benefits to phloem feeding crop pests such as aphids.

In the wheat growing regions of the world the cosmopolitan cereal aphid (*Sitobion avenae*) can be a major pest, reducing grain quality and yield directly by phloem feeding and indirectly as a vector for plant viruses (Watt *et al.* 1984, Foster *et al.* 2014). For example, this cereal aphid is a known vector for barley yellow dwarf virus, which can lead to a total loss of crop yield (Kolbe & Linke 1974). A reduction in grain size, grain nitrogen concentration and grain yield have all been attributed to *S. avenae* (Wratten 1978). Wheat yields can be reduced by 20-40% from direct damage caused by removal of plant nutrients by phloem feeding (Kolbe & linke 1974, Kieckhefer & Gellner 1992). Consequently phloem feeding insects reduce the nitrogen uptake efficiency of plants (Ladha *et al.* 2005). Therefore, a reduction in phloem feeding insects on wheat crops can potentially increase nitrogen uptake efficiency and consequently grain yield and quality. There is currently little understanding of how plants with higher nitrogen uptake efficiencies will influence aphid performance.

The salient variables for measuring aphid performance on different host plant genotypes in the laboratory are the development time from nymph to reproduction, the fecundity (Lamb *et al.* 2009), and the theoretical rate of increase (Dixon 1998, He *et al.* 2013). Measuring aphids' intrinsic rate of increase can infer if plant resistance traits are being expressed, which are associated with a decrease in aphid performance (Bruce *et al.* 2003, Meihls *et al.* 2013). Aphid performance on different genotypes in the laboratory can be indicative of the infestation potential of these genotypes when grown in a field setting (Dahlin & Ninkovic 2013). An important determinant of aphid performance is host plant quality in terms of nutritional quality for aphids (Karley *et al.* 2002).

Host plant quality for aphids is influenced by intrinsic and extrinsic plant factors that correlate with aphid performance traits, such as fecundity (Awmack & Leather 2002). The intrinsic plant factors include but are not limited to, nutritional aspects of the plant and also secondary metabolites such as those synthesised by the plant. These include, for example, the various stages of the jasmonic acid pathway (Bruce *et al.* 2003) and defensive compounds derived from benzoxazinoid synthesis and metabolism (Meihls *et al.* 2013). The key determinants of host plant quality for aphids in terms of nutrition, are nitrogen content (Kahn & Port 2008), chlorophyll concentration (Machado-Assefh *et al.* 2014) and amino acid concentration in the phloem (Karley *et al.* 2002). The application of nitrogen fertiliser increases host plant quality, in terms of chlorophyll concentration in the plant leaves (Bojović &

Marković 2009), and the concentration of amino acids in plant phloem (Caputo & Barneix 1997). These relationships underpin the prediction that increasing nitrogen uptake efficiency in wheat genotypes may have the cost of increasing the risk of infestation of pests such as aphids.

The indirect effects on aphid populations of nitrogen fertiliser application via changes in their host plants are well documented (Duffield *et al.* 1997; Khan & Port 2008; Aqueel and Leather 2011, Wang *et al.* 2015). Increasing nitrogen fertiliser application to wheat crops positively influences *S. avenae* performance, in terms of peak density reached in the field (Duffield *et al.* 1997*)*. The development time of *S. avenae* was not affected by nitrogen application, but adult body weight was positively correlated with nitrogen fertiliser application (Aqueel and Leather 2011). Moreover, the fecundity and longevity of *S. avenae* was higher on wheat plants that received higher volumes of ammonium nitrate fertiliser in the laboratory (Aqueel and Leather 2011). The intrinsic rate of increase (r_m) was higher for *S. avenae* cultivated on wheat plants with higher nitrogen content (Khan & Port 2008). Two important determinants of aphid performance are the concentration of nitrogen in plant phloem and also the profile of amino acids in the phloem (Ponder *et al.* 2000, Nowak & Komor 2010), which further reinforce the prediction that wheat genotypes with higher nitrogen uptake efficiencies may benefit aphid performance traits, and increase infestation potential.

The potential for infestation of wheat genotypes in the field by *S. avenae* can be inferred by observing settling behaviour on wheat genotypes in the laboratory. Settling behaviour is defined as the acceptance of a host plant by either apterous or alatae aphids (Castle *et al.* 1998; Powell *et al.* 2006). During aphid settling a sequence of behaviours can be observed. During initial contact aphids will assess the leaf surface cues before stylet penetration (Prado & Tjallingii 1994). This behaviour can be observed as aphids move their antennae back and forth enabling detection of morphological and chemical cues on the leaf surface (Storer *et al.* 1996). Later stages in aphid settling behaviour include probing the epidermis with their stylet, and consequently phloem acceptance and sustained ingestion (Prado & Tjallingii 1994).

During sustained phloem ingestion, aphids are stationary with antennae swept back over their bodies (Prado & Tjallingii 1994). Aphids are considered to have accepted and settled on a plant once food ingestion begins (Schoonhoven *et al.* 1998). Although it is alatae forms of aphids that disperse between crops, apterous *S. avenae* have been shown to leave their natal host to settle on different plants in response to stimuli other than overcrowding, predation and climate (Holmes 1988). The settling behaviour of *S. avenae* on

wheat genotypes can have direct economic impacts in agricultural systems, due to the reduction in yield caused by aphid infestation and also the potential spread of their associated viruses.

The post settling fecundity of aphids on wheat genotypes can be assessed by recording the number of nymphs born on that genotype following aphid settling (Powell *et al.* 2006). The term post settling fecundity is defined as the number of offspring produced on a host plant following settling and reproduction by insects (Singer 2000). The post settling fecundity of *S. avenae* on wheat genotypes influences the infestation potential of these preferred genotypes relative to other genotypes. The factors influencing post settling fecundity of aphids on their host plants include defensive compounds produced by the plant (Meihls *et al.* 2013), and the nutritional quality of the host plant (Powell *et al.* 2006). Adult viviparae reproduce in-situ parthenogenetically during phloem ingestion (Prado & Tjallingii 1994). The post settling fecundity of aphids on host plant genotypes can indicate the susceptibility of these genotypes to aphid infestation in the field.

The performance of *S. avenae* on a subset of nine SavRia genotypes and both the parental cultivars are investigated for this study. The SavRia population was produced by crossing the winter wheat varieties Savannah and Rialto. It comprises 94 doubled haploid genotypes, which have been shown to vary in nitrogen uptake efficiencies in John Innes field trials (JIC archive 2011- unpublished data). The SavRia population is described in detail in chapter two. Nitrogen content in the plant biomass was measured as a proxy for nitrogen uptake efficiency for this investigation.

The rational for this investigation is to increase our understanding of how aphid infestation will be affected by plants with higher nitrogen uptake efficiencies. Breeding plants with higher nitrogen uptake efficiencies contributes to wheat improvement under the agronomy pillar. The broad aim addressed in this study is to determine if breeding to increase nitrogen uptake efficiencies in winter wheat will increase aphid performance. The primary objective is to identify if SavRia genotypes with higher nitrogen uptake are associated with higher aphid performance. The ancillary objectives include identifying if the results from this laboratory study can be extrapolated to aphid populations on wheat crops in a field setting. In addition, defining the relationship between nitrogen content in the plant biomass with chlorophyll concentration in the plant leaves, and discovering if either of these factors are correlated with aphid performance traits. Quantifying *S. avenae* settling behaviour and post settling fecundity on SavRia genotypes that are associated with high and low aphid performance measures are also objectives.

4.1.1 Hypotheses

1. There will be a significant positive correlation between nitrogen content in plant biomass of the SavRia genotypes in the field and laboratory.

2. The chlorophyll concentration in SavRia plant leaves will be positively correlated with the nitrogen content in the total above ground plant biomass of SavRia genotypes.

3. *S. avenae* performance will be higher as indicated by a) shorter development time, b) higher fecundity, and c) higher intrinsic rate of increase when cultivated on SavRia genotypes that have higher nitrogen concentration in the total above ground plant biomass.

4. *S. avenae* performance parameters a) development time, b) fecundity, and c) the intrinsic rate of increase will be positively correlated with chlorophyll concentration in SavRia plant leaves.

5. A higher number of apterous *S. avenae* will settle on the SavRia genotype on which the *S. avenae* performance trait fecundity was previously found to be higher.

6. Post settling fecundity of *S. avenae* will be higher on the genotype on which the *S. avenae* performance parameter of fecundity is higher.

4.2 Material and Methods

4.2.1 Aphid performance experiment – Experiment one

4.2.2 Experimental design

The first laboratory experiment with a random assortment design was used to monitor the aphid performance parameters of development time, seven day fecundity and the intrinsic rate of increase on nine SavRia genotypes and both parental cultivars. The SavRia genotypes and parental cultivars had different nitrogen uptake efficiencies in a field trial (Gaju *et al.* 2011). In the first experiment nine SavRia genotypes were replicated seven times and the parental cultivars three times (Table 4.1).

The dependent variables for the first experiment monitoring aphid performance were development time from nymph to reproduction, aphid fecundity over a seven day period and the intrinsic rate of increase (r_M) of the aphids. The independent variables were SavRia genotype, plant height, number of tillers, chlorophyll concentration (SPAD reading - index of relative chlorophyll concentration), plant biomass and nitrogen concentration in the aerial part of the plants including the leaves and stems.

Table 4.1. Experimental design for monitoring aphid performance and settling behaviour on different SavRia genotypes (GTs) and parental cultivars (Parents) with replication (Reps), the dependent and independent variables.

Experiment	GTs	Reps	Parents	Dependent variables	Independent variables
1- aphid performance	9	7	Both 3-reps	Development time, fecundity 7 day^{-1}, Intrinsic rate of increase.	Plant GT and N-content, plant traits, Chlorophyll content.
2- aphid settling behaviour	2	12	none	Aphid settling, Fecundity 24 h^{-1}.	Plant GT, Chlorophyll content.
3- confirmation of aphid performance	2	6	none	Development time, fecundity , Intrinsic rate of increase.	Plant GT

4.2.3 Plant genotypes

The SavRia genotypes used in these two experiments were selected to capture variation in nitrogen uptake efficiency (Table 4.2). The genetic background was controlled by selecting genotypes with high allele similarity based on Euclidian distance (unpublished data provided by Luzie Wingen, John Innes centre).

Table 4.2. Traits of the SavRia genotypes being used for the two experiments, genotypes ordered by decreasing nitrogen content, data supplied by JIC field trials. Abbreviations; DTAD: days till anthesis date; nitrogen content (gN kg Biomass^{-1}).

SavRia genotype	N-content (gN kg BM^{-1})	Height (cm)	DTAD (d)
46	10.15	81.08	35.41
25	11.05	81.66	38.41
92	11.32	80.81	37.5
SAV	11.51	-	38.25
13	11.53	74.81	35.75
12	11.77	90.53	40.41
RIA	11.89	-	36.08
59	11.92	80.15	39.25
43	11.96	77.84	35.91
90	12.33	87.02	35.16
31	12.47	60.98	35.75

4.2.4 Experimental procedure

4.2.5 Plant cultivation

The plants were cultivated and treated using the same protocol for all three experiments. SavRia winter wheat genotypes were sown in John Innes No. 2 substrate (Khan & Port 2008) in two 60 well seed trays (dimensions: 52 cm x 29 cm, Cells: 4 cm^2 x 5 cm^2) known as P60 trays. The seedlings were grown on in a controlled environment (day length 18 h light: 6 h dark, light intensity 245 μmol m^{-2} S^{-1}, 22 ^{o}C ±3 ^{o}C, humidity 30% ±5%) and grown for 7 days. The plants were vernalised for 56 d in a controlled environment (day length 18 h light: 6 h dark, light intensity 120 μmol m^{-2} s^{-1}, temperature 5 ^{o}C ±1 ^{o}C , humidity 55% ±5%) (Brooking 1996). Following vernalisation the plants were then potted into 7*7*8 cm pots that were arranged randomly in 3 trays (52 cm x 42 cm x 9 cm).

Plants were then grown for a week in a controlled environment (day length 18 h light, 6 h dark, light intensity 245 μmol m^{-2} s^{-1}, 22 °C ±3 °C , humidity 50% ±5%) in insect enclosures (dimensions: 10 mm thick Perspex, 550 mm high X 530 mm wide, two access windows 210 mm X 210 mm in the front and a 390 mm x 110 mm vent in each side guarded by 50 μm mesh, a 70 mm deep watering tray is housed in the bottom of the enclosure). The plants were grown until growth stage 25 (GS 25) on the Zadoks scale (Zadoks *et al*. 1974), and watered from below; each tray had the same volume (500-1000 mL) of water applied every 24-72 hours depending upon the growth stage throughout vegetation. During the aphid settling experiment all plants were transferred to insect enclosures adapted for use as aphid choice arenas (Figure 4.1).

4.2.6 Plant traits

Data on plant variables were collected following development time of aphids (8±1 d) when fecundity and the intrinsic rate of increase of aphids were being recorded.

Plant height (cm) was measured from the base of the plant to the tip of the flag leaf. The number of tillers on each plant were counted and recorded.

The chlorophyll concentration in the plant leaves was measured with a soil plant analysis development (SPAD) meter. The chlorophyll concentration values were collected using a "Minolta 502 Plus" SPAD meter at the base, midsection and tip of the flag leaf. These data were used for regression against nitrogen content in the above ground plant biomass, including the leaves and stems. These SPAD data from the leaf midsection (2/3 along the leaf toward the tip) were used to investigate the relationship between chlorophyll concentration and aphid fecundity 7 d^{-1}, as this area is where the aphids were cultivated in clip cages.

Plant biomass was measured at the end of the experiment when the plants were cut at their base. The harvested plant material was dried in a LTE-Scientific Swallow drying oven at 80 °C for 24 h. To measure total content of elemental nitrogen in the plant leaves and stems. Dried plant material was ground up using a Spex 6770 freezer-mill cooled with liquid nitrogen (1 minute precool, 2 minute cooling cycle: 3 minute grinding at 13 CPS). The sub samples of dry powdered plant material were weighed using a Sartorious SE2-microbalance to between 4-5 micrograms and placed in 8x5 mm aluminium foil cups (supplied by elemental micro analysis) in 90 well cassette trays (brand: Nunclon) before analysis in a Carlo-Erba 1108 CHN analyser.

4.2.7 Aphids

The cereal aphid *Sitobion avenae* was used as a model phloem feeder for these experiments. The population of *Sitobion avenae* used for these experiments has been described in Chapter 2. Aphids were cultivated and maintained on oat plants (*Avena sativa* cv. Dula) in insect enclosures (as used for the plant cultivation) in a controlled environment (day length 16 h light, 8 h dark, light intensity 245 μmol m^{-2} S^{-1}, 18 ^{o}C, humidity 50%) before inoculating the experimental wheat genotypes. This protocol was to avoid confounding effects of previously exposing aphids to wheat plants which can later influence aphid performance (Leather *et al.* 1981). The plants were inoculated with the cereal aphid *Sitobion avenae* at growth stage 25 (GS25) on the Zadoks scale (Zadoks *et al.* 1974).

Each experimental plant replicate had one clip cage (25 mm diameter x 15 mm deep) placed two thirds of the way along a wheat leaf (MacGillivray & Anderson 1957). The clip cages were placed on the midsection of young leaves, which is the most suitable plant organ at this growth stage to assess aphid performance (Watt 1979). Individual *Sitobion avenae* adults were caged in the clip cages and checked every 24 hours until nymphs were produced. The adults were removed post reproduction leaving one nymph of the same age (produced within 24h of each other) on each of the experimental plants. The clip cages were checked and aphids observed every 24 h to monitor aphid performance.

The aphid performance traits monitored were development time, seven day fecundity and the intrinsic rate of increase. **Development time** was recorded as the time from nymph birth to reproduction as an adult (Lamb 1992).

Aphid fecundity 7d^{-1} was calculated following parthenogenetic reproduction of an apterous adult, by recording the number of nymphs removed every 24 h for a period of seven days (Lamb *et al.* 2009; Pointeau *et al.* 2015).

The **intrinsic rate of increase** was calculated using the equation: $\boldsymbol{R_m = 0.738}$ ($\mathbf{ln}\ \boldsymbol{M_d / d}$), where **d** is the development time in days and $\boldsymbol{M_d}$ is the number of progeny produced in a period equal to ***d***, the constant 0.738 is an approximation of the proportion of the total fecundity produced by a viviparous female in the first ***d*** days of reproduction (Wyatt & White 1977).

4.2.8 Aphid settling behaviour and post settling fecundity experiment – Experiment two

4.2.9 Experiment design

The second experiment was used to monitor aphid settling behaviour and post settling fecundity on two contrasting SavRia genotypes in three replicated choice arenas (Table 4.1). Aphid settling behaviour was observed on the two SavRia genotypes on which there was the highest and lowest aphid performance during the first experiment. The dependent variables for the second experiment were aphid settling behaviour and post settling fecundity after 24 h. The independent variables were SavRia genotype and chlorophyll concentration (Table 4.1).

During this experiment, aphid settling behaviour and post settling fecundity was observed on genotypes 25 and 43 which had the lowest and highest aphid performance in the first performance experiment respectively. Aphid settling behaviour was defined as aphids being stationary on a leaf with their antennae swept back over their bodies indicative of phloem acceptance and ingestion (Prado & Tjallingii 1994).

Post settling fecundity was the number of nymphs produced on a genotype in a 24 h period, which is indicative of aphid post settling fecundity on that genotype (Singer 2000, Powell, Tosh & Hardie 2006). The dependent variables were aphid settling behaviour and post settling fecundity. The independent variables were plant genotype and chlorophyll concentration.

4.2.10 Experimental procedure

There were three choice arenas each with four replicates of the SavRia genotypes (Table 4.1). The plants were grown using the same protocol for the first performance experiment and inoculated with aphids when the SavRia genotypes were at GS25-Zadoks scale (Zadoks *et al.* 1974). One hundred and twenty adult apterous aphids were introduced into each choice arena on a Petri-dish base that was placed on top of the lid that was fitted into the choice arena platform (Figure 4.1).

The aphids used for the observations on settling behaviour and post settling fecundity were adult apterae of the same age, cultivated using the following protocol. Aphids were collected from the stock population and cultivated for 24 h in insect enclosures (as described for the first aphid performance experiment) on oat plants (*Avena sativa* cv. Dula) that were grown for three weeks in the same controlled environment as the first aphid performance

experiment. The apterous adults were then removed from the plants and the nymphs that were produced within 24 h of each other cultivated for a further 8 days. The average development time for aphids under these conditions was 8 days ± 1 day (*personal observations*). This protocol was used so that all experimental aphids belonged to the same age class, and to avoid confounding maternal effects of previously exposing aphids to wheat plants. This new population of apterous adult aphids of the same age (produced < 24 h of each other) were starved for 12 h in empty Petri dishes (Powell 1993), before being introduced into the insect choice arena in the base of a Petri dish (Figure 4.1).

For observing aphid settling behaviour the choice arena had a Petri dish lid in the centre of the platform, with plant leaves equidistant from the centre of the Petri dish. Each genotype had the flag leaf inserted through the platform alternately. The aphids were introduced to the choice arena on a Petri dish base that was placed on top of the lid, which was already fitted in the platform (Figure 4.1). The number of adult aphids settled on each genotype was observed and recorded. Observations were made 5, 10, 15, 30 60, 240, 480, 720 and 1440 minutes after release into the choice arena. Aphids initiate probing and feeding behaviour faster following a period of starvation (Powell 1993). Differences in aphid settling behaviour are most evident during the first 24 h following inoculation of the host plant (Pompon & Pelletier 2012). One observation was made 24 hours after aphids were released into the choice arena, at which time the number of nymphs produced on each genotype after 24 h was recorded. The plant chlorophyll content (SPAD reading) was also recorded 24 h following inoculation with aphids.

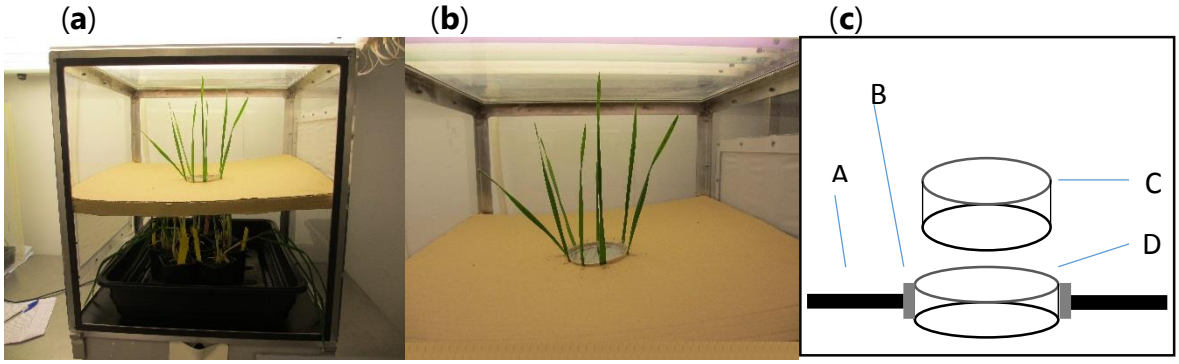

Figure 4.1. **a**) The layout of the aphid choice arena comprising a simple replicate arena. **b**) Each plant genotype had the flag leaf inserted alternately through the platform, equidistant from the centre of the Petri dish. **c**) The layout of the Petri dish. A: platform, B: rubber band, C: Petri dish base where aphids were introduced, D: Petri dish lid which was fitted into the platform for the base to be placed on.

4.2.11 Confirmation of aphid performance on two contrasting SavRia genotypes –experiment 3

Observations on aphid performance were repeated in a third experiment on two contrasting SavRia genotypes replicated six times to confirm aphid performance on SavRia GT 25 & GT 43 following the settling experiment. The dependent variables were as described for the first aphid performance experiment, while the independent variable was SavRia genotype (Table 4.1). The experimental procedure for the plants and aphids was identical to that described for the first aphid performance experiment.

4.2.12 Statistical analysis

4.2.13 Plant traits

A Pearson product-moment correlation coefficient was computed to assess the relationship between nitrogen content of plant biomass grown in a field trial and the nitrogen content of plants grown in this laboratory study.

All data that were analysed using ANOVA were first tested using Levene's test for equality of variances (Levene 1960). A Shapiro-Wilk test was used to test data for normal distributions (Shapiro & Wilk 1965). The test for equality of variances was found to be violated for the plant trait: height (cm). Therefore a non-parametric Kruskal Wallis test was conducted. A one-way ANOVA comparing plant traits between genotypes was conducted on number of tillers, plant biomass (g) and nitrogen uptake in genotypes that were grown in the laboratory with genotype as a fixed factor. Post hoc comparisons using the Tukey HSD test indicated differences between genotypes analysed using ANOVA.

The Pearson product-moment correlation coefficients were computed for the plant traits: height, number of tillers, biomass, chlorophyll concentration (SPAD reading), and nitrogen content. A bivariate linear regression was conducted to test if nitrogen content in the plant biomass predicted the chlorophyll concentration in the plant leaves.

4.2.14 Aphid performance traits

The Levene test for equality of variance was used to test aphid performance traits development time, fecundity and intrinsic rate of increase (Levene 1960). A Shapiro-Wilk test of normality was used to test the aphid performance traits (Shapiro & Wilk 1965). A non-parametric Kruskal Wallis

test was conducted on non-normally distributed data. The *S. avenae* performance parameters of fecundity and the intrinsic rate of increase were dependent variables analysed using one-way ANOVA with genotype as a fixed factor. A bivariate regression of aphid fecundity on chlorophyll concentration in the mid-section of the leaves where the aphids were cultivated in clip cages was performed. All statistical analyses on plant traits and aphid performance parameters were performed using SPSS (2013). Figures were produced in Microsoft Excel version (2013).

4.2.15 Aphid settling behaviour

The aphid settling data were modelled using a generalised linear model (GLM) with a log link and a Poisson error structure for the aphids settled. The residuals were plotted for the different error structures, and the Poisson error structure offered the best fit for these data. SavRia genotype and time were the predictor variables with aphids settled as the response variable. A Hosmer and Lemeshow goodness of fit test was used to compare the modelled values with observed values (Pulkstenis & Robinson 2002). A Wilcoxon ranked sum test was used to compare aphid fecundity between the two genotypes. These analyses were performed using R environment for statistical computing (R Core Team 2015). Figures for the modelled GLM were produced in R environment using GGplot (Wickham 2009).

4.3 Results

4.3.1 Differences in plant nitrogen uptake by SavRia genotypes between field and laboratory studies.

The rank order of genotypes based on increasing nitrogen content in the plant biomass between the field data and laboratory data are very different (Figures 4.2 & 4.3).

There was no significant correlation between these data (r=0.219, n=11, P=0.082). The plants grown in the laboratory had a consistently higher nitrogen content in the plant biomass with a higher range (mean±1SE: 45.62±0.477 gN kg BM^{-1}, variance=14.6, range=18.34) than the plants grown in the field ($\bar{X}$±1SE: 11.75±0.053 gN kg BM^{-1}, variance=0.27, range=3). The nitrogen content (gN kg BM^{-1}) of the dry plant biomass (BM) grown in the laboratory for this study was significantly different between genotypes ($F_{\{10,53\}}$ =3.403, P =0.02; Fig 3). The SavRia genotype 43 has the lowest average nitrogen content in the above ground plant biomass ($\bar{X}$±1SE: 41.94±1.1), and was significantly different from genotype 90 (49.76±1.33), and the Rialto parent which had the highest nitrogen content (50.95±0.81) (Figure 4.3).

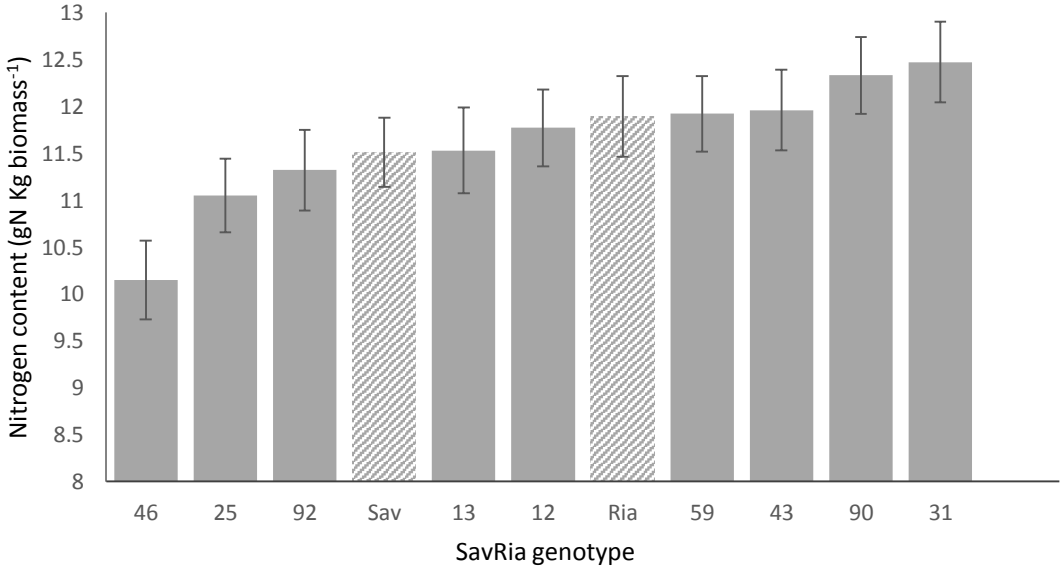

Figure 4.2. The mean nitrogen content of the plants (gN kg BM^{-1}) grown in a field trial for each of the nine genotypes used in this study and both of the parental cultivars Savannah (Sav) and Rialto (Ria). Genotypes arranged from left to right by increasing nitrogen content in the plant biomass (gN kg $biomass^{-1}$) from a field trial. Unpublished data supplied by Luzie Wingden from a JIC field study. $\bar{X}$±1SE.

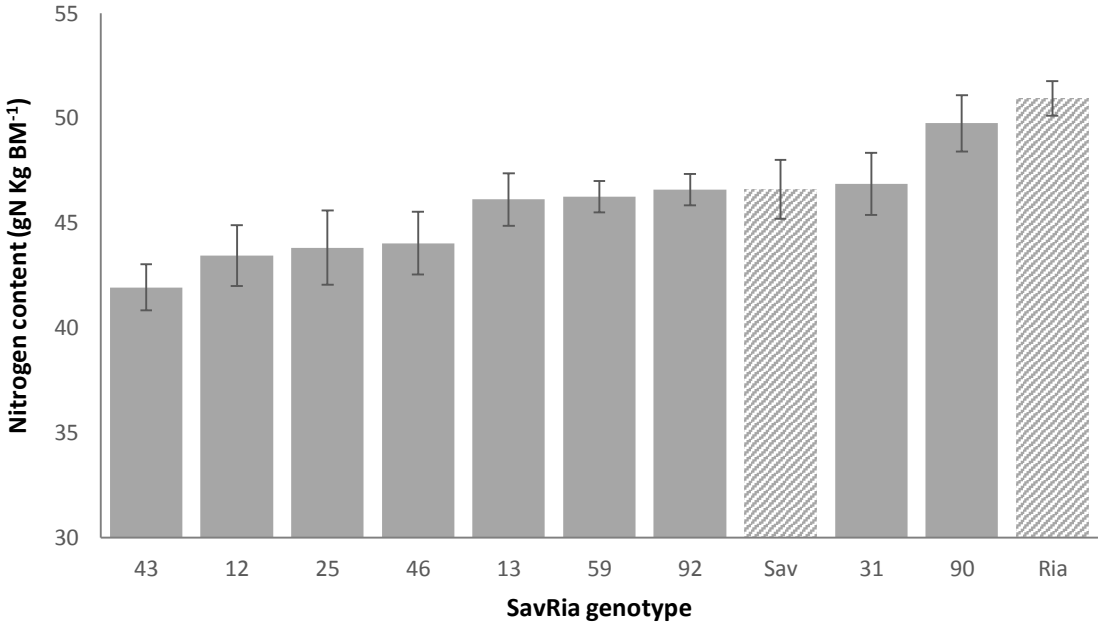

Figure 4.3. Then mean nitrogen content (gN kg BM^{-1}) of the aerial plant tissue grown in the laboratory (Y-axis) for each SavRia genotype (X-axis) arranged from left to right by increasing nitrogen content (gN kg BM^{-1}) in the plants. The parental varieties Savannah (Sav) and Rialto (Ria) are displayed with hatched bars ▨. $\bar{X}$±1SE.

4.3.2 Variation in plant traits grown in the laboratory

All of the plant traits were normally distributed (W=0.9, *P*>0.05), with the exception of the number of tillers $plant^{-1}$ which was approximately normal (Appendix II). The test for equality of variances was found to be violated for the plant trait: height (cm) (Levene's statistic=3.296, d.f 10, 57, P<0.05). The Kruskal-Wallis test showed the distribution of plant height was not different between genotypes (*P*>0.05). All of the remaining plant traits were analysed using ANOVA, and had equal variances.

There was a significant difference in chlorophyll concentration in the plant leaves, and nitrogen content in the plant biomass between SavRia genotypes (Table 4.3, Figure 4.4). The chlorophyll concentration in the parental variety Rialto ($\bar{X}$±1SE: 44.63±1.44), was significantly higher than SavRia GT 25 (37.16 ±1.52), and GT 43 (37.25 ±0.85). The nitrogen content in the plant biomass of SavRia GT 43 ($\bar{X}$±1SE: 41.94 ±1.12) was significantly lower than the Rialto parent (50.95±0.81). There was no significant difference in the number of tillers $plant^{-1}$ or in plant biomass between SavRia genotypes (Table 4.3).

Table 4.3. The one-way ANOVA results testing for differences in plant traits between SavRia genotypes. The sample size (n) and the mean sum of squares between groups are displayed. Significant differences are in italics ($P<0.05$), and bold italics ($P<0.01$).

Plant trait	n	sum of squares	F-stat	d.f.	*P*
No. tillers	11	10.76	0.812	10,57	0.61
Biomass (g)	11	142.66	1.41	10,57	0.2
Chlorophyll concentration	11	201.60	2.169	10,57	*0.03*
N-content (gN kgBM^{-1})	11	359.67	3.403	10,53	***0.002***

There was a significant correlation between plant height and biomass ($P<0.05$), number of tillers and biomass ($P<0.01$), and nitrogen content and biomass ($P<0.01$). There was also a significant correlation between chlorophyll concentration and nitrogen content ($P<0.01$) (Table 4.4).

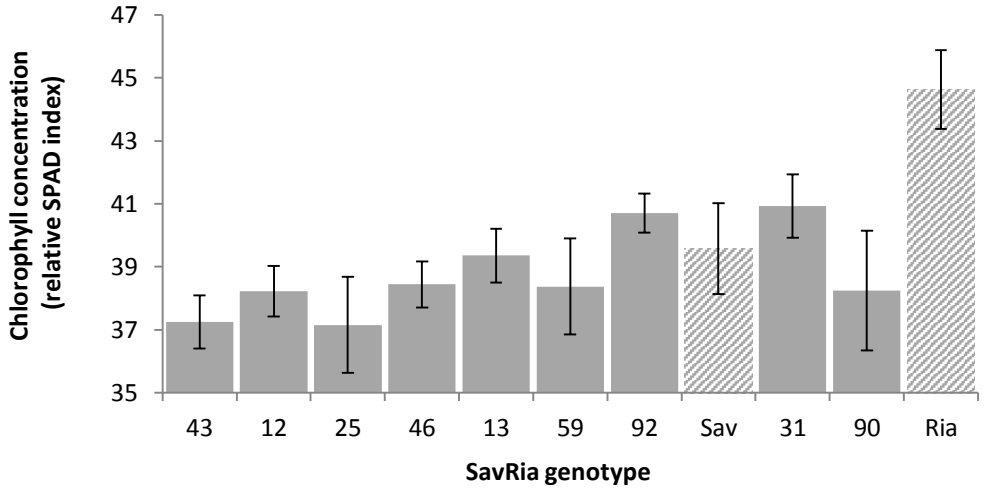

Figure 4.4. The chlorophyll concentration (relative SPAD index - no units) in plant leaves (Y-axis) of the SavRia genotypes and their parental varieties ▨ Savannah (Sav), and Rialto (Ria) (X-axis). SavRia genotypes arranged from left to right ordered by increasing nitrogen content (gN kg BM^{-1}) in the plant biomass that were grown in the laboratory (as in Figure 4.3). Error bars ± 1 standard error.

Table 4.4. A correlation matrix of plant traits. Values are Pearson correlation coefficients (r). Sample size for all plant traits were n=11, Significant correlations are in italics ($P<0.05$), and bold italics ($P<0.01$).

	Plant height (cm)	No. of tillers	Biomass (g)	chlorophyll concentration
Plant height (cm)				
Number of tillers	0.135			
Biomass (g)	*0.244*	***0.565***		
Chlorophyll concentration	-0.193	0.001	0.032	
N-content (gN kg BM^{-1})	-0.156	-0.041	***-0.306***	***0.671***

4.3.3 Plant nitrogen uptake in this laboratory study.

Variation in the nitrogen content of the plant biomass was analysed in relation to association with *S. avenae* performance (Figure 4.3). The nitrogen content in the plant biomass explained 45% of the variation in the plant's chlorophyll concentration (Figure 4.5).

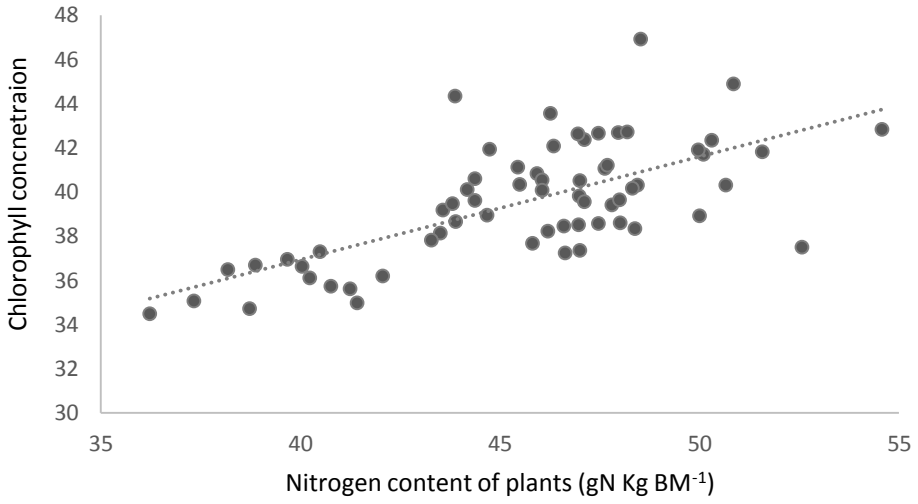

Figure 4.5. The chlorophyll concentration (relative SPAD index - no units) in plant leaves (Y-axis) against nitrogen content in the above ground plant biomass (gN kg BM^{-1}) (X-axis) Laboratory data. ($R^2=0.45$, $F_{(1,62)}=50.84$, $P<0.01$).

4.3.4 Measuring S. avenae *performance cultivated on nine SavRia genotypes - Experiment 1*

The aphid performance traits fecundity and the intrinsic rate of increase were normally distributed. However, the development times of aphids were non-normally distributed (W=0.690, d.f=10, $P<0.05$) (Appendix II).

The development time of aphids was not significantly different between SavRia genotypes or parental varieties ($P>0.05$). It is notable that the highest fecundity and intrinsic rate of increase of *S. avenae* are observed on genotype 43 with the lowest nitrogen content in the plant biomass, and the lowest *S. avenae* performance was observed on the Rialto parent which had the highest nitrogen content in the plant biomass (Figs 4.3, 4.6 a. & b). The *S. avenae* performance parameters a) fecundity $7d^{-1}$ and b) intrinsic rate of increase (r_M) were significantly different between genotypes (Table 4.5; Figure 4.6 a. & b). SavRia genotype 25 had the lowest fecundity ($\bar{X}$±1SE: 15.15±0.76) except for the parental variety Rialto ($\bar{X}$±1SE: 13.07±2.87), genotype 43 had the highest fecundity (27±2.1). A similar pattern to aphid fecundity is observed for the intrinsic rate of increase, whereby GT25 has the lowest intrinsic rate of increase (0.266±0.006) except the Rialto parent (0. 240±0.024) with genotype 43 having the highest rate of increase (0.343±0.014).

Table 4.5. Comparison of mean aphid performance traits between nine SavRia genotypes and both parental cultivars. Kruskal-Wallis test results for development time. One-way ANOVA test results for fecundity $7d^{-1}$ and intrinsic rate of increase. Significant *P*-values in italics ($P<0.05$), bold italics ($P\leq0.01$).

Aphid performance trait	n	Range	X^2	Mean sum of squares	F-stat	d.f.	P-value
Development time	11	6.00	13.4	_	_	10	0.202
Fecundity $7d^{-1}$	11	25.48	_	88.05	2.594	10,57	***0.01***
Intrinsic rate of increase	11	0.19	_	0.004	2.361	10,57	*0.02*

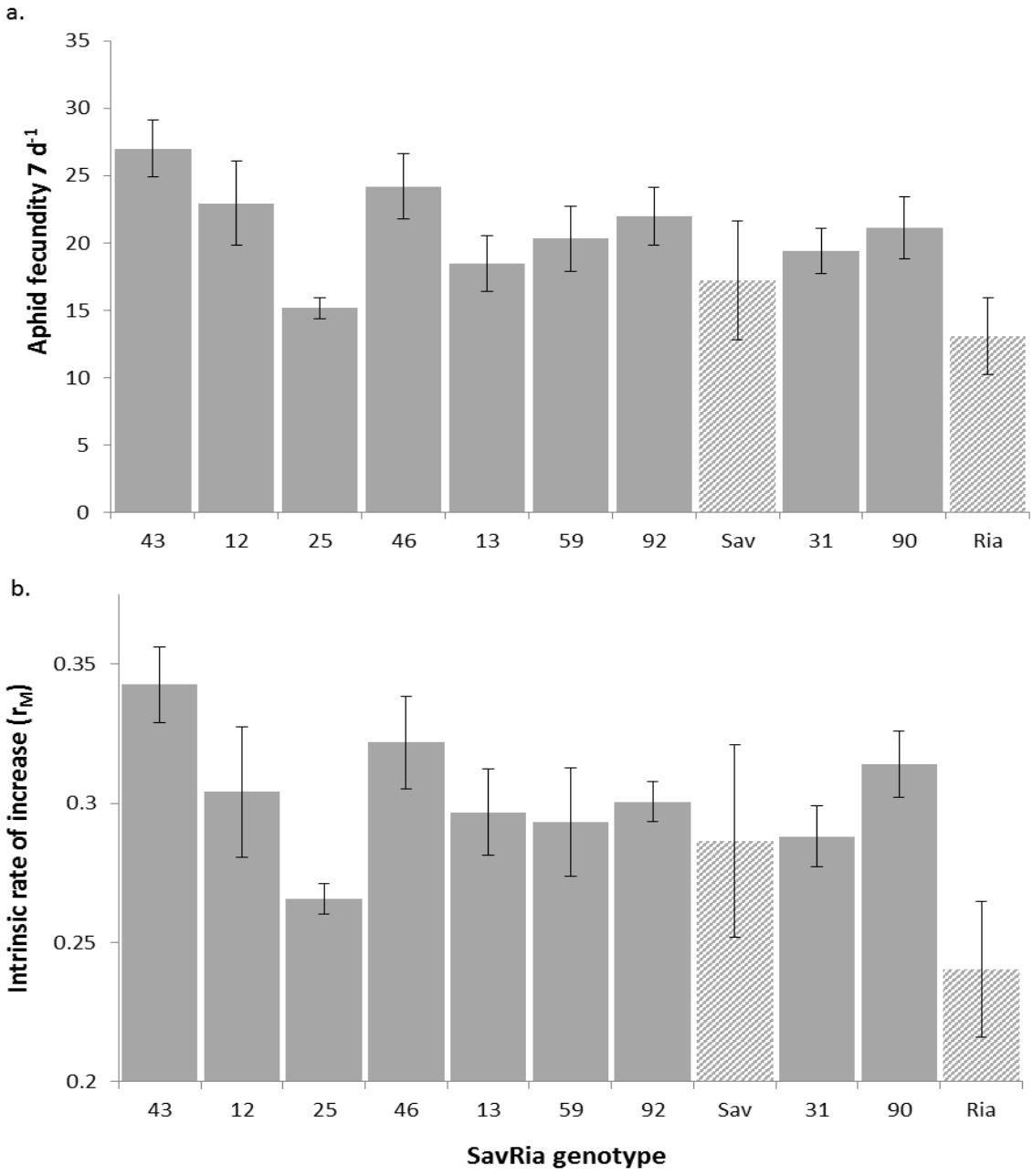

Figure 4.6. **a**: Mean ±1SE *Sitobion avenae* fecundity $7d^{-1}$. **b**. The mean intrinsic rate of increase (r_M) of *S. avenae*. X-axes categories are the nine SavRia winter wheat genotypes and their parental varieties (▨ Savannah (Sav), and Rialto (Ria)) on which aphids were cultivated. SavRia genotypes ordered from left to right by increasing nitrogen content (gN kg BM^{-1}) in the plant biomass that were grown in the laboratory (as in Figure 4.3).

The chlorophyll concentration in SavRia plants explains 9% of the variation in fecundity (Figure 4.7). Regression analysis on the intrinsic rate of increase for aphid populations against the nitrogen content in the above ground plant biomass was not significant (R^2 = 0.001, $F_{(1,52)}$ =0.69, *P*=0.794).

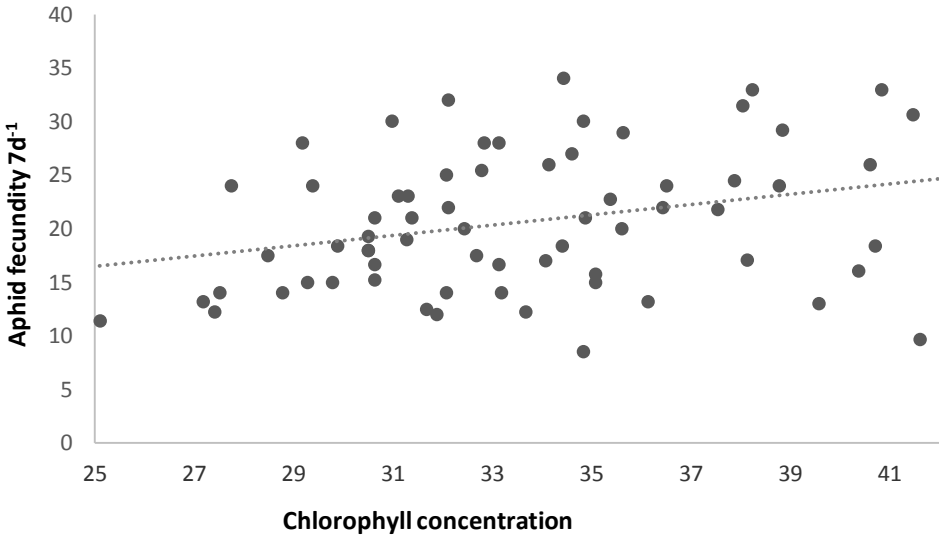

Figure 4.7. The mean fecundity $7d^{-1}$ of aphids cultivated on the nine SavRia genotypes and both parental cultivars against chlorophyll concentration (relative SPAD index - no units) (X-axis). The chlorophyll concentration data were measured in the mid-section of leaves where the aphids were cultivated in clip cages. (R^2 = 0.09, $F_{(1.66)}$ =6.559 *P*=0.01).

4.3.5 Measuring Sitobion avenae *settling behaviour and post settling fecundity on two SavRia genotypes*

Results of aphid settling behaviour observed on two SavRia genotypes chosen to represent genotypes associated with the highest (GT 43) and lowest (GT 25) *S. avenae* performance parameter of fecundity measured in experiment one are given in Figure 4.6a. There was a significant negative effect of genotype on aphids settled $leaf^{-1}$ (Figure 4.8; Table 4.6). A Hosmer and Lemeshow goodness of fit test showed the fitted model values were not significantly different from the observed data (X^2 = -0.35, d.f = 8, *P*-value = 1). Consistently more aphids settled on genotype 25 at each time point. Higher aphid performance parameters of fecundity and the intrinsic rate of increase had been observed on genotype 43 in the first aphid performance experiment (Figs 4.6a & b). However, settling behaviour did not follow the same trend. There were fewer aphids settled on the leaves of GT 43 than GT

25 over time in the aphid settling experiment. Post settling fecundity of *S. avenae* on GT 25 ($\bar{X}$±1SE: 2.88±0.49) was significantly higher than GT 43 (1.95±0.32) after 24h in the aphid settling experiment (Figure 4.9). The chlorophyll concentration in the wheat leaves was not significantly different between genotypes 25 & 43 in the aphid settling experiment (Table 4.7).

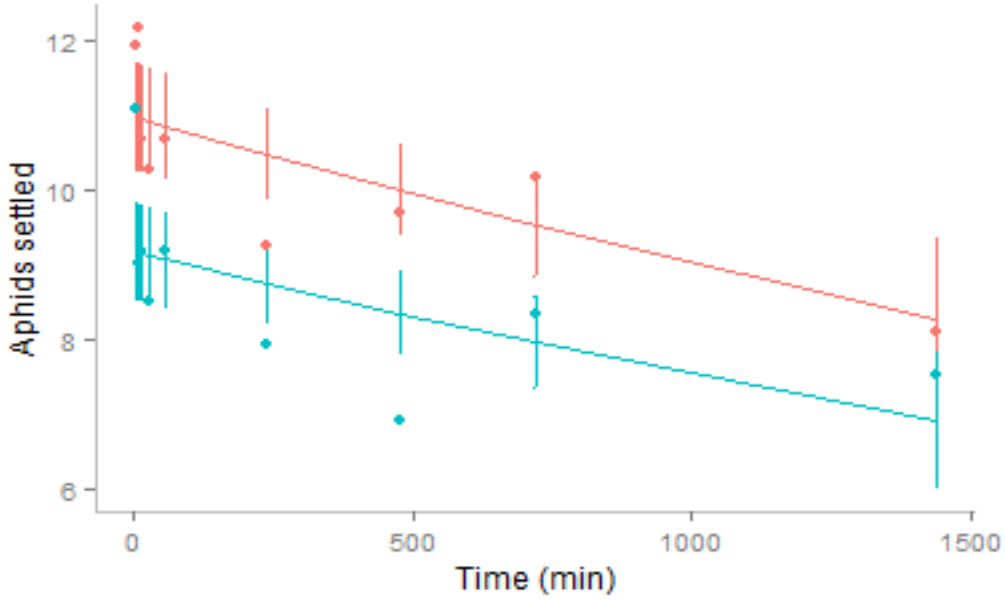

Figure 4.8. Observed mean number of aphids settled leaf^{-1} on SavRia GT 25 and GT 43 (Y-axis).The lines are predicted model values fitted for SavRia GT 25 and GT 43 from a Poisson generalised linear model. Time in minutes (X-axis). The bars indicate (upper and lower) 95% confidence intervals.

Table 4.6. The Poisson generalised linear model results. The Response variable was aphids settled leaf^{-1}. Poisson regression coefficients beta (*ß*) for each predictor variable, standard error, 95% confidence intervals for the coefficients, z-statistic and *P*-values.

			95% confidence interval			
Predictor	***ß***	**S.E**	Lower	upper	**Z**	***P*-Value**
Genotype	-0.009970	0.0050	0.981	0.999	-2.021	0.043
Time (min)	-0.000197	0.0001	0.999	1.000	-1.907	0.056

Table 4.7. Chlorophyll concentration (relative SPAD index-no units) in the leaves of each genotype during the aphid settling experiment. ANOVA test results.

GT	Trait	n	Mean	SE	F-stat	d.f.	*P*-value
25	Chlorophyll concentration	2	44	1.04			
43	Chlorophyll concentration	2	47	0.8	4.12	1,22	0.055

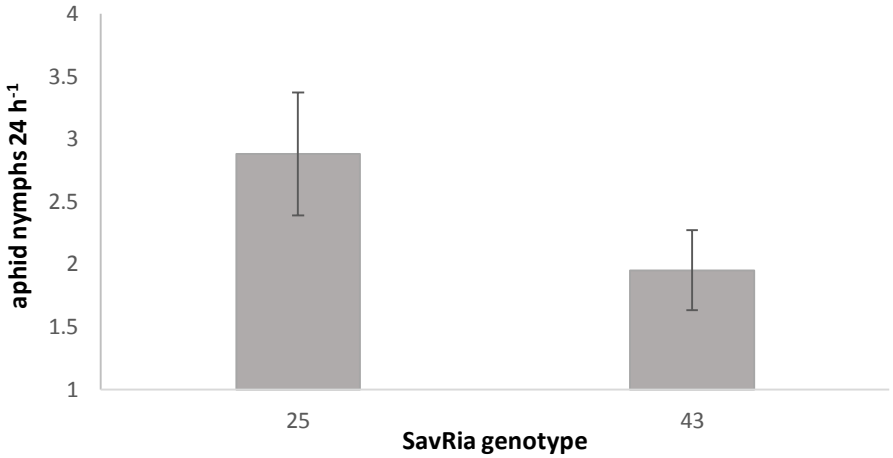

Figure 4.9 Post settling fecundity of *S. avenae* indicated by aphid nymphs produced adult^{-1} after 24 h (Y-axis) on SavRia genotypes (X-axis). (Wilcoxon ranked sum W = 0, *P* = 0.03) Error bars ± 1 standard error.

4.3.6 Confirmation of aphid performance on two SavRia genotypes- experiment three

The aphid performance parameters of development time, fecundity 7d^{-1} and the intrinsic rate of increase were not significantly different between the SavRia genotypes 25 and 43 (Table 4.8).

Table 4.8. Comparison of mean aphid performance parameters of development time, fecundity 7d^{-1} and the intrinsic rate of increase between two SavRia genotypes. One-way ANOVA test results.

Aphid performance trait	n	Range	Mean sum of squares	F-stat	d.f.	*P*-value
Development time	2	6	6.75	3.716	1,10	0.083
Fecundity 7d^{-1}	2	13.13	0.676	0.047	1,10	0.833
Intrinsic rate of increase	2	0.25	0.049	0.039	1,10	0.848

4.4 Discussion

The winter wheat genotypes used in this experiment had different nitrogen uptake efficiencies in the field. It was hypothesised that there would be a significant positive correlation between nitrogen content in plant biomass of the SavRia genotypes in the field and laboratory. It was also predicted that aphid performance parameters would be significantly higher when aphids were cultivated on wheat genotypes with higher nitrogen uptake efficiencies, as indicated by nitrogen content in the above ground plant biomass. These predictions reflect the assumption that plants with higher nitrogen uptake would have a higher concentration of nitrogen in the plant phloem. Sucking insect herbivores are strongly influenced by nitrogen content in their host plants (White 1984). Cereal aphid populations are positively affected by nitrogen application in the field (Gash 2012, Wang *et al.* 2015) and in the laboratory (Aqueel and Leather 2011). Aphid performance is positively influenced by the amino acid profile and nitrogen concentration in plant phloem (Ponder *et al.* 2000, Karley *et al.* 2002). Therefore aphid performance on wheat genotypes with higher nitrogen uptake can be predicted to reflect the effects of increasing nitrogen fertiliser application.

Nitrogen uptake during the laboratory experiment was very different form the nitrogen uptake during the JIC field trial. The nitrogen content of plants in the laboratory was several times higher than that in the field (Figs 4.2 & 4.3), with a much higher range of values. However hypothesis one that there will be a significant positive correlation between nitrogen content in plant biomass of the SavRia genotypes in the field and laboratory was not supported. Nitrogen uptake efficiency is a variable trait that is influenced by the amount of nitrogen in the soil (Moll *et al.* 1982, Gaju *et al.* 2011). The different edaphic conditions between the field soil and soil in plant pots may account for some of these differences. The rank orders of SavRia genotypes based on nitrogen content in the plant biomass between the field and laboratory plants were not significantly associated (Figs 4.2 & 4.3). There is a genotype/environment interaction for the trait nitrogen uptake in wheat (Gaju *et al.* 2011), an interaction that has also been observed in other crops (Barraclough *et al.* 2009). The interaction between wheat genotype and environmental conditions could have affected the rank ordering of SavRia genotypes based on nitrogen content in plants, which had been grown in the different environments.

A possible explanation is that the growth stage at which the nitrogen content in the plant biomass was sampled may have affected the results. The field plants were sampled at harvest when nitrogen had been remobilised and

utilised for grain production (Simpson *et al.* 1983, Karrou & Nachit 2015). The nitrogen content in laboratory grown plants was measured at growth stage 25 when plants are taking up nitrogen (Zadoks *et al.* 1974, Simpson *et al.* 1982). A second possible explanation of the differences in nitrogen content of field and laboratory grown plants is that wheat is very efficient at remobilising nitrogen in the plant tissue and utilising it for grain production (Ladha 2005, Barraclough *et al.* 2010). Following grain production very little nitrogen remains in the plant biomass (Gaju *et al.* 2011). This could explain the substantially lower amount of nitrogen in the plant biomass in the field samples. These factors suggest it is not appropriate to extrapolate the results of this laboratory data to a field situation, without first investigating the nitrogen content of plant biomass in both environments.

There was no significant difference in plant biomass or the number of tillers between the SavRia genotypes grown in the laboratory (Table 4.3) when the growth stage of plants was controlled. Cereal aphid performance can be affected by plant growth stage for *R. padi* (Leather & Dixon 1981) and *S. avenae* (Watt 1979). Therefore, particular care was taken to grow plants under the same environmental conditions in the same soil type so homogenising growth stage and nitrogen availability between genotypes.

The cereal aphid *S. avenae* is predominantly an ear feeding species but will also feed on wheat leaves (Watt 1979). The part of the plant on which aphids are cultivated also affects aphid performance. For *S. avenae* cultivated on wheat plants the development time was shortest and fecundity $7d^{-1}$ was highest when cultivated on the ears of plants followed by the young leaves (Watt 1979). During this experiment, aphids were confined in clip cages on young plant leaves (MacGillivray & Anderson 1957), as the most suitable part of the plant at this growth stage for assessing aphid performance (Watt 1979). The aphids were restricted from the whole plant with nymphs regularly removed from clip cages to reduce effects of spatial and density dependent constraints (Watt 1981). Therefore it was predicted that plant size variables would not be related to aphid performance, as was found to be the case.

The JIC field trial showed SavRia genotypes had different nitrogen uptake efficiencies in the field (JIC archive 2011). Differences in nitrogen content in the plant biomass in the laboratory between SavRia genotypes was significant (Table 4.3). These differences could be due to the genetic background of the SavRia genotypes, which may have different genes influencing physiological plant traits that control nitrogen uptake between genotypes (Barraclough *et al.* 2010). The winter wheat *cv.* Rialto and SavRia genotype 43 had the highest and lowest nitrogen content in the plant biomass respectively (Figure 4.3). The Rialto parent has been shown to have

high nitrogen uptake at all growth stages (HGCA project report 1998). The chlorophyll concentration in the plant leaves was the highest in the Rialto parent and was significantly different from that in SavRia genotypes 25 and 43 which had the lowest chlorophyll concentrations in the leaves (Figure 4.4).

Notably there was a significant correlation between nitrogen content in the plant biomass and chlorophyll concentration in the plant leaves (Table 4.4). These results support hypothesis two that the chlorophyll concentration in SavRia plant leaves will be positively correlated with the nitrogen content in the total above ground plant biomass of SavRia genotypes. The nitrogen content in the plant biomass explained 45% of the variation in chlorophyll concentration in the plant leaves (Figure 4.5). There is a significant linear relationship between nitrogen content and chlorophyll concentration in cereal leaves (Dwyer *et al.* 1991; Markwell *et al.* 1995). The aerial plant organs including leaves and stems were used for measuring nitrogen content in plant tissue. The chlorophyll measurements were taken from plant leaves only. A higher amount of variation in chlorophyll concentration may have been explained by nitrogen content if nitrogen content had been measured in the plant leaves only. There was ample variation in nitrogen and chlorophyll concentration to investigate their effects on *S. avenae* performance.

The third hypothesis that, *S. avenae* performance will be higher as indicated by a shorter development time was not supported. The development times of *S. avenae* were not significantly different between genotypes in this experiment (Table 4.5). The development time of aphids from birth to reproduction is influenced by many factors including abiotic conditions (Mcvean & Dixon 2001) and plant growth stage (Watt & Dixon 1981) which were both standardised and controlled during the experiments. The nitrogen content in the plant tissue from the SavRia genotypes were significantly different (Figure 4.3; Table 4.3). This difference in nitrogen content may not have influenced the nutritional quality of the SavRia genotypes in a way which effected aphid development time, because this is also affected by the profile as well as the concentration of amino acids in the plant phloem (Ponder *et al.* 2000). The development time of *S. avenae* in the laboratory was similarly not affected by application of nitrogen fertiliser in laboratory experiments (Aqueel and Leather 2011). It is well established that the nutritional quality of host plants has a greater effect on fecundity than on the development time of aphids (Karley *et al.* 2002).

The highest aphid performance, in terms of both seven day fecundity and the intrinsic rate of increase, was observed on SavRia genotype 43, and the lowest values of these performance traits on the Rialto parent (Figure 4.6 a &

b). The lowest nitrogen content in the plant biomass was measured on SavRia GT 43, and the highest nitrogen content was measured in the Rialto parent (Figure 4.3). These results do not support the third hypothesis that, *S. avenae* performance will be higher with higher fecundity, and a higher intrinsic rate of increase, when cultivated on SavRia genotypes that have higher nitrogen concentration in the total plant biomass. Aphid performance in terms of fecundity has been found to benefit from increased nitrogen application rates (Aqueel and Leather 2011), and higher amino acid concentration in plant phloem (Ponder *et al.* 2000). Plants treated with higher nitrogen application rates have higher amino acid concentrations in the plant phloem (Caputo and Barneix 1997). Hypothesis three was underpinned by the assumption that plant genotypes with higher nitrogen uptake efficiencies would have higher nitrogen content in the plant biomass and benefit aphid performance in a similar way to plants that had more nitrogen fertiliser applied, or higher nitrogen content in the plant phloem.

Plant genotypes with higher nitrogen uptake efficiencies may not have higher amino acid concentration in the plant phloem, therefore they may not benefit aphid performance in a similar way to the increase in phloem amino acid concentrations resulting from higher nitrogen application rates (Karley *et al.* 2002, Nowak & Komor 2010). Aphid performance can be measured in a variety of different ways depending on the host plant, the growing environment and the aims of the study. In a field setting aphid population abundance may be the most important factor (Dixon 1998, Honek *et al.* 2006). In laboratory conditions aphid fecundity and the intrinsic rate of increase can be measured more accurately and therefore can be used to investigate the effects of host plant nutritional quality or plant resistance traits on aphid performance. In this laboratory study Wyatt & White's (1977) equation was used to calculate the intrinsic rate of increase. Aphid fecundity and development time are components of this index. The similar pattern observed between aphid fecundity $7d^{-1}$ and the theoretical intrinsic rate of increase could be an artefact of the equation used to calculate the rate of increase (Wyatt & Whites 1977) (Figure 4.6 a & b). SavRia genotypes with higher nitrogen content in the plant biomass do not have higher aphid performance parameters.

There was a positive correlation between chlorophyll concentration in the SavRia plant leaves and aphid fecundity $7d^{-1}$ (Figure 4.7). Chlorophyll concentration in the plant leaves explained 9% of the variation in aphid fecundity $7d^{-1}$ (Figure 4.7). Hypothesis four that *S. avenae* performance parameters a) development time, b) fecundity, and c) the intrinsic rate of increase will be positively correlated with chlorophyll concentration in SavRia plant leaves was supported for aphid fecundity. However, hypothesis five was

not supported for development time and the intrinsic rate of increase. The intrinsic rate of increase considers both development time and fecundity of aphids (Wyatt & White 1977). The nutritional quality of the host plant has greater effects on fecundity than on development time of aphids (Karley *et al.* 2002). Furthermore aphid development time varied little between the SavRia genotypes (Table 4.5). This may explain why only aphid fecundity was correlated with chlorophyll concentration in the midsection of plant leaves.

Chlorophyll concentration in plant leaves is positively correlated to nitrogenous compounds in the plant phloem (Lima *et al* 2006), and nitrogen fertiliser application rate (Caputo and Barneix 1997). Aphid fecundity is also positively correlated with these two factors (Karley *et al.* 2002, Aqueel and Leather 2011). These studies suggest that higher chlorophyll concentration in plants can benefit aphids, or it is positively correlated with plant traits shown to benefit aphid performance. A potential explanation for the low aphid fecundity on the Rialto parent, which had the highest chlorophyll concentration in the plant leaves at this growth stage (Figure 4.4) is the high number of leaf hairs present on this variety that have been shown to negatively influence aphid performance (Zarpas *et al.* 2006). The results from this study show chlorophyll concentration in plant leaves is positively correlated with aphid fecundity, and has a more positive effect than nitrogen content in the plant biomass.

The highest and lowest aphid performance parameters on SavRia genotypes were measured on SavRia GT 43 and GT25 respectively (Figure 4.6a & b). Aphid settling behaviour was significantly different between the two SavRia genotypes GT25 and GT43 (Figure 4.9, Table 4.6). Hypothesis five that a higher number of apterous *S. avenae* will settle on the SavRia genotype on which the *S. avenae* performance trait fecundity was previously found to be higher was not supported. More aphids settled on SavRia genotype 25 (Figure 4.9), which was previously associated with the lowest aphid fecundity relative to GT 43 on which aphid fecundity was the highest during the first aphid performance experiment (Figure 4.6a & b).

Aphids use a suite of complex plant cues that influence settling behaviour, which include morphological traits and chemical signals detected on the leaf surface (Prado & Tjallingii 1994, Wojcicka 2015). Aphids also use leaf colour and therefore chlorophyll concentration as a cue for settling and feeding behaviour (Doering *et al.* 2009). The chlorophyll concentration in the plant leaves was higher in SavRia genotype 43 (47±0.8) but was not significantly different from genotype 25 (44±1.04) during the settling experiment (Table 4.7). There was no relationship between aphid settling and chlorophyll concentration in the plant leaves of SavRia genotypes.

In natural aphid populations it is the alatae (winged) morphs that migrate and settle on new host plants (Watt & Dixon 1981, Dixon 1998). Therefore settling behaviour in aphids may be better assessed using alatae morphs, which weren't available in the number needed for this experiment. However, apterous *S. avenae* have been shown to disperse between plants, leaving their natal host in response to intrinsic plant factors and not as a response to spatial constraints or deteriorating plant quality (Holmes 1988).

The sixth hypothesis that post settling fecundity of S. avenae will be higher on the genotype on which the *S. avenae* performance parameter of fecundity is higher was not supported. There were significantly more nymphs produced on SavRia genotype 25 compared with GT 43 after 24 h (Figure 4.9). Higher post settling fecundity of aphids indicates aphid preference for that plant genotype (Powell, Tosh & Hardie 2006). Aphids produced more nymphs on GT 25 during the settling and post settling fecundity experiment. This is the converse of observations made on fecundity during the first performance experiment (Figs 4.6a & 4.9).

Given the incongruence between aphid settling and previous aphid performance on these two SavRia genotypes, a third experiment was used to confirm aphid performance parameters on SavRia genotypes 25 and 43 previously observed to have the lowest and highest performance parameters respectively during the first aphid performance experiment. The results of this experiment showed no significant differences in aphid performance parameters between SavRia genotypes 25 and 43 (Table 4.8). The lack of consistency in aphid performance between each of the aphid performance experiments could be due to plant factors that weren't measured. For example genotype 25 could have a defensive response to aphid infestation that takes longer than 24 h to influence aphid fecundity. Moreover, aphid settling and post settling fecundity may not be related to a defence response of the plant. These results suggest that intrinsic or extrinsic plant factors that weren't measured during these experiments may be more important in determining settling behaviour and post settling fecundity of apterous *S. avenae* than previous differences between genotypes that were associated with higher aphid performance.

In summary the absence of a correlation between nitrogen content in the plant biomass between the laboratory and field data suggests the findings of this study may not be extrapolated to a field situation. The lack of a positive relationship between aphid performance and the nitrogen content in the SavRia genotypes can be explained if plant nitrogen uptake does not increase concentration of essential amino acids in the plant phloem, or offer

other nutritional benefits to aphids. The results of this study supported the hypothesis that aphid fecundity will be positively correlated with the chlorophyll concentration in SavRia plant leaves. This suggests that leaf chlorophyll concentration which is correlated to nitrogen content in the leaves of cereals (Dwyer *et al.* 1991; Markwell *et al.* 1995) and to amino acid concentration in plant phloem (Caputo & Barneix 1997) may be a more important determinant of aphid performance than nitrogen uptake in wheat plants.

The wider implications of these findings for the other sections of this PhD are that the nitrogen concentration in plant biomass in the laboratory may not affect aphid performance in a similar way to increasing nitrogen application. The conclusions of this study have implications for the experiment described in thesis chapter five, in which the components of nitrogen use efficiency of six SavRia genotypes are quantified under different nitrogen application rates following the plants entire growth cycle. The results from quantifying nitrogen use efficiencies of SavRia genotypes will be used as a basis to investigate relationships between the components of nitrogen use efficiency and *Sitobion avenae* densities tiller^{-1} in chapter 6. A further wider-ranging implication of these results, is that breeding to increase nitrogen uptake efficiency in wheat genotypes may not have the predicted cost of increasing the risk of infestation of pests such as aphids.

Therefore these findings could have significant economic implications, because new varieties with increased nitrogen uptake efficiency may not be more prone to pest infestation as they would be if nitrogen content was increased by the application of nitrogen fertilisers. Wheat varieties with increased nitrogen uptake efficiency may therefore contribute to improving agronomic practice with apparently few costs in terms of increased aphid infestation potential.

5 Phenotypic components of nitrogen use efficiency in winter wheat genotypes under different nitrogen application rates

5.1 Introduction

A central question addressed in this thesis is "do differences in the nitrogen use efficiency of wheat alter the potential for infestation by aphids?" In order to address this question in the next chapter it is first necessary to quantify nitrogen use efficiency and its components of nitrogen uptake efficiency and nitrogen utilisation efficiency for the six SavRia genotypes and their parental cultivars 'Savannah' and 'Rialto' that are being used as a model system for examining genotypic variation in nitrogen use efficiency.

The growth and subsequent yield of plants can be sustainably improved with the addition of chemical nitrogen fertilisers (Chardon *et al.* 2010) the environmental costs of which are outlined in Chapter 1.

The nitrogen use efficiency of wheat is the amount of dry matter grain yield per unit nitrogen in the soil and applied as fertiliser (NUE: kg dry matter grain yield kg^{-1} N available) (Moll *et al.* 1982). In this context, nitrogen availability is estimated as the sum of the amount of nitrogen measured in the soil and the amount of nitrogen applied as fertilisers (Moll *et al.* 1982, Gaju *et al.* 2011). Nitrogen use efficiency and its components are introduced and described in Chapter 1 & 2. Wheat genotypes that have higher nitrogen use efficiencies produce higher yields from lower inputs of nitrogen fertiliser (Gaju *et al.* 2011; Karrou & Nachit 2015).

The components of nitrogen use efficiency are nitrogen uptake efficiency (NupE), which is the capacity of a plant to acquire nitrogen from the soil (NupE: kg above ground (AG) plant N content kg^{-1} N available) and nitrogen utilisation efficiency (NutE), which is the fraction of nitrogen content in the above ground plant tissue at harvest that is subsequently converted to grain yield (kg grain dry matter kg^{-1} AGN content the plant at harvest) (Moll *et al.* 1982). Increasing the components of nitrogen use efficiency can result in crops which are more efficient at taking up nitrogen and converting that nitrogen into grain yield (Moll *et al.* 1982; Gaju *et al.* 2011; Karrou & Nachit 2015).

The way in which plants respond to nitrogen availability is dependent both on the plant genotype and on the rate at which nitrogen fertiliser is applied. (Chardon *et al.* 2010). The factors that influence nitrogen uptake efficiency in wheat include features of root architecture, water availability, temperature (Powlson *et al.* 1992; Xu *et al.* 2012; Sedlar *et al.* 2015), developmental stage of the plant (Ladha 2005), the efficiency of absorption and assimilation of NH_4^+ and other nitrogen species in the soil (Kundu *et al.* 1996). The nitrate and ammonium transporter activity and their different affinities for nitrogen species in the rhizosphere are important determinants of the nitrogen uptake efficiencies of wheat genotypes (Xu *et al.* 2012). The availability of carbon provided by photosynthesis is also a key determinant of the efficiency of the root system in taking up nitrogen (Xu *et al.* 2012).

Possibly the most salient factors influencing nitrogen uptake efficiency in wheat are related to features of the root system such as, root mass, the age of roots and the distribution of roots (Sedlar *et al.* 2015). The level of soil nitrogen plays a significant role in the expression of traits associated with nitrogen uptake efficiency of wheat crops, as there is an interaction between wheat genotype and nitrogen application rate on the nitrogen uptake and utilisation efficiency of wheat crops (Powlson *et al.* 1992, Xu *et al.* 2012).

The factors influencing the nitrogen uptake efficiency are different from those that influence nitrogen utilisation efficiency of wheat plants (Karrou & Nachit 2015). The plant leaves are a sink for nitrogen during the vegetative stage; later in the plant's life cycle during leaf senescence this nitrogen is remobilised mainly as amino acids for grain production (Okumoto & Pilot 2011). The differences in a plants ability to mobilise, translocate and distribute absorbed nitrogen to and from plant organs in part defines nitrogen utilisation efficiency (Raun & Johnson 1999, Xu *et al.* 2012).

The import and export of nitrogen from the flag leaf and leaf senescence patterns play an important role in the nitrogen utilisation efficiency of wheat genotypes (Kundu & Ladha 1997). A recent study identified that the quantitative trait loci (QTL) associated with nitrogen remobilisation traits are also QTL associated with leaf senescence traits (Coque *et al.* 2008). Glutamine synthetase is an enzyme involved in catalysing the condensation of glutamate and ammonia to glutamine; it is essential for the nitrogen metabolism in wheat (Eisenberg *et al.* 2000). The importance of glutamine synthetase activity during the remobilisation of nitrogen as amino acids following anthesis was emphasised using quantitative trait loci (QTL) approaches (Bernard & Habash 2009). Efficient nitrogen remobilisation and assimilation post anthesis is required to achieve higher nitrogen use efficiency in wheat (Cox *et al.* 1985;

Karrou & Nachit 2015). Therefore, 'stay-green' phenotypes (wheat plants that senesce later in the life cycle) benefit nitrogen uptake capacity and subsequently utilisation of nitrogen into grain yield and quality (Hörtensteiner 2009, Distelfeld *et al.* 2014). The factors influencing the nitrogen uptake and nitrogen utilisation efficiency of wheat are both genetic and environmental (Ladha 2005; Xu *et al.* 2012; Karrou & Nachit 2015). Furthermore, these factors can interact with nitrogen application rate (Chardon *et al.* 2010), adding complexity to the selective breeding programs that aim to improve traits contributing to an increase in the nitrogen use efficiency of wheat.

Wheat improvement strategies that aim to increase nitrogen use efficiency are relevant to both pillars of breeding and agronomy. The components of nitrogen use efficiency, nitrogen uptake and nitrogen utilisation efficiency, are complex polygenic traits influenced by many different factors that can interact with the environment and nitrogen application rate (Ladha 2005, Xu *et al.* 2012).The genes that code for all the separate traits involved in nitrogen use efficiency are currently unknown in wheat (Ladha 2005, Okumoto & Pilot 2011). Many of the traits involved in nitrogen uptake and nitrogen utilisation efficiency appear to be independently inherited (Coque *et al.* 2008). Considerable effort must be dedicated to phenotyping these traits, at different nitrogen application rates and in different environments, particularly due to the interaction between environmental factors and nitrogen application rates (Karrou & Nachit 2015; Sedlar *et al.* 2015). The results of these studies can be used in wheat improvement programs to increase understanding of how nitrogen use efficiency can be increased, and how novel genes involved in the regulation of nitrogen use can be identified and incorporated into breeding programs.

There are clear prospects for increasing nitrogen use efficiency through improved agronomic practice (Sedlar *et al.* 2015). Providing better nitrogen and water management regimes, such as timing nitrogen applications with the plant requirements based on the developmental stage of the crop (Powlson *et al.* 1992; Ladha 2005; Sedlar *et al.* 2015). Meeting the crops water requirements will also improve nitrogen use efficiency (Xu *et al.* 2012; Karrou & Nachit 2015). Improving agronomic practice to increase nitrogen use efficiency can have many environmental benefits including a decrease in the leaching of nitrogen into the wider environment (Powlson *et al.* 1992; Karrou & Nachit 2015).

It is desirable to breed wheat genotypes with higher nitrogen use efficiencies. However, there are potential drawbacks in breeding to increase nitrogen use efficiency. One drawback is the trade-off between increasing wheat yields and

reducing the nitrogen concentration in the grain (Ortiz-Monasterio *et al.* 1997), consequently reducing the nutritional benefit and market value (Acuna *et al.* 2005, Ma *et al.* 2007). Increasing the components of nitrogen use efficiency in wheat genotypes also has the potential risk of increasing the infestation potential of nitrogen sensitive crop pests such as cereal aphids (Ladha 2005).

The aim of this study is to investigate the influence of nitrogen fertiliser application on the components of nitrogen use efficiency and yield characteristics of SavRia genotypes. The key objective is to quantify the components of nitrogen use efficiency as a basis for the next thesis chapter, which will aim to explore how cereal aphid densities respond to genotypes with higher nitrogen use efficiencies.

5.1.1 Hypotheses

1. Plant nitrogen content, grain nitrogen content, grain yield and grain nitrogen content will all be higher in plants grown under higher nitrogen application rates.

2. The nitrogen utilisation efficiencies of SavRia genotypes will increase with nitrogen application rate.

3. Different genotypes in the SavRia population will differ in nitrogen content, nitrogen uptake efficiency, nitrogen utilization efficiency, grain nitrogen content and grain yield.

4. The nitrogen uptake, utilisation and use efficiency of different SavRia genotypes will respond differently under different nitrogen application rates.

5. The variation in nitrogen use efficiency will be explained by the variation in nitrogen utilisation efficiency in the low and high nitrogen application rates.

5.2 Methods

5.2.1 SavRia genotypes

Doubled haploid SavRia winter wheat populations used in this field trial are described in detail in chapter 2. A subset of six of the nine SavRia genotypes used in the laboratory experiment reported in chapter 4 were selected to capture variation in nitrogen use efficiency (Table 5. 1). Of these genotypes 43 and 25 were characterised by the highest and lowest aphid performance respectively during the laboratory experiment (chapter 4).

Table 5. 1. The SavRia genotypes selected for this field experiment. Genotypes ordered by decreasing nitrogen content in the plant biomass. The units of Nitrogen use efficiency (kg dry matter grain yield kg^{-1} N available). Unpublished data supplied by JIC field trials (JIC archive 2011).

SavRia genotype	N-content (gN kg BM^{-1})	Nitrogen use efficiency
46	10.15	50.73
25	11.05	51.42
92	11.32	49.82
SAV	11.51	47.74
13	11.53	54.62
RIA	11.89	48.99
43	11.96	48.97
31	12.47	46.61

5.2.2 Experimental design

A randomised split plot design was used with three different nitrogen treatments (Table 5. 2). There were six SavRia genotypes and both parental varieties 'Savannah' & 'Rialto'. The genotypes were sown in 1 m^2 plots replicated five times in each nitrogen treatment (Table 5. 2). All 1 m^2 plots were inoculated with a laboratory reared population of *Sitobion avenae* as described in chapter 6. A five m^2 buffer area was sown with the winter wheat variety 'Paragon' between the nitrogen treatments to separate the experimental plots from the different nitrogen application rates (Figure 5.1).

Table 5. 2. Details of the site, soil type, and experimental design used for this field experiment.

Site		Soil		Drilling		Experimental Design				
location	coordinates	type	N content	depth (mm)	Density (Seed m^{-2})	N treatments	N application rates (kg N ha^{-1})	N.O SavRia genotypes	Plot size (m^2)	SavRia GT plot reps
Church Farm Norwich	5 2°37'40''N, 1°10'46''E	sandy clay loam	56.5 kg ha^{-1}	50	180	Control	0	6	1	5
						Low N	120	6	1	5
						High N	240	6	1	5

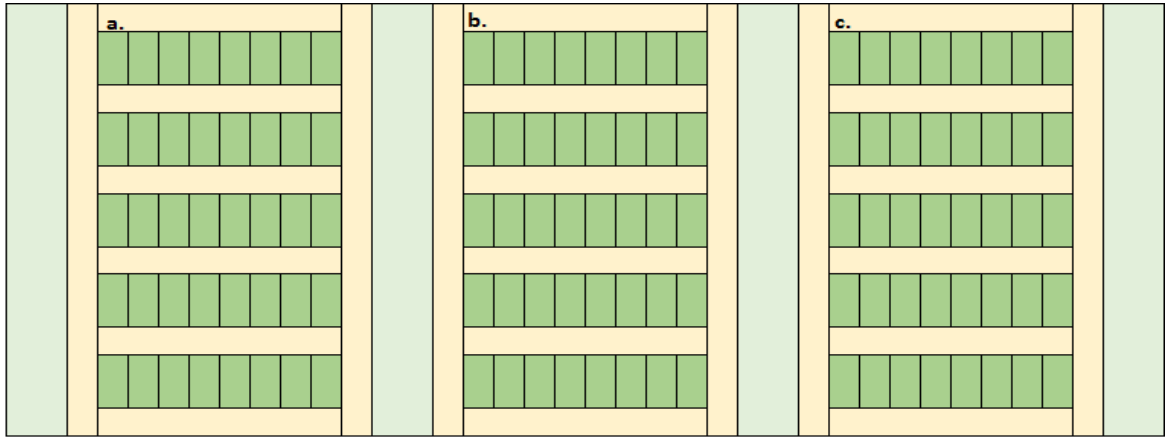

Figure 5.1. The layout of the experimental wheat plots □, planted with SavRia genotypes randomly arranged under each nitrogen application rate **a**). Low (120 kg N ha^{-1}), **b**). Control (0 kg N ha^{-1}), **c**). High (240 kg N ha^{-1}). The 5 meter wide buffer zones that separate the N application rates were sown with 'Paragon' □, the 80 cm wide walkways between plots □. This entire section of plots was also surrounded with a 5 meter wide buffer zone to prevent any adjacent insecticide treatments influencing aphid densities.

5.2.3 Location & drilling

The field site was the Rookery Field, Church Farm, Bawburgh, Norfolk (5 $2^0$37'40''N, $1^0$10'46''E). The field was unirrigated. The soil type is a sandy clay loam. The 1 m^2 plots were drilled with 180 seeds m^{-2} on November 22nd 2012 (Table 5. 2).

5.2.4 Nitrogen treatments

There were three nitrogen applications rates, the control treatment (0 kg N ha^{-1} applied), the low nitrogen treatment (120 kg N ha^{-1}), and the high nitrogen treatment (240 kg N ha^{-1}). No nitrogen was applied to the control treatment. The nitrogen was applied as ammonium nitrate in a split application to the high and low nitrogen treatments. The first application of 40 kg N ha^{-1} was applied to the low and high nitrogen treatments (03/03/13) when plants were at growth stage GS30-31 on the Zadoks scale (Zadoks *et al.* 1974). A further application was made (19/04/13) at GS34-39 of 80 kg N ha^{-1} to the low nitrogen treatment, and 200 kg N ha^{-1} to the high nitrogen treatment. Nitrogen application rates of 120 kg N $ha^{-1}$are typical in the UK for growing thatching wheat, and rates of 240 kg N ha^{-1} for growing commercial bread wheat (Barraclough *et al.* 2010). The soil nitrogen analysis showed an average nitrogen content of 56.5 kg N ha^{-1} measured with cores (30-90cm); these data were collected late February 2007 and 2008 by Envirofield agronomic analysis team (data from John Innes archives, provided by Simon Orford).

5.2.5 Phenotypic plant traits measured

The phenotypic plant traits including components of nitrogen use efficiency that were measured are given in Table 5. 3. The plot height was measured using a meter ruler with a cane at right angle over the plot, and the measurement was taken when >85% of the top of the plants were level with the cane, i.e. not the tallest plant but the average height of all plants in the 1m^2 plot.

Table 5. 3. The plant traits measured for this field experiment. The abbreviations: biomass (BM), above ground nitrogen (AGN).

Measured plant traits		
Crop development	**Nitrogen components**	**Yield characteristics**
Plot height (cm)	plant N content (g N kg DBM-1)	Yield (kg ha^{-1})
Canopy density	NupE (kg AG N kg^{-1} N available)	Grain protein (%)
Heading date	NutE (kg grain kg^{-1} N uptake)	
Senescence (%)	Grain N content (%)	
	NUE (kg yield kg^{-1} N available)	

The **canopy density** was assessed by observing the percentage ground cover (Duffield *et al.* 1997). For example if only 5% ground area was visible when standing over each plot then canopy density was recorded as 95% canopy density.

Each plot was assessed for **heading date** at GS57, recorded when wheat ears were 50% emerged from the flag leaf in >50% of plants in the plot.

Senescence was recorded in each plot when the flag leaf and plant peduncle in each plot began yellowing. A 0-100 score was given with 0 denoting completely green flag leaf and peduncle, and 100 being loss of green colour in 100% of flag leaves and the plant peduncle in each plot (Distelfeld *et al.* 2014).

Grain weight and **grain protein content** were assessed following harvest. Plots were harvested with a 1 m^2 combine harvester. The grain yield for each plot was weighed on a Sartorious SIWADCP-V3 (15 kg x 0.1 g) balance.

Grain protein concentration was measured using a CropScan 1000B Whole Grain Analyser, which is a near infrared transmission analyser. The grains (500 ml) were poured into the grain hopper which grinds the grain to flour before measuring protein content with near infrared fluorescence at 720-1100 nm. The weight of the grain along with moisture and dry protein content were also recorded. These data were used to calculate grain nitrogen content. The equation used to calculate grain nitrogen content from grain protein content was: Grain protein content ÷ 5.83 = grain nitrogen content (Mosse 1990).

5.2.6 Nitrogen analysis

The **plant nitrogen contents** were measured at harvest, when whole plant grab samples were taken on 14/08/13 from each experimental plot. The harvested plant material was dried in a LTE-Scientific Swallow drying oven at 80 °C for 24 h. The complete dry above ground plant material was cut up into 25-40 mm long sections and mixed to get a tissue sample representative of the whole plant. The dried cut up plant material was ground into a fine powder using a Spex 6770 freezer-mill cooled with liquid nitrogen (1 minute precool, 2 minute cooling cycle: 3 minute grinding at 13CPS).

To measure total **content of elemental nitrogen** in the above ground (AG) plant material a sub sample of dry powdered plant material from each experimental wheat plot was weighed using a Sartorious SE2-microbalance to between 4-5 micrograms. The samples (4-5 µg) were placed in 8x5 mm aluminium foil cups (supplied by elemental micro analysis) in a 90 well cassette tray (brand: Nunclon) before analysis in a Carlo-Erba 1108 CHN analyser.

Grain nitrogen content was measured from a sub-sample of grain randomly selected from three different nitrogen application rates analysed with the Carlo-Erba 1108 CHN analyser using the same protocol described for the plant material. These data were used to confirm the relationship between grain protein content and grain nitrogen content (data are not displayed).

5.2.7 Calculating nitrogen use efficiency

The equation used to calculate nitrogen uptake efficiency from plant nitrogen content was: total above ground plant nitrogen content at harvest (kg AG N ha^{-1}) ÷ total nitrogen supply (soil nitrogen content + total volume nitrogen applied (kg N ha^{-1}) (Moll *et al.* 1982, Gaju *et al.* 2011).

The nitrogen utilisation efficiencies of the SavRia genotypes were calculated using the equation: Grain yield dry matter (kg ha^{-1}) ÷ plant nitrogen uptake at harvest (kg AG N kg^{-1} N ha^{-1}) (Moll *et al.* 1982, Gaju *et al.* 2011).

The nitrogen use efficiencies of the SavRia genotypes were calculated using the equation: Grain yield (kg ha^{-1}) ÷ total nitrogen supply (soil nitrogen content + total volume nitrogen applied (kg N ha^{-1})) (Moll *et al.* 1982).

5.2.8 Statistical analyses

All statistical analyses were performed using SPSS (2013). The differences in the means of plot height and canopy cover were tested using one-way ANOVA, with nitrogen application rate as a factor. The Tukey honest significant difference (HSD) test was used to identify significant differences ($P \leq 0.05$) between traits. A multivariate general linear model was used to test if the plant traits (plant N content, NupE, grain yield, grain N content, NutE and NUE) were statistically different between the two factors, nitrogen application rate and SavRia genotype. The interaction between nitrogen application rates and SavRia genotypes were included in the model. A multivariate general linear model was used to test for differences in mean plant senescence between the two factors nitrogen application rate and SavRia genotype. Time (weeks) was the co-variate in the model and the interaction between SavRia genotype and nitrogen application rate was also tested in this model.

The Pearson product-moment correlation coefficients were computed for the plant traits: plant nitrogen content, nitrogen uptake efficiency, plant senescence, grain yields, grain nitrogen content, grain protein content, nitrogen utilisation efficiency, and nitrogen use efficiency using bivariate correlations due to auto-correlated variables. Bivariate regression analyses were used to quantify the relationship between the variation in nitrogen use efficiency and its components. The significance levels of the regression analyses were tested using ANOVA.

5.3 Results

The results are presented in four sections: 1) each phenotypic plant trait is presented to show the relationship with nitrogen application rate; 2) the component traits of nitrogen use efficiency and yield characteristics are displayed in relation to effects of SavRia genotype; 3) the components of nitrogen use efficiency identified as having a significant interaction between SavRia genotype and nitrogen application rate are displayed; 4) the relationships between the components of nitrogen use efficiency and yield characteristics are presented.

5.3.1 Phenotypic traits in relation to nitrogen application rates

The significance of both nitrogen application rates and genotypic differences in phenotypic plant traits are displayed in Table 5. 4. The SavRia genotypes have been aggregated within each nitrogen application rate to show the relationship between the phenotypic plant traits and nitrogen application (Figure 5.2). The plant traits that were recorded were significantly different between nitrogen application rates (Table 5. 4, Figure 5.2).

Table 5. 4. The multivariate GLM results for the plant nitrogen efficiencies and yield characteristics (plant traits). Significant effects are in italics ($P<0.05$), and bold italics ($P<0.01$).

Plant trait	Factors	Sum of squares	F statistic	D.o.F	*P*-value
Plot height	Nitrogen treatment	54012.7	1039.58	2,39	***0.001***
canopy density	Nitrogen treatment	169757.9	2126.6	2,39	***0.001***
Plant nitrogen content	SavRia Genotype	12.48	3.523	7,118	*0.01*
	Nitrogen treatment	70.29	69.456	2,39	***0.001***
	genotype x treatment	6.03	0.851	14,118	0.6
N uptake efficiency	SavRia Genotype	20.90	2.069	7,118	*0.05*
	Nitrogen treatment	4710.99	1631.89	2,39	***0.001***
	genotype x treatment	32.64	1.615	14,118	0.09
Yield	SavRia Genotype	1.85	11.646	7,118	***0.001***
	Nitrogen treatment	4.38	96.619	2,39	***0.001***
	genotype x treatment	0.38	1.184	14,118	0.3
N content grain	SavRia Genotype	1.48	31.338	7,118	***0.001***
	Nitrogen treatment	5.46	404.613	2,39	***0.001***
	genotype x treatment	0.14	1.476	14,118	0.1
N utilisation efficiency	SavRia Genotype	3253.47	9.927	7,118	***0.001***
	Nitrogen treatment	35306.43	377.037	2,39	***0.001***
	genotype x treatment	1337.03	2.04	14,118	*0.05*
N use efficiency	SavRia Genotype	12946.98	14.946	7,118	***0.001***
	Nitrogen treatment	178366.24	720.678	2,39	***0.001***
	genotype x treatment	7065.66	4.078	14,118	***0.001***

The winter wheat plot (1 m^2) heights were shortest in the control treatment (0 kg N ha^{-1}), and highest in the low nitrogen (120 kg N ha^{-1}) treatment (Figure 5.2.a). The canopy densities (%) of the plots (1 m^2) were significantly different between the three nitrogen application rates. The wheat plots (1 m^2) had the highest canopy densities in the high nitrogen (240 kg N ha^{-1}) treatment (Figure 5.2.b).

The plant nitrogen content (g N kg^{-1} biomass) of SavRia genotypes were significantly different between the three different nitrogen application rates (Figure 5.2.c; Table 5. 4). The plant nitrogen contents (g N kg^{-1} biomass) were significantly higher in plants grown in the high nitrogen treatment ($\bar{X}$ ±1 SE: 6.2±0.14) (Figure 5.2c). The nitrogen uptake efficiencies (NupE) of SavRia genotypes were also significantly different between nitrogen application rates, and were highest in the control treatment (Figure 5.2.d; Table 5. 4).

The grain yields were significantly different between nitrogen application rates (Figure 5.2.e, Table 5. 4). The grain yield of SavRia genotypes (kg m^{-2}) was highest in the low (120 kg N ha^{-1}) nitrogen application rate ($\bar{X}$ ±1 SE: 11400±240), but it was not significantly different (Tukey HSD $P<0.05$) from grain yield in the high (240 kg N ha^{-1}) nitrogen treatment ($\bar{X}$ ±1 SE: 10600±240) (Figure 5.2.e; Table 5. 4).

The grain nitrogen contents (%) were significantly different between nitrogen application rates (Figure 5.2.f; Table 5. 4). The SavRia genotypes grain nitrogen concentrations were highest when grown in the high nitrogen treatment (Figure 5.2.f).

The nitrogen utilisation efficiency (NutE) of SavRia plants were significantly different between nitrogen application rates, and was highest under the high nitrogen application rate (Figure 5.2.g; Table 5. 4).

The nitrogen use efficiencies (NUE) were significantly different between nitrogen application rates, with NUE being highest in the control treatment (0 kg N ha^{-1}) (Figure 5.2.h; Table 5. 4).

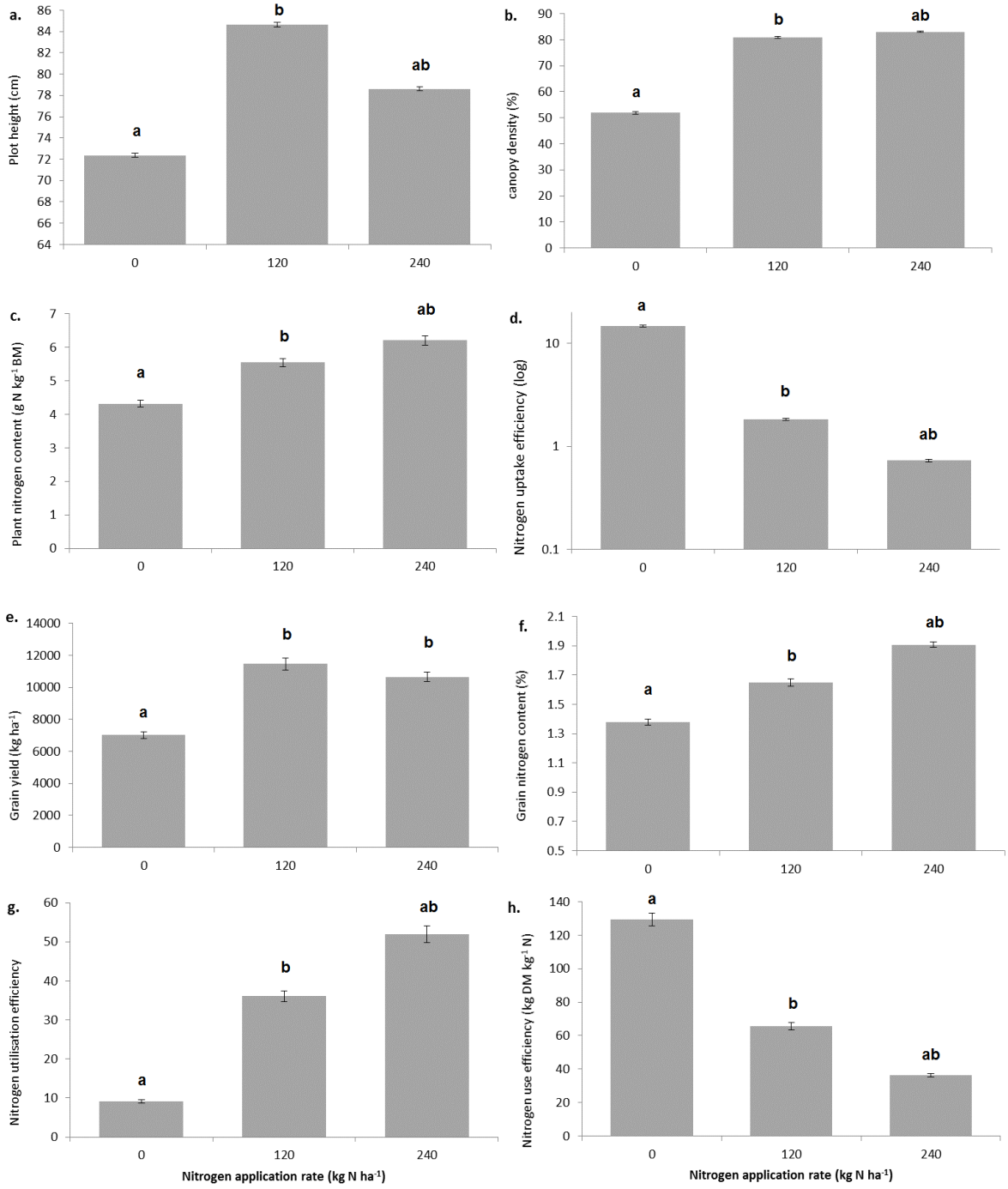

Figure 5.2. The mean phenotypic plant traits **a**). height (cm) of wheat plots m^2, **b**). canopy density (%) of plots (m^2), **c**). plant nitrogen content, **d**). nitrogen uptake efficiency (Log_{10} scale kg AGN kg^{-1} N available), **e**). dry matter grain yield (kg ha^{-1}), **f**). grain nitrogen content (%), **g**). nitrogen utilisation efficiency (kg dry matter (DM) grain kg^{-1} plant N uptake at harvest), **h**). nitrogen use efficiency (kg dry matter grain yield kg^{-1} N available) under each nitrogen application rate (0, 120, 240 kg N ha^{-1} common X axes).SavRia genotypes aggregated within nitrogen application rates. Means followed by the same letters were not significantly different for each plant trait (Tukey HSD, P <0.05). Error bars ±1 standard error.

5.3.2 The component traits of nitrogen use efficiency and yield characteristics in relation to effects of SavRia genotype

Yield characteristics and components of nitrogen use efficiencies were significantly different between the SavRia genotypes (Table 5. 4, Figure 5.3).

The plant nitrogen contents were significantly different between SavRia genotypes (Figure 5.3.a; Table 5. 4). The nitrogen uptake efficiencies (NupE) were significantly different between SavRia genotypes (Figure 5.3.b; Table 5. 4). SavRia genotype 31 and the Rialto parent had the highest and lowest plant nitrogen content and the highest and lowest nitrogen uptake efficiencies respectively (Figure 5.3.a & b).

The grain yields were significantly different between SavRia genotypes; the Savannah parent and SavRia GT92 had the highest and GT31 the lowest grain yields in the control treatment (Figures 5.3.c, Table 5. 4).

The grain nitrogen contents (%) were significantly different between SavRia genotypes, with SavRia GT31 having the highest grain nitrogen content (Figure 5.3.d; Table 5. 4).

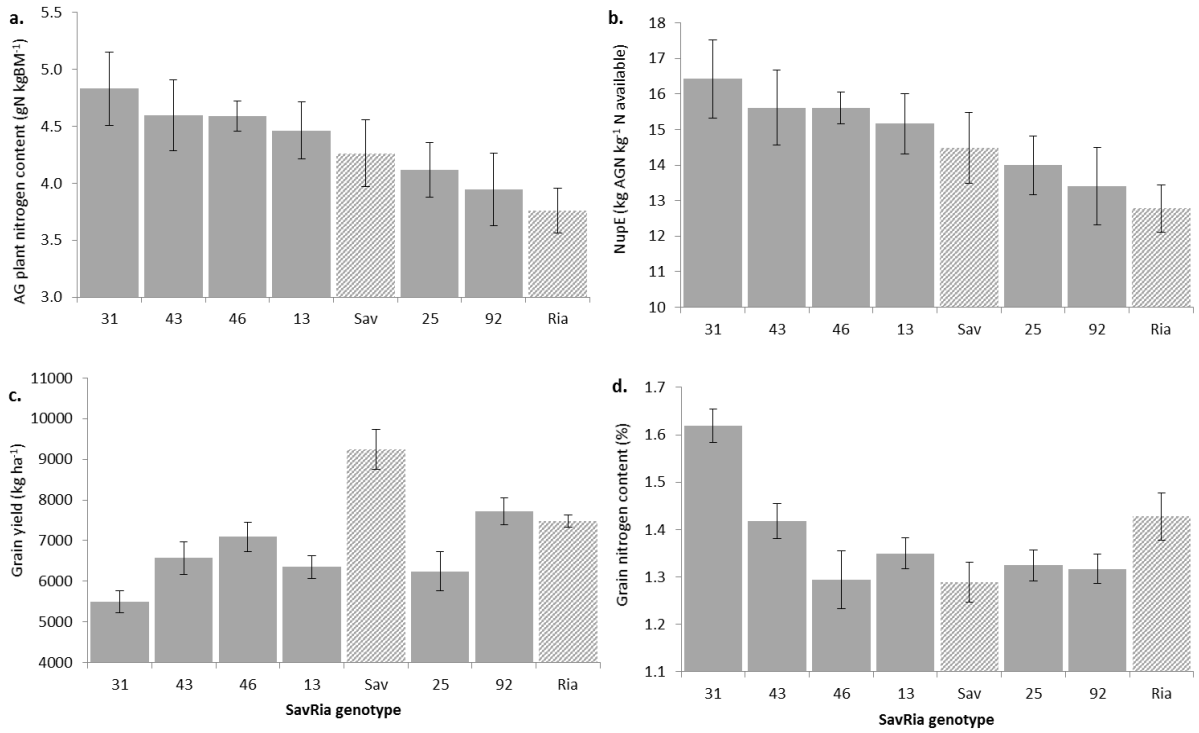

Figure 5.3. The mean average **a**). plant nitrogen content **b**). nitrogen uptake efficiency (NupE: kg above ground (AG) plant N content kg^{-1} N available), **c**). dry matter grain yield (kg ha^{-1}), **d**). grain nitrogen content (%) for each SavRia genotype and parental cultivars ▨ (Ria) and (Sav), grown in the control treatment (0 kg N ha^{-1}). Genotypes arranged from left to right ranked according to decreasing plant nitrogen content (Figure 5.3.a). (X-axis) Error bars ±1 standard error.

5.3.3 The interaction between SavRia genotype and nitrogen application rate on components of nitrogen use efficiency

There were significant interactions between SavRia genotype and nitrogen application rate on nitrogen utilisation efficiency (Table 5. 4, Figure 5.4) and nitrogen use efficiency (Table 5. 4, Figure 5.4).

The nitrogen utilisation efficiency (NutE) was significantly different between SavRia genotypes (Figure 5.4.a; Table 5. 4). There was a significant interaction effect between SavRia genotype with nitrogen application rates on the nitrogen utilisation efficiency of the SavRia genotypes (Figure 5.4.a, b, c; Table 5. 4). The SavRia genotypes responded differently to the different nitrogen application rates. SavRia genotypes 92 and GT31 had the highest and lowest nitrogen utilisation efficiencies respectively in both the high and low nitrogen application rates.

The nitrogen use efficiencies (NUE) were significantly different between SavRia genotypes (Figures 5.4.d; Table 5. 4). There was a significant interaction effect between SavRia genotype with nitrogen application rates on the nitrogen use efficiency of SavRia genotypes (Table 5. 4). The SavRia genotypes had different nitrogen use efficiencies within and between the different nitrogen application rates (Figures 5.2.h & 5.4.d, e, f; Table 5. 4).

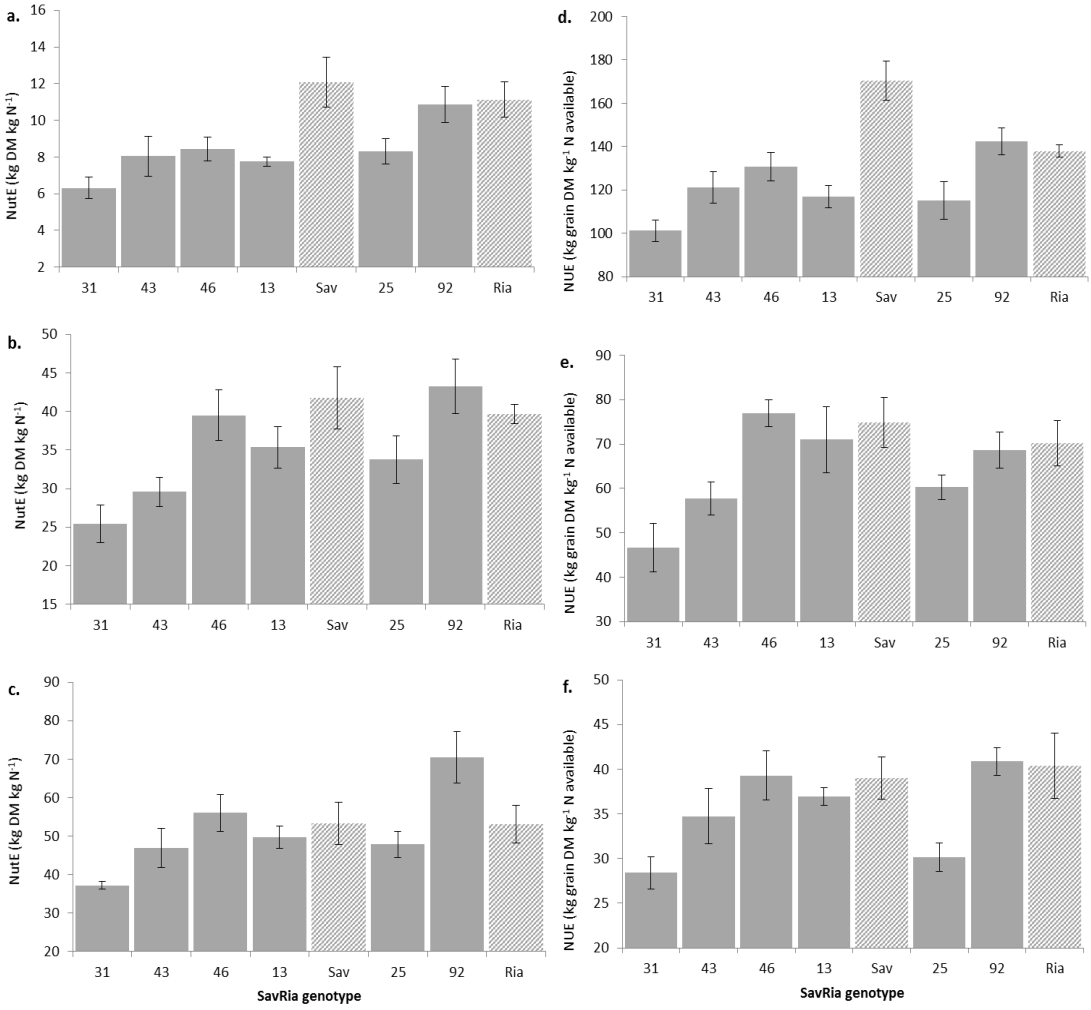

Figure 5.4. The mean average nitrogen utilisation efficiency (NutE: kg dry matter (DM) grain kg^{-1} AG plant N content at harvest) and nitrogen use efficiency (NUE: (kg dry matter grain yield kg^{-1} N available) of each SavRia genotype and parental cultivars ▨ Rialto (Ria) and Savannah (Sav) grown with **a** & **d**). **0** kg N ha^{-1} applied, **b** & **e**). **120** kg N ha^{-1}, and **c** & **f**). **240** kg N ha^{-1}. Genotypes arranged from left to right by decreasing plant nitrogen content as in Figure 5.3.a (X-axis). Error bars ±1 standard error.

5.3.4 The relationships between the components of nitrogen use efficiency and yield characteristics

Plant senescence was significantly different between SavRia genotypes (Table 5. 5; Figure 5.5 a), and between the three nitrogen application rates (Table 5. 5; Figure 5.5). There was a significant interaction identified between SavRia genotype with nitrogen application rate on the plant senescence (Table 5. 5; Figure 5.5). The SavRia genotypes and parental varieties senesced at different rates between each nitrogen application rate. The Rialto and Savannah parents were predominantly in the higher and lower range of senescence values respectively.

The plants grown with a low nitrogen application rate (120 kg N ha^{-1}) had a higher level of senescence from week 4- 6 (Figure 5.5). The senescence of plants grown with the low nitrogen application rate was negatively correlated with grain yield (n=40, r= -0.37, $P<0.05$) and with nitrogen use efficiency (n=40, r= -0.37, $P<0.05$) (Table 5. 6).

Table 5. 5. The multivariate GLM results testing the difference in plant senescence between genotypes and nitrogen application rates. The interaction effect was tested in a GLM, and time (weeks) was the significant co-variate. Significant *P*-value in italics ($P<0.05$), and bold italics ($P<0.01$).

Plant trait	Factors / co-variates	Sum of squares	F statistic	d.f	*P*-value
Senescence	weeks	4987399	16042.2	1,118	***0.001***
	SavRia Genotype	52552	24.148	7,118	***0.001***
	Nitrogen treatment	417572	671.57	2,118	***0.001***
	genotype x treatment	14886	3.42	14,118	***0.001***

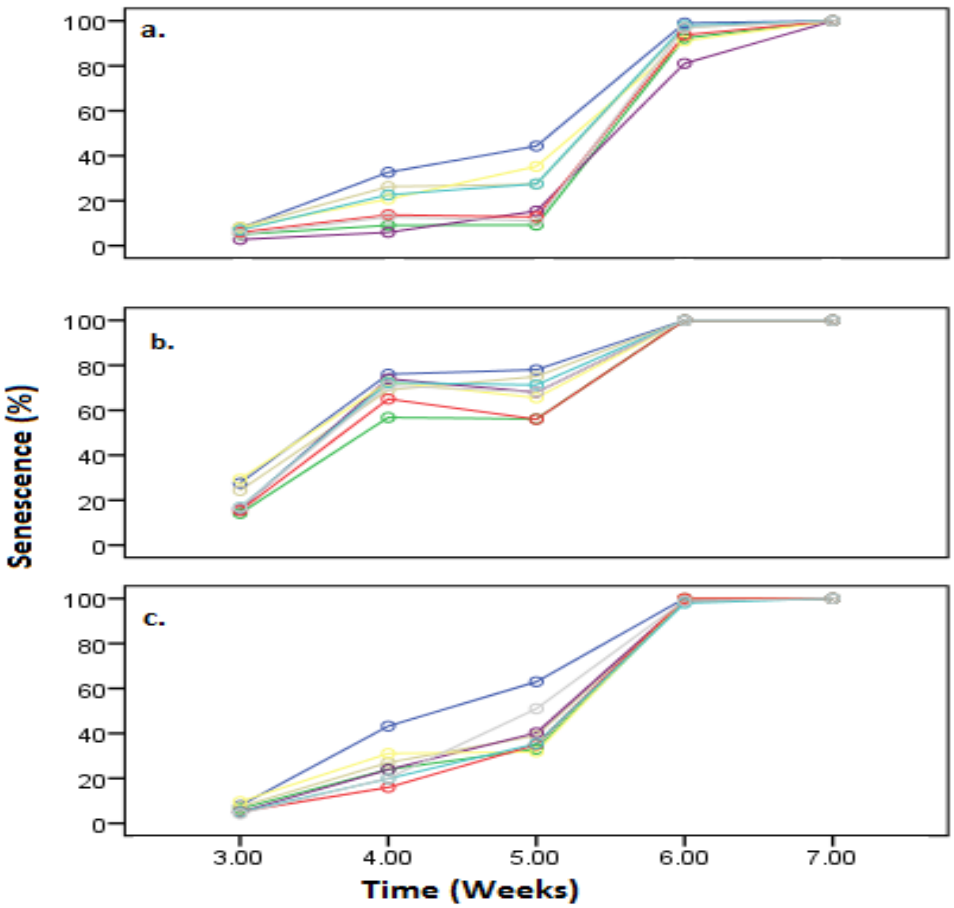

Figure 5.5. The average plant senescence for each SavRia genotype (Rialto, Savannah, 13, 25, 31, 43, 46, 92) grown in the three different nitrogen application rates **a**). 0 kg N ha^{-1} **b**). 120 kg N ha^{-1} **c**). 240 kg N ha^{-1}.

Plant nitrogen contents were significantly correlated (n=40, r= 1.00, $P<0.001$) with the nitrogen uptake efficiency of SavRia genotypes in both the low and high nitrogen application rates (Tables 5.6 & 5.7). The **nitrogen uptake efficiencies** were negatively correlated with the **nitrogen utilisation efficiencies** of SavRia genotypes in both the low (n=40, r= -0.5, $P<0.001$) and high (n=40, r= -0.65, $P<0.001$) nitrogen application rates (Table 5.6 & 5.7).

The **grain yields** of SavRia genotypes were positively correlated with the nitrogen utilisation efficiencies, and with the nitrogen use efficiencies of the SavRia genotypes in both the high and low nitrogen treatments (Table 5.6 & 5.7)

There is a significant negative correlation between grain yield and grain nitrogen content when the high and low nitrogen application rates are aggregated (Figure 5.6).

Table 5. 6. A correlation matrix of plant traits grown at the low application rate (**120** kg N ha^{-1}) including the components of nitrogen use efficiency and yield characteristics (n=40). The values are the Pearson correlation coefficient (r). Correlation coefficients (r) significant at $P \leq 0.05$ italics, significant at $P \leq 0.01$ bold italics.

Variables	Plant N content	N uptake efficiency	Senescence	Grain yield	Grain N content	Grain protein content	N utilisation efficiency
Plant nitrogen content (g N kg BM^{-1})	1						
Nitrogen uptake efficiency	***1.00***	1					
Senescence (%)	-0.21	-0.21	1				
Grain yield (kg m^{-2})	0.06	0.06	*-0.37*	1			
Grain nitrogen content (%)	-0.12	-0.12	0.10	-0.31	1		
Grain protein content (%)	-0.12	-0.12	0.10	-0.31	***1.00***	1	
Nitrogen utilisation efficiency	***-0.50***	***-0.50***	-0.22	***0.83***	-0.22	-0.22	1
Nitrogen use efficiency (kg DM kg N^{-1})	0.06	0.06	*-0.37*	***1.00***	-0.31	-0.31	***0.83***

Table 5. 7. A correlation matrix of plant traits grown at the high application rate (**240** kg N ha^{-1}) (n=40). The values are the Pearson correlation coefficient (r). Correlation coefficients (r) significant at $P \leq 0.05$ italics, significant at $P \leq 0.01$ bold italics.

Variables	Plant N content	N uptake efficiency	Senescence	Grain yield	Grain N content	Grain protein content	N utilisation efficiency
Plant nitrogen content (g N kg BM^{-1})	1						
Nitrogen uptake efficiency	***1.00***	1					
Senescence (%)	0.09	0.09	1				
Grain yield (kg m^{-2})	0.01	0.01	0.22	1			
Grain nitrogen content (%)	0.02	0.02	0.23	-0.19	1		
Grain protein content (%)	0.02	0.02	0.23	-0.19	***1.00***	1	
Nitrogen utilisation efficiency	***-0.65***	***-0.65***	0.09	***0.73***	-0.18	-0.18	1
Nitrogen use efficiency (kg DM kg N^{-1})	0.01	0.01	0.22	***1.00***	-0.19	-0.19	***0.73***

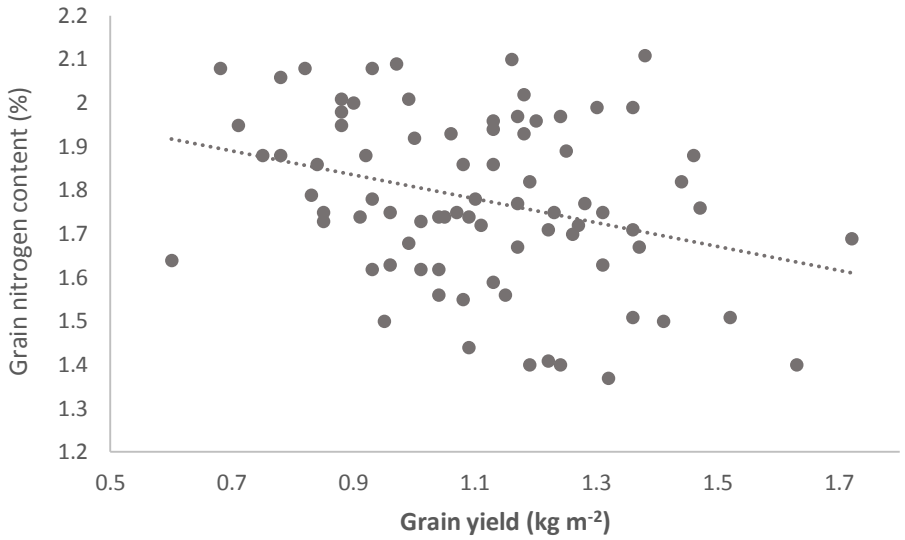

Figure 5.6. The grain nitrogen content of all SavRia genotypes in the low (120 kg N ha^{-1}) and high (240 kg N ha^{-1}) nitrogen application rates (Y-axis) against the grain yield (kg m^{-2}). The regression line (R^2=0.09, β=-0.317, n=80, $F_{(1, 79)}$ =8.539, P<0.05).

There was a negative relationship between the nitrogen uptake efficiency at harvest with the nitrogen utilisation efficiency of SavRia genotypes grown in the low nitrogen application rate (Figure 5.7.a) and the high nitrogen application rate (Figure 5.7.d). With the low and high nitrogen application rates nitrogen uptake efficiency at harvest explained 25% and 42% of the variation respectively in nitrogen utilisation efficiency (Figure 5.7.a & d).

There was a positive relationship between the nitrogen utilisation efficiency and the grain yields of SavRia genotypes grown with the low nitrogen application rate (Figure 5.7.b) and the high nitrogen application rate (Figure 5.7.e). In the low and high nitrogen treatments nitrogen utilisation efficiency explained 68% and 54% of the variation in grain yields respectively (Figure 5.7.b & e).

There was a positive relationship between the nitrogen utilisation efficiency and the nitrogen use efficiency of SavRia genotypes grown with the low nitrogen application rate (Figure 5.7.c) and the high nitrogen application rate (Figure 5.7.f). In the low and high nitrogen treatments nitrogen utilisation efficiency explained 68% and 54% of the variation respectively in nitrogen use efficiency (Figure 5.7.c & f).

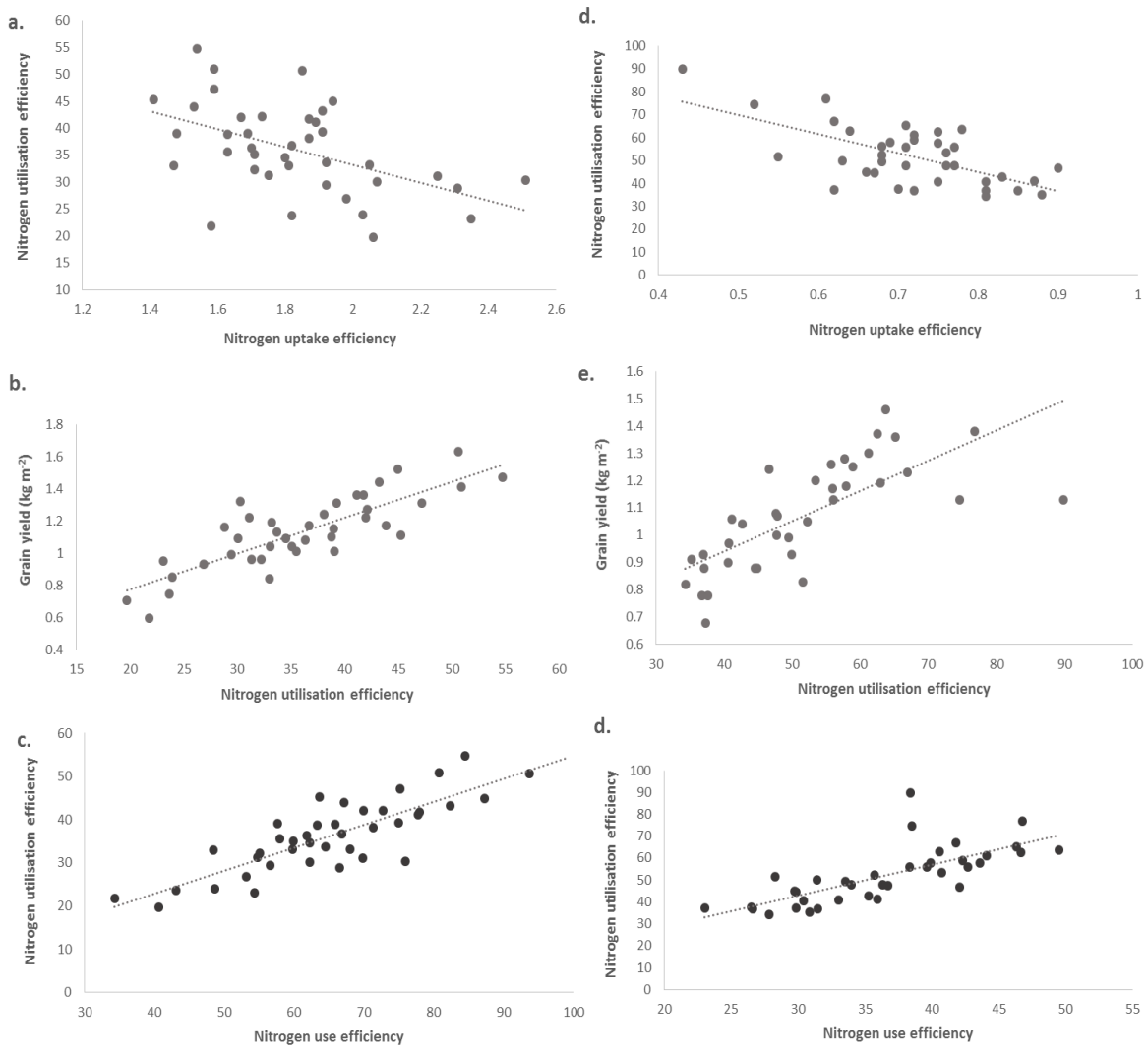

Figure 5.7. The components of the nitrogen use efficiency of all SavRia genotypes (aggregated) grown with the **low** (**a**, **b**, **c**: 120 kg N ha^{-1}) and **high** (**d**, **e**, **f**: 240 kg N ha^{-1}) nitrogen application rates (n=40 for each N application rate). **a**). the nitrogen utilisation efficiency (Y-axis) against the nitrogen uptake efficiency (X-axis), (R^2=0.25, β=-0.5, $F_{(1, 39)}$ =12.13, P<0.01). **b**). the grain yield (Y-axis) against the nitrogen utilisation efficiency (X-axis), (R^2=0.68, β=0.02, $F_{(1, 39)}$ =79.21, P<0.001). **c**). the nitrogen utilisation efficiency (Y-axis) against the nitrogen use efficiency (X-axis), (R^2=0.68, β=0.53, $F_{(1, 39)}$ =79.21, P<0.001). **d**). the nitrogen utilisation efficiency (Y-axis) against the nitrogen uptake efficiency (X-axis), (R^2=0.42, β=-0.65, $F_{(1, 39)}$ =25.09, P<0.001). **e**). the grain yield (Y-axis) against the nitrogen utilisation efficiency (X-axis), (R^2=0.54, β=0.73, $F_{(1, 39)}$ =40.38, P<0.001). **f**). the nitrogen utilisation efficiency (Y-axis) against the nitrogen use efficiency (X-axis), (R^2=0.54, β=1.4, $F_{(1, 39)}$ =40.38, P<0.001).

5.4 Discussion

The way in which wheat plants respond to increased nitrogen application is well documented (Powlson *et al.* 1992; Chardon *et al.* 2010). As an essential element for plant growth, nitrogen applied as fertiliser can increase metabolic function including photosynthesis in plants (Chardon *et al.* 2010). In general plants grown with higher nitrogen application rates will be larger and attain higher yields (Powlson *et al.* 1992; Chardon *et al.* 2010; Sedlar *et al.* 2015).

The plant nitrogen content (g N kg biomass^{-1}) in SavRia genotypes was significantly higher when grown at the high nitrogen application rate (Figure 5.2.c, Table 5. 4). These data support the hypothesis that plant nitrogen content will be higher in plants grown under higher nitrogen application rates. SavRia genotype 31 had the highest plant nitrogen content in the control treatment (Figure 5.3.a) and the second highest after the Rialto parent with the high nitrogen application rate. These results are consistent with John Innes field trial data (Table 5. 1) and the results of the laboratory study reported in chapter 4. SavRia genotype 31 may have a larger root mass compared with other SavRia genotypes and this trait may contribute to the high nitrogen content in the plant biomass (Xu *et al.* 2012).

The nitrogen uptake efficiencies of the SavRia genotypes aggregated within each nitrogen treatment were significantly different between the three nitrogen application rates (Figure 5.2.d, Table 5. 4). The nitrogen uptake efficiencies were also significantly different between SavRia genotypes (Figure 5.3.b, Table 5. 4). SavRia genotype 31 and genotype 92 had the highest and lowest nitrogen uptake efficiency respectively when grown at the high nitrogen application rate. There are different regulatory genes for high and low affinity nitrate transporter responses, which in part determine plant nitrogen uptake at different nitrogen application rates (Sedlar *et al.* 2015); these genes can be inherited independently (Obara *et al.* 2004, Coque *et al.* 2008). Therefore, nitrogen uptake efficiency can be variable between different nitrogen application rates, and between different genotypes within a population.

The grain yields of the SavRia genotypes were significantly different between the three nitrogen application rates (Figure 5.2.e, Table 5. 4). The grain yields of the SavRia genotypes (aggregated within nitrogen application rates) were highest when grown in the low (120 kg N ha^{-1}) nitrogen application rate

(Figure 5.2.e). The hypothesis that the grain yields of wheat plots will be higher under higher nitrogen application rates was unsupported by these results. The taller plants (Figure 5.2.a) grown under the low nitrogen application rate produced on average 800 g more grains than were produced with the high nitrogen application rate. However, grain quality as defined by nitrogen content in the grain was higher when the nitrogen application was increased.

A key aspect of grain quality is defined by grain protein content, which is highly correlated to grain nitrogen content (Table 5.6 & 5.7); this correlation is due to nitrogen being a major constituent of all proteins (Mosse 1999). The grain nitrogen contents were significantly different between the three nitrogen application rates (Figure 5.2.f, Table 5. 4). The grain nitrogen contents of SavRia genotypes were significantly higher in the highest (240 kg N ha^{-1}) nitrogen application rate (Figure 5.2.f). The hypothesis that the grain nitrogen content of SavRia genotypes will be higher under the highest nitrogen application rate was supported. The application of nitrogen fertiliser on wheat crops increases the grain nitrogen content (Xu *et al.* 2012).

There is a trade off in wheat between increasing yield and sustaining grain quality (Ortiz-Monasterio 1997). The hypothesis that the grain nitrogen content of all SavRia genotypes will be negatively correlated with grain yield was supported. There was a negative correlation between grain yield and grain nitrogen content (Figure 5.6). Increasing wheat yields are imperative to sustaining food security, but a decrease in the nutritional quality of grain, which is associated with a decrease in grain nitrogen content is an undesirable trade-off. Increasing the way in which applied nitrogen is utilised not only for grain production but also for grain protein concentration is an important wheat improvement strategy.

The nitrogen utilisation efficiencies of SavRia genotypes were significantly different between the three nitrogen application rates; they were highest when grown at the high nitrogen application rate (Figure 5.2.g, Table 5. 4). These results support the hypothesis that the nitrogen utilisation efficiencies of SavRia genotypes will increase with nitrogen application rate. The nitrogen utilisation efficiencies of wheat plants are related to leaf senescence patterns (Kundu & Ladha 1997). The plant senescence of SavRia genotypes was significantly different between the nitrogen application rates (Figure 5.5, Table 5. 5).

The plant senescence during weeks 4 & 5 (17-31th July 2013) of the growing season was lower at the high nitrogen application rate relative to the low

nitrogen application rate. A study by Coque *et al.* (2008) identified that quantitative trait loci (QTL) associated with leaf senescence patterns corresponded with QTL associated with nitrogen mobilisation and therefore utilisation efficiency. So called 'stay-green' phenotypes, which are plants that senesce later in their life cycle (staying green for longer) can increase nitrogen utilisation efficiency (Kundu & Ladha 1997, Coque *et al.* 2008). It has been shown that leaf senescence patterns can be influenced by different nitrogen application rates and different wheat genotypes within a single population (Powlson *et al.* 1992).

The nitrogen utilisation efficiencies were significantly different between SavRia genotypes (Figure 5.4.a, b, c, Table 5. 4). SavRia genotype 31 had the lowest nitrogen utilisation efficiency at the low and high nitrogen application rates (Figure 5.4.b & c). SavRia genotype 92 had the highest nitrogen utilisation efficiency when grown at the low and high nitrogen application rates (Figure 5.4.b & c). The QTL identified by Coque *et al.* (2008) were associated with nitrogen utilisation traits, and can segregate in a population. Therefore, those traits can be expressed differentially by genotypes within a population (Powlson *et al.* 1992, Coque *et al.* 2008).

There was also a significant interaction between SavRia genotype with nitrogen application rate on nitrogen utilisation efficiency. The glutamine synthetase activity in plants is associated with nitrogen utilisation efficiency (Obara *et al.* 2004). The glutamine synthetase activity is influenced by different genes dependent on nitrogen application rate (Obara *et al.* 2004). The nitrogen utilisation efficiency in wheat is heavily influenced by the amount of nitrogen that is remobilised mainly as constituents of amino acids for grain production post anthesis (Obara *et al.* 2004, Hörtensteiner 2009). The differential expression of these different traits under different nitrogen application rates offers potential explanation for the interaction between SavRia genotypes with nitrogen application rate on their nitrogen utilisation efficiencies.

The components of nitrogen use efficiency; nitrogen uptake and utilisation efficiency appear to be complex polygenic traits (Coque *et al.* 2008, Chardon *et al.* 2010). The nitrogen use efficiencies of SavRia genotypes were significantly different between nitrogen application rates (Figure 5.4.d, e, f, Table 5. 4). Its components, nitrogen uptake and utilisation efficiency were also different between the nitrogen application rates (Figure 5.2, Table 5. 4). The nitrogen use efficiencies were different between SavRia genotypes, as were the nitrogen utilisation efficiencies (Figure 5.4, Table 5. 4). The SavRia genotypes significantly interacted with nitrogen application rate influencing

the nitrogen use efficiencies of the different SavRia genotypes (Figure 5.4, Table 5. 4). The genes involved in regulating nitrogen uptake and utilisation efficiencies in plants, and therefore nitrogen use efficiency may have segregated in the SavRia population; many of these genes can also interact with nitrogen application rate (Coque *et al.* 2008, Chardon *et al.* 2010).

The interaction between SavRia genotypes with nitrogen application rates on nitrogen use efficiency is clear (Figure 5.4.d, e, f, & Table 5. 4). SavRia genotype 31 had the lowest nitrogen use efficiencies at all three nitrogen application rates (Figure 5.4.d, e, f,). SavRia genotype 46 had the highest nitrogen use efficiency in the low nitrogen application rate, and genotype 92 had the highest nitrogen use efficiency in the high nitrogen application rate (Figure 5.4.d, e, f). These results were reflective of the pattern observed for nitrogen utilisation efficiency, which was also influenced by a significant interaction between SavRia genotype and nitrogen application rate (Figure 5.4.a, b, c, Table 5. 4). There was no significant interaction between SavRia genotype and N application rate on nitrogen uptake efficiency. The hypothesis that the nitrogen uptake, utilisation and use efficiency of different SavRia genotypes will respond differently under different nitrogen application rates was supported for nitrogen utilisation and use efficiency, but was unsupported for nitrogen uptake efficiency. These results were similar to data from a John Innes field trial (Table 5. 1), with SavRia genotypes 46 and 92 in the higher spectrum of nitrogen use efficiencies.

The nitrogen use efficiencies were positively correlated with the nitrogen utilisation efficiencies of SavRia genotypes in both the low and high nitrogen application rates (Table 5.6 & 5.7). In modern wheat varieties it can be nitrogen utilisation efficiency that contributes most strongly to nitrogen use efficiency and consequently grain yields (Haile *et al.* 2012, Karrou & Nachit 2015). A significant amount of the variation in nitrogen use efficiencies (54%) was explained by the variation in nitrogen utilisation efficiencies of SavRia genotypes when grown at the high nitrogen application rate (Figure 5.7.f). Nitrogen uptake did not explain a significant amount of the variation in nitrogen use efficiencies under the high nitrogen rate. The same patterns between nitrogen use components were observed when plants were grown at the low nitrogen application rate (Figure 5.7.c, Table 5.6 & 5.7). These results support the hypothesis that variation in nitrogen use efficiency will be explained by the variation in nitrogen utilisation efficiency in the low and high nitrogen application rates. These results also highlight the inherent complexity in the components of nitrogen use efficiency of wheat.

The components of nitrogen use efficiency and the yield characteristics of these SavRia genotypes have been quantified using different nitrogen application rates. The results are in agreement with other studies on nitrogen use efficiency in wheat (Barraclough *et al.* 2010, Gaju *et al.* 2011). These studies show there are clear prospects for improving nitrogen use efficiency in wheat, using better agronomic practice and breeding wheat genotypes with higher nitrogen use efficiencies. However, there may be potential drawbacks from increasing nitrogen use efficiency in wheat. For example, one important drawback of increasing nitrogen use efficiency in wheat comes from the trade-off between decreases in grain protein content as grain yields are increased (Ortiz-Monasterio 1997). A decrease in grain quality is an undesirable outcome that is associated with an increase in grain yields. Therefore, wheat genotypes that use nitrogen more efficiently for increased grain yield and concomitantly grain protein will be superior. Another potential drawback of improving nitrogen use efficiency in wheat maybe that it also increases the infestation potential of nitrogen sensitive crop pests.

Populations of *Sitobion avenae* are sensitive to nitrogen application, with population increases associated with higher nitrogen application rates (Aqueel & Leather 2011). Populations of *S. avenae* also benefit from and an increase in amino acids in the plant phloem (Ponder *et al.* 2000). The efficient remobilisation of amino acids post anthesis leads to an increase in nitrogen utilisation efficiency (Okumoto & Pilot 2011). Therefore, wheat genotypes with higher nitrogen utilisation efficiencies could increase the potential for infestation by cereal aphids. This prediction will be investigated in chapter 6, by means of quantifying cereal aphid densities on SavRia genotypes in relation to the different components of their nitrogen use efficiencies.

6 Effects on *Sitobion avenae* densities of winter wheat genotypes with different nitrogen use efficiencies under different nitrogen application rates

6.1 Introduction

A central question addressed in this thesis is "do differences in the nitrogen use efficiency of wheat influence aphid densities, therefore altering the potential for infestation by aphids?" In order to address this question the nitrogen use efficiency and its components of nitrogen uptake efficiency and nitrogen utilisation efficiency for the six SavRia genotypes and their parental cultivars 'Savannah' and 'Rialto, ' were quantified in chapter 5. In this chapter aphid densities on the tillers of these SavRia genotypes will be related to variation in the components of nitrogen use efficiency of their host plants.

It is well recognised that nitrogen dynamics within the host plants on which insects feed can limit their population growth (White 2003, Dixon 1998). Phytophagous insects damage plants as a result of a variety of feeding strategies that include chewing by mandibulate insects or sucking by haustellate insects. The sap sucking, or phloem feeding, insects are found mainly in the order Hemiptera, including the suborders Sternorrhyncha and Auchenorrhyncha. Phloem feeders damage their host plants directly by ingesting nitrogenous compounds from the plant phloem (Kuhlmann *et al.* 2013). The phloem feeders have specially adapted mouthparts for piercing plant tissue to locate phloem sieve tubes in the plant, where they feed from the plant phloem fluids (Gash 2012, Kuhlmann *et al.* 2013). Host plant nutritional quality is of particular importance to phloem feeding insects (Awmack & Leather 2002). The amino acids transported in the plant phloem are essential for protein production in the plant; they are also an essential for the growth and reproduction of phloem feeding insects (Karley *et al.* 2002, Kuhlmann *et al.* 2013).

The phloem feeders are a diverse group with numerous species that have a range of strategies and adaptations to exploit host plants. Scale insects, like many phloem feeders, can be influenced by host plant nitrogen content. The growth rate and fecundity of *Fiorinia externa* are positively correlated with the concentration of nitrogen in the new growing needles of eastern hemlock

(*Tsuga Canadensis*) on which it feeds in North America (McClure 1980). The leafhopper *Nephotettix cincticeps* has faster growth rates and improved reproduction on rice plants with a higher concentration of plant nitrogen (Andow 1984). Paine and Hanlon (2010) showed that fertiliser application resulted in increased numbers of the red gum lerp psyllid *(Glycaspis brimblecombei)* feeding on eucalyptus in California. Aphids are phloem feeders that can also be influenced by host plant nutritional quality (Awmack & Leather 2002, Karley *et al.* 2002). Populations of different species of aphids have been shown to increase on host plants with higher plant nitrogen content (Khan & Port 2008, Nowak & Komor 2010), and in relation to nitrogen fertiliser application (Duffield *et al.* 1997, Aqueel & Leather 2011).

The nitrogen content and senescence dynamics of aphid host plants are important determinants of aphid population growth (Dixon 1998, Khan & Port 2008). Aphid reproduction and population growth can be influenced by changes in plant phenology (Leather & Dixon 1981). Aphids that are flush feeders will colonise leaves in the early plant growth stage to access the highly mobile nitrogen in the new growth. In contrast, aphids that are senescence feeders will colonise and feed on leaves that are starting to senesce when nitrogen is being remobilised from leaves into other plant tissues. The sycamore aphid (*Drepanosiphum platanoidis*) is both a senescence and a flush feeder. New sycamore growth in the spring supports its reproduction during spring (Dixon 1998), whereas the senescing autumn leaves support the autumnal reproductive phase (Dixon 1998). During summer *Drepanosiphum platanoidis* can survive on sycamore leaves but cannot grow or reproduce (Dixon 1998).

The aphid species *Myzus persicae* and *Macrosiphum euphorbiae* both had shorter development times and higher fecundity on potatoe plants (*Solanum tuberosum*) in their early developmental stage ('pre-tuber-filling') relative to older plants during tuber-filling (Karley *et al.* 2002). Cereal aphid populations are also influenced by plant phenology and nitrogen content of their host plants (Leather & Dixon 1981, Khan & Port 2008). Therefore, it is important to monitor aphid populations on cereal crops at different growth stages, under different nitrogen application rates to elucidate the relationship between aphid populations and nitrogen application rate.

Cereal aphid populations have been shown to increase on host plants receiving increased nitrogen application rates both in the laboratory (Aqueel

& Leather 2011) and in field studies (Gash 2012, Wang *et al.* 2015). Aphid longevity, adult weight and fecundity were positively correlated with nitrogen fertiliser application for both *Rhopalosiphum padi* and *Sitobion avenae* on several wheat cultivars grown in laboratory conditions (Aqueel & Leather 2011). The cereal aphid *Metopolophium dirhodum* had higher fecundity and intrinsic rate of increase on winter wheat plants grown in the field and glasshouse respectively when higher volumes of nitrogen fertiliser were applied (Gash 2012). When nitrogen application was increased on field grown winter wheat, larger populations of *Metopolophium dirhodum* and *S. avenae* were recorded (Duffield *et al.* 1997). Increasing nitrogen application rate on wheat was found to be associated with increases in aphid population densities (Gash 2012), thus increasing the potential for infestation by aphid crop pests (Honek *et al.* 2006). Therefore variation in nitrogen application rates was chosen as a key variable in the experimental design used in this chapter.

It has been demonstrated that aphids may be more sensitive to amino acid composition in the plant phloem contents rather than the nitrogen concentration *per se* (Hale *et al.* 2003, Nowak & Komor 2010). Karley *et al.* (2002) related the increase in performance of *M. persicae* and *Macrosiphum euphorbiae* to changes in amino acid composition in the plant phloem. The intrinsic rate of increase of the cereal aphid *R. padi* was correlated with the concentration and composition of essential amino acids in plant phloem (Hale *et al.* 2003). The amino acid concentration and composition in plant phloem may in part define nutritional quality of aphid host plants (Ponder *et al.* 2000, Hale *et al.* 2003).

Aphid populations in the field are not just influenced by intrinsic host plant factors such as amino acid composition. The production of secondary plant metabolites such as benzoxazinoids are important plant defence strategies that reduce aphid population growth rates (Meihls *et al.* 2013). Plant morphology and surface waxes that are variable within plant populations also play a role in defending plants against aphid attack (Zarpas *et al.* 2006, Wojcicka 2015).

The canopy density of winter wheat plots in the field also influences aphid infestations. *Sitobion avenae* populations persisted for longer and had higher peak densities in less dense wheat plots (Honek & Martinkova 1999). Other extrinsic factors unrelated to host plant quality can regulate aphid population

growth and abundance. Environmental conditions, particularly temperature, play a central role in regulating aphid population growth (Brabec *et al.* 2014, Ma *et al.* 2015).

Aphid abundance on field grown crops can also be influenced by natural enemies, for instance predators, such as coccinellids, carabids, and spiders which together can rapidly decimate aphid populations (Dixon 2000, Winder *et al.* 2013). Hymenopteran parasitoids, such as *Aphidius ervi,* can also cause a significant reduction in aphid populations at lower densities (Aslam *et al.* 2012). These natural enemies can reduce aphid densities on wheat crops grown in the field. Insecticide applications can reduce populations of natural enemies' (Foster *et al.* 2014). They are also a strong selection pressure for the evolution of pesticide resistance in aphids, especially to commonly applied pesticides such as Pyrethroids (Foster *et al.* 2014). Hence, it is important to breed wheat varieties with novel aphid resistance genes. Plant resistance is an important part of integrated pest management programs (Crespo-Herrera *et al* 2013). It is equally important to ensure other wheat breeding goals do not increase the potential for crop infestations by aphids.

One aim of wheat improvement strategies is to increase the nitrogen use efficiency of wheat. To achieve this aim, the components of nitrogen use efficiency can be increased by breeding wheat genotypes with higher nitrogen uptake and nitrogen utilisation efficiencies (Barraclough *et al.* 2010, Karrou & Nachit 2015). Breeding wheat varieties to increase the components of nitrogen use efficiency can result in wheat genotypes that have higher yields from lower application rates of nitrogen fertiliser (Karrou & Nachit 2015). A potential risk of increasing the components of nitrogen use efficiency in wheat genotypes may be that the potential for infestation by aphids may be increased. Currently very little is known about the relationship between cereal aphid densities and the nitrogen use efficiency of winter wheat.

The nitrogen use efficiency of wheat is the amount of dry matter grain produced kg^{-1} N available. In this context available nitrogen refers to the amount of nitrogen measured in the soil and applied as fertiliser (Moll *et al.* 1982, Gaju *et al.* 2011). Nitrogen use efficiency has two main components of nitrogen uptake (kg above ground plant N content kg^{-1} N available) and nitrogen utilisation efficiency, which is the amount of nitrogen in the above ground plant tissue that is converted into grain yield (kg grain dry matter kg^{-1}

above ground N content in the plant tissue at harvest). In modern wheat cultivars it is nitrogen utilisation efficiency that contributes most strongly to overall nitrogen use efficiency (Haile *et al.* 2012, Karrou & Nachit 2015). The plant nitrogen utilised for grain production is remobilised in the plant phloem mainly as constituents of amino acids (Xu *et al.* 2012). Therefore, plants that have higher nitrogen utilisation efficiency can have higher concentrations of amino acids in the plant phloem during grain fill (Hörtensteiner 2009, Karrou & Nachit 2015).

It is intuitive that aphids reduce nitrogen use efficiency by removing nitrogen from plant phloem and by reducing grain yield (Ladha *et al.* 2005). Aphid populations have been shown to increase in response to increases in amino acid concentration in plant phloem (Karley *et al.* 2002, Nowak & Komor 2010). Accordingly, increasing the nitrogen utilisation efficiency of wheat genotypes may increase aphid densities. A conspicuous "gap in the science" appears to be that few studies have investigated aphid densities in relation to the components of nitrogen use efficiency in wheat.

For wheat crops in the UK three cereal aphids, *Metopolophium dirhodum*, *Rhopalosiphum padi* and *S. avenae,* are common pests (Foster *et al.* 2014). Of these the grain aphid *S. avenae* is considered the most serious due to the reduction in grain yield it can cause (Wratten 1975, Kieckhefer & Gellner 1995) and as a known vector for several plant viruses, including the devastating barley yellow dwarf virus (Foster *et al.* 2014). *S. avenae* is an ear feeding species, feeding on wheat ears as the grain ripens, post anthesis (Wratten 1975). Plant traits that are involved in nitrogen utilisation efficiency will be functioning at this crucial growth stage, such as those involved with remobilising nitrogen from wheat leaves to the ripening grain (Hörtensteiner 2009, Xu *et al.* 2012).

The SavRia winter wheat population has novel genotypes which vary in their nitrogen utilisation efficiency and other components of nitrogen use efficiency. The important agronomic components of nitrogen use efficiency for six SavRia genotypes and the parental cultivars are described and quantified in chapter 5. In this chapter the densities of aphids on these SavRia genotypes are compared with the components of nitrogen use efficiency with particular focus on the relationship between aphid densities and nitrogen utilisation efficiency. The aim of this study is to identify if

breeding wheat genotypes with higher nitrogen use efficiency increases the potential for infestation by *S. avenae*.

6.1.1 Hypotheses

1. Wheat plants receiving higher nitrogen application rates will have higher aphid densities tiller^{-1}.

2. Aphid densities will be higher on SavRia genotypes that have higher nitrogen uptake efficiencies.

3. Aphid densities will be higher on SavRia genotypes that have higher nitrogen utilisation efficiencies.

4. Aphid densities on the SavRia genotypes will be positively correlated to their components of nitrogen use efficiency under the different nitrogen application rates.

6.2 Methods

Experimental wheat plots located at Church Farm, Bawburgh that were used to quantify the nitrogen use efficiencies of six SavRia genotypes and the parental varieties 'Savannah' and Rialto, as described in chapter 5, were monitored concomitantly for aphid density tiller^{-1}. The experimental design including positioning of the experimental 1m^2 plots, the nitrogen applications, harvesting techniques and nitrogen analyses that were used are all as detailed in the methods section in chapter 5.

Each of the experimental wheat plots was assessed for aphids by counting aphids on seven random wheat tillers in each plot. Each plot of SavRia genotypes was replicated five times in each of the three nitrogen application rates (Table 6.1).

Table 6.1. Experimental design. Treatments: the SavRia genotypes (together with parental cultivars) and three nitrogen application rates. Replication: the number of experimental wheat plots, the number of times the SavRia genotypes were replicated within them, the number of aphids (*S. avenae*) used to inoculate each plot, the number of tillers monitored for aphids 1m^{-2} plot and the interval between aphid counts.

Experimental design	No. of GTs	N application rates (kg N ha^{-1})	No. of 1m^2 plots	No. of GT reps	No. of aphids inoculated 1m^{-2} plot (±1SD)	No. tillers counted 1m^{-2}plot	count interval (days)
Randomised split plot	8	Control - 0	40	5	85.9 ± 5.3	7	7
		Low - 120	40				
		High - 240	40				

Monitoring aphid numbers before inoculating the field plots with S. avenae

The number of aphids naturally infesting experimental wheat plots was monitored weekly, from the last week in April (23/4/2013) until the first week in June (7/6/2013). There were no aphids counted on the experimental wheat plots during April, May and early June 2013. The Rothamsted aphid bulletin was checked for aphid occurrence at their monitoring sites (Rothamsted

2013). The zero aphid counts on the experimental plots in Bawburgh between April and early June were in agreement with aphid sample data from Rothamsted monitoring sites, which showed no *S. avenae* present at any monitoring site during this period (Rothamsted 2013).

6.2.1 Aphid cultivation in the laboratory

It was not possible to find any cereal aphids naturally infesting the experimental field plots. Therefore, laboratory reared populations of *S. avenae* were used to inoculate the experimental wheat plots in the field (Duffield *et al.* 1997). The source population of *S. avenae* used to inoculate the field plots is described in chapter 2. The aphid populations that were used to inoculate the field were cultivated on oat plants (*A. sativa –cv*. Dula) in 120 insect cages (19 cm x 10 cm x 7 cm) (Figure 6.1), one cage for each experimental wheat plot ($1m^2$). The insect cages were adapted from Polyethylene Terephthalate insect containers with perforated sides. These containers are commonly used to transport live insects.

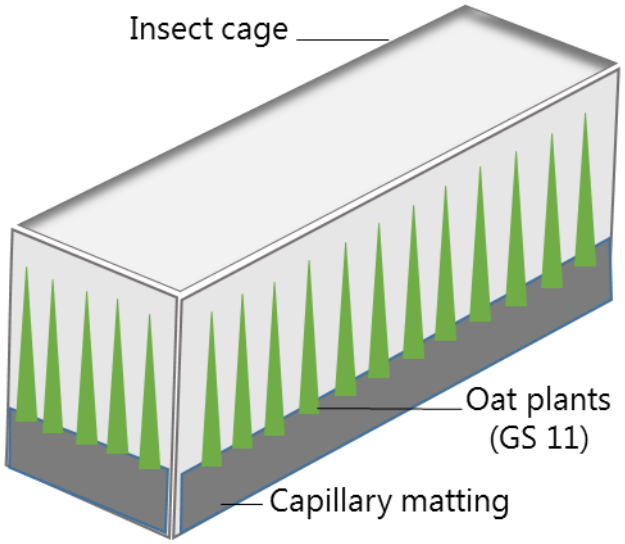

Figure 6.1. An insect cage with capillary matting in the base and the oat plants (growth stage 11- Zadoks scale), which were used to cultivate aphid populations prior to inoculating the experimental wheat plots in the field.

The inside base of each insect cage was lined with a 15 mm think layer of damp capillary matting (Figure 6.1). The oat seeds (*Avena sativa –cv*. Dula)

were evenly sprinkled over the capillary mat in the base of each insect cage using a full measuring beaker (120 ml). The oat seeds were germinated in each insect cage in a controlled environment (day length 18 h light: 6 h dark, light intensity 245 μmol m^{-2} S^{-1}, Day temperature 22 ^{o}C ±3 ^{o}C, night temperature 18 ^{o}C humidity 50 % ±5 %) and grown for 10 days before inoculation with aphids. Each of the 120 insect cages with 10 day old oat plants (GS11- Zadoks scale) were inoculated with seven *S. avenae* adults in the same controlled environment (Zadoks *et al.* 1974). The aphids were cultivated in the insect cages in the same controlled environment for 10 days, allowing enough time for viviparous production of nymphs (*personal observations*).

These 120 aphid populations, each in an individual insect cage, were moved to a colder controlled environment for seven days (day length 18 h light: 6 h dark, light intensity 245 μmol m^{-2} S^{-1}, Day temperature 18 ^{o}C, night temperature 16 ^{o}C humidity 48 % ±5 %). This protocol was adopted to reduce the potentially negative impact of temperature shock involved in relocating laboratory reared populations of aphids into the colder field environment (Parish & Bale 1993).

6.2.2 Inoculating the experimental wheat plots in the field

One population of aphids from one individual insect cage was used to inoculate each of the 120 experimental wheat plots (12/06/2013). The wheat plots in the field were inoculated by removing the capillary mat complete with the rooted oat plants (GS 11). The oat plants that were infested with aphids were cut and sprinkled downwind over each wheat plot, making sure the *S. avenae* infested plant fragments landed in the wheat plant canopy within each experimental plot (Duffield *et al.* 1997).

Monitoring aphids on the experimental wheat plots in the field

Counting aphids on the experimental wheat plots began on June 26th 2013, 14 days after inoculation. This allowed enough time for populations to establish and begin producing nymphs that may also have produced progeny. The aphid counts were performed weekly throughout their

population growth and decline, until no aphids could be found on the experimental wheat plots (Duffield *et al* 1997, Gash 2012). Seven random tillers plot^{-1} were selected to monitor aphid density each week. To select the seven random tillers plot^{-1} a simple map was made to split each plot into numbered co-ordinates. A random number generator was used to allocate co-ordinates of the seven random tillers plot^{-1} (R Core Team 2013).

The life stage of all aphids observed, which included nymphs, adult apterae and alatae, were counted and recorded on the seven random tillers in the 1m^2 plot^{-1} every seven days by three people starting at 9am. The position of the feeding sites of aphids on the wheat plants (leaves, stems or ears) was also recorded (Wratten 1975). Any aphids that appeared to be a different species were collected for identification. (There were very few, only 9 individuals of *Metopolophium dirhodum* in total). Any natural enemies of aphids observed on the plants were recorded and identified to order. Aphids that had obviously been parasitized by hymenoptera were also recorded (Duffield *et al.* 1997).

6.2.3 Statistical analysis

The different aphid morphs were aggregated to produce average aphid densities tiller^{-1}. The aphid counts (density tiller^{-1}) were tested using Levene's test for equality of variances (Levene 1960). The aphid counts for each morph (adult, nymph and alate) had unequal variances. A Shapiro-Wilk test was used to test these data for normality (Shapiro & Wilk 1965). The aphid count data for each aphid life stages were non-normally distributed. The peak aphid densities were calculated for week two, when aphid density was highest. Mean seasonal aphid densities were calculated by taking the average aphid densities tiller^{-1} and averaging them over all weeks (Carter & Ankersmit 1981); these data were used to display the interaction effect between SavRia genotype and nitrogen application rate on aphid densities and for the correlation analyses only.

Due to unequal variances, a non-parametric Kruskal Wallis test was conducted to test if the mean peak aphid density tiller^{-1} was significantly different between weeks (1-3), nitrogen application rates and SavRia genotype which were factors in the analyses (Kruskal Wallis 1952).

A visual comparison of the frequency distribution of aphid densities tiller^{-1} exhibited a Poisson distribution, commonly found in count data (Zuur *et al.* 2010). The aphid densities tiller^{-1} were modelled using a generalised linear model (GLM) with a log link and a Poisson error structure (Zuur *et al.* 2010). The residuals were plotted for the different error structures; the Poisson error structure offered the best fit for these data. The non-significant predictors tested were plot height and canopy density; these were removed from the model (Zuur *et al.* 2010). The variance inflation factors (VIF) were calculated for the covariates used in the model to test for collinearity (Carroll *et al.* 2006). Montgomery & Peck (1992) suggested collinear variables will have a VIF value >10, whereas a more stringent VIF value of >5 was suggested by Carroll *et al.* (2006). All covariates used in the Poisson GLM had a VIF value <5 revealing no significant collinearity between covariates.

Aphid density tiller^{-1} was the response variable in the Poisson GLM. SavRia genotype and nitrogen application rate were the predictor variables, with weeks and plant senescence as the covariates in the model. The interaction between SavRia genotype and nitrogen application rate and the interaction between plant senescence and nitrogen application rate were included in the model. These data analyses were performed using R environment for statistical computing (R Core Team 2015).

The Pearson product-moment correlation coefficients were computed to assess the relationship between seasonal aphid densities tiller^{-1} and the components of nitrogen use efficiency under the different nitrogen application rates. The Kruskal Wallis test and the Pearson product-moment correlation analyses were performed using SPSS (2013).

6.3 Results

The results are presented in four sections: 1) the average aphid densities tiller^{-1} on SavRia genotypes and parental varieties during each week of the summer growing season 2) the aphid densities tiller^{-1} in relation to nitrogen application rates; 3) the aphid densities tiller^{-1} in relation to SavRia genotype and the interaction between SavRia genotype and nitrogen application rate; 4) the relationship between aphid densities tiller^{-1} with plant senescence and the correlation between aphid densities and components of nitrogen use efficiency.

6.3.1 Changes in Aphid Densities tiller^{-1} on SavRia Genotypes during the Summer Growing Season

The aphid populations reached peak densities tiller^{-1} in week 2 (03/07/13) and subsequently declined until week 7 (07/08/13) as the wheat plants matured and senesced (Figure 6.2). The peak aphid densities were significantly different between weeks 1 - 3 (Table 6.2). The crop growth stage is displayed relative to aphid count date (Table 6.3). The aphid densities on SavRia genotypes during each week of the field trial were analysed using a GLM with a Poisson distribution. The covariate weeks was a significant predictor associated with a decline in aphid densities tiller^{-1} (β -0.203, 95% CI, -0.15 to -0.25, P<0.001) (Figure 6.2, Tables 6.4 & 6.5).

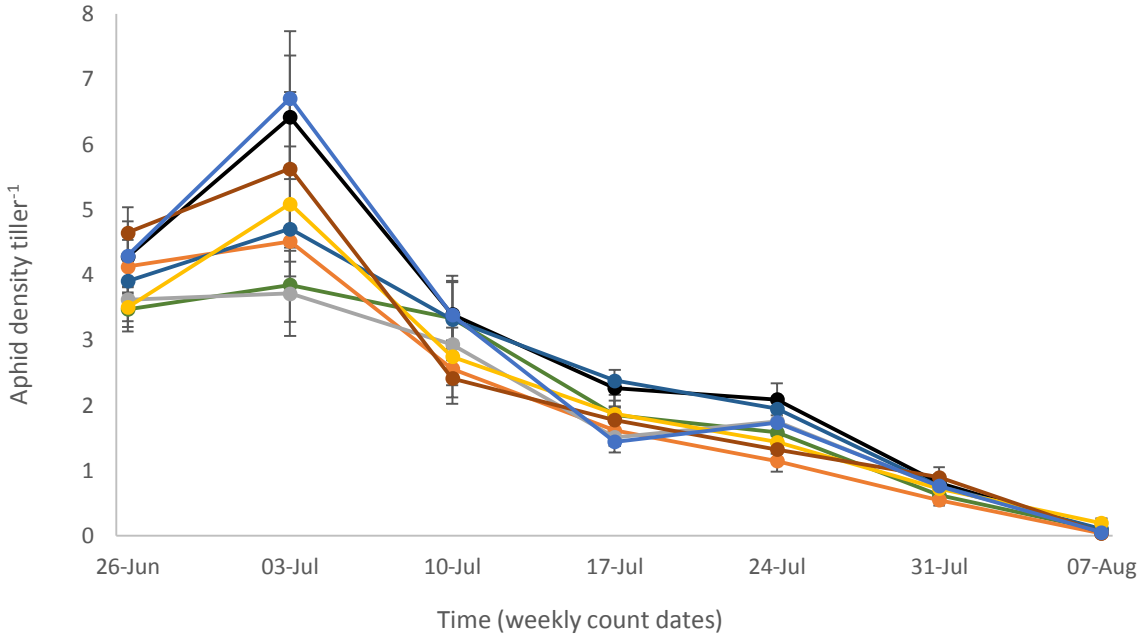

Figure 6.2. The mean aphid density tiller^{-1} on six SavRia genotypes and both parental cultivars sav ria 13 25 31 43 46 92 grown under the aggregated nitrogen treatments (y axis). The date of weekly aphid counts (X axis). Error bars ± 1SE of mean aphid counts.

Table 6.2. The results of testing peak aphid densities tiller^{-1} between factors. The factors were weeks (1-3), SavRia genotype and nitrogen application rate. Chi-square (χ^2) and *P*-values (≤0.01 bold italics).

Factor	d.f	Chi-Square	*P*- value
Weeks (1 - 3)	2	37.7	***0.01***
SavRia Genotype	7	5.61	0.56
Nitrogen application rate	2	4.27	0.11

Table 6.3. Observations on the approximate timing of crop developmental stage and growth stage (Zadoks *et al.* 1974) relative to aphid count dates.

Aphid count dates	26-Jun	03-Jul	10-Jul	17-Jul	24-Jul	31-Jul	07-Aug
Crop growth stage (Zadoks)	GS-61	GS-69	GS-71	GS-75	GS-77	GS-91	GS-92
Crop developmental stage	Anthesis	Grain set	Grain fill			Ripening	
S. avenae feeding site on plants	Leaves	Leaf/ear	Leaf/ear	Ears		Ears	

Table 6.4. Poisson generalised linear model results. The Response variable was aphid density tiller^{-1}. Weeks and senescence were covariates in the model. The likelihood ratio Chi-square (χ^2) for each predictor variable and interaction term (*) included in the model with their *P*-values (<0.05 italics; <0.01 bold italics). The variance inflation factor (VIF) for each covariate in the model were weeks=1.1, senescence=4.8.

Predictor	Likelihood ratio χ^2	*d.f*	*P*-value
Weeks	63.017	1,118	***0.001***
Nitrogen treatment	0.93	2,118	0.628
Genotype	28.241	7,118	***0.001***
genotype * N treatment	26.494	14,118	*0.05*
plant senescence	59.476	1,118	***0.001***
plant senescence * N treatment	7.187	2,118	*0.05*

Table 6.5. The Poisson generalised linear model parameter estimates. The Response variable was aphid density tiller^{-1}. Weeks and senescence were the covariates in the model. Poisson regression coefficients beta (*ß*) for each significant predictor variable and interaction term (*) included in the model, standard error, 95% confidence intervals for the coefficients, Wald Chi-square (χ^2) and their *P*-values (<0.05 italics; <0.01 bold italics).

			95% Confidence Interval			
Predictor parameter	*ß*	1 S.E	Lower	Upper	Wald χ^2	*P*-value
weeks	-0.203	0.026	-0.253	-0.152	61.34	***0.001***
Nitrogen treatment (Low)	-0.351	0.153	-0.652	-0.051	5.268	*0.05*
Nitrogen treatment (High)	-0.169	0.142	-0.449	0.11	1.407	0.22
Savannah	-0.474	0.15	-0.769	-0.179	9.91	***0.01***
SavRia GT13	-0.481	0.153	-0.782	-0.181	9.85	***0.01***
SavRia GT25	-0.44	0.149	-0.732	-0.149	8.76	***0.01***
SavRia GT43	-0.444	0.15	-0.738	-0.151	8.79	***0.01***
GT13 * Low N rate	0.612	0.221	0.178	1.046	7.62	***0.01***
GT43 * Low N rate	0.625	0.217	0.199	1.05	8.28	***0.01***
GT46 * Low N rate	0.401	0.209	-0.01	0.811	3.66	*0.05*
Plant senescence	-0.013	0.002	-0.017	-0.009	37.88	***0.001***
senescence * Low N rate	0.004	0.002	0.0005	0.008	3.94	*0.05*

6.3.2 Aphid densities tiller^{-1} in relation to nitrogen application rates

Testing the hypothesis that wheat plants receiving higher nitrogen application rates will have higher aphid densities tiller^{-1}.

Nitrogen application rate was not a significant predictor of aphid densities (Table 6.4). The aphid densities tiller^{-1} over time were lower under the low nitrogen application rate relative to the zero nitrogen application rate (Table 6.5, Figure 6.3). The only week during the growing season that aphid densities tiller^{-1} were higher on plants under the low nitrogen application rate relative to the zero nitrogen application rate was during week four (Figure 6.3). The seasonal aphid densities tiller^{-1} (aphid densities averaged over all weeks) were higher under the zero nitrogen application rate ($\bar{X}$±1SE: 2.46 ± 0.16) relative to the low ($\bar{X}$±1SE: 2.29 ± 0.14) and high nitrogen application rates ($\bar{X}$±1SE: 2.25 ± 0.14). The peak aphid densities tiller^{-1} (week 2: 03/07/13) were not significantly different between nitrogen application rates (Table 6.2, Figure 6.3).

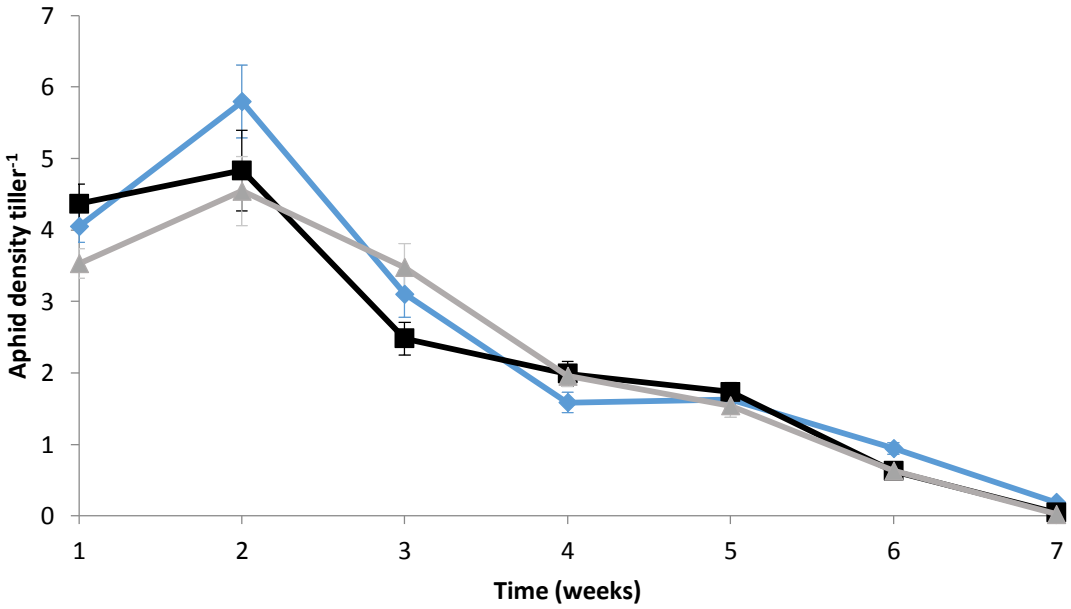

Figure 6.3. Aphid density tiller^{-1} on SavRia genotypes and parental varieties aggregated (y axis) against time in weeks (x axis). Nitrogen application rates: zero 0 kg N ha^{-1} ♦, low 120 kg N ha^{-1} ■ and high 240 kg N ha^{-1} ▲. Error bars are ±1 SE.

6.3.3 Aphid densities tiller^{-1} in relation to SavRia genotype and the interaction between SavRia genotype and nitrogen application rate

Quantifying aphid densities tiller^{-1} on the SavRia genotypes to test the hypotheses that aphid densities will be higher on SavRia genotypes that have higher nitrogen uptake and nitrogen utilisation efficiencies.

SavRia genotype was a significant predictor of aphid density tiller^{-1} (Figure 6.2, Tables 6.4 & 6.5). There was a significant negative effect of the 'Savannah' parent and SavRia genotypes 13, 25, 43 on aphid density tiller^{-1} relative to genotype 92, which had the highest aphid density tiller^{-1} under the zero nitrogen application rate (Figure 6.4.a, Tables 6.5 & 6.6). The peak aphid densities tiller^{-1} (week 2) were not significantly different between SavRia genotypes (Table 6.2, Figure 6.2).

There was a significant interaction effect between SavRia genotype and nitrogen application rate on aphid densities tiller^{-1} (Table 6.4). The aphid densities tiller^{-1} responded differently to the SavRia genotypes under the different nitrogen application rates. SavRia genotypes 13, 43, and 46 grown under the low N application rate (120 kg N ha^{-1}) were associated with an increase in aphid densities tiller^{-1} over time, relative to the zero N application rate (0 kg N ha^{-1}) (Figure 6.4.a, b, c, Table 6.5).

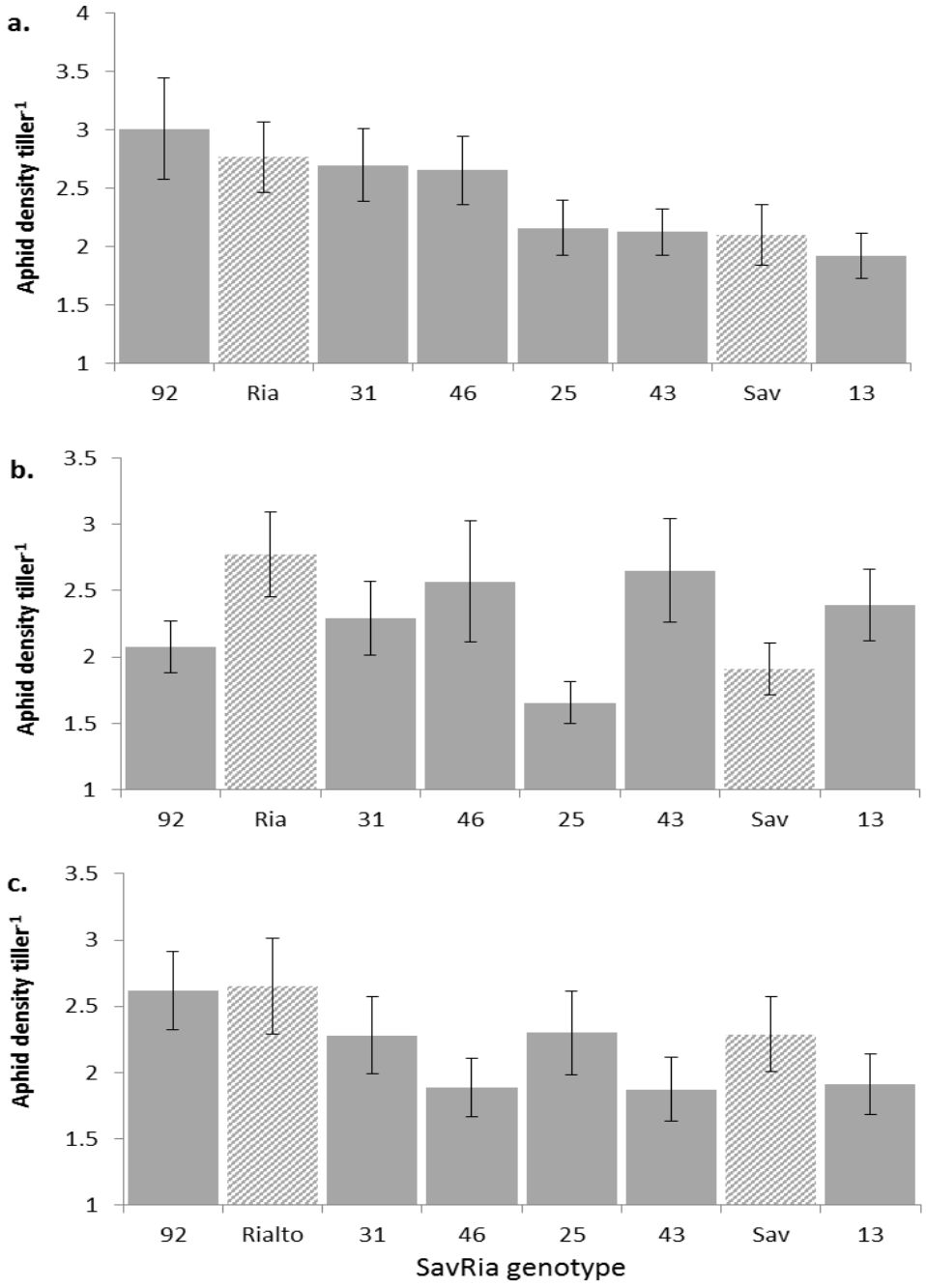

Figure 6.4. The seasonal aphid density tiller^{-1} (averaged over the growing season) on each SavRia genotype and parental cultivars ▨ for each nitrogen application rate **a**). 0 kg N ha^{-1} applied, **b**). 120 kg N ha^{-1} applied **c**). 240 kg N ha^{-1} applied. SavRia genotypes arranged by aphid densities from high to low in the control treatment Figure 6.4.a. (X-axis). Error bars ±1S.E.

6.3.4 The relationship between aphid density tiller^{-1} with plant senescence and the components of nitrogen use efficiency

Plant senescence (%) was associated with a decline in aphid densities tiller^{-1} (β -0.013, 95% CI, -0.017 to -0.009, P<0.001) (Tables 6.4 & 6.5).

There was a significant interaction between plant senescence and nitrogen application rate on aphid densities tiller^{-1}. Plant senescence in the low nitrogen application rate was associated with an increase in aphid densities tiller^{-1} relative to the zero N application rate (Tables 6.4 & 6.5). During week four of the field trial plant senescence was highest under the low nitrogen application rate (Chapter 5: Figure 5.5, Table 5.5). Aphid densities tiller^{-1} during week 4 were higher under the low nitrogen application rate relative to the zero application rate (Figure 6.3).

Testing the hypothesis that aphid densities on the SavRia genotypes will be positively correlated to components of their nitrogen use efficiency under the different nitrogen application rates.

Neither the seasonal aphid densities tiller^{-1} nor the peak aphid densities tiller^{-1} were correlated with the components of nitrogen use efficiency under the different nitrogen application rates (Table 6.6). There was no correlation between seasonal aphid densities tiller^{-1} with the nitrogen uptake efficiencies (Figure 6.5, Table 6.6) nor nitrogen utilisation efficiencies of SavRia genotypes under the different nitrogen application rates (Figure 6.6, Table 6.6).

Table 6.6. A correlation matrix of aphid densities tiller^{-1} with components of plant nitrogen use, and grain yields under each nitrogen application rate **a**). Control (0 kg N ha^{-1}) (n=40), **b**). Low (120 kg N ha^{-1}) (n=40) and **c**). High (240 kg N ha^{-1}) (n=40). The values are the Pearson correlation coefficient (r). Correlation coefficients (r) significant at $P \leq 0.01$ bold italics.

	Nitrogen application rates					
	a). Control (0 kg N ha^{-1})		**b**). Low (120 kg N ha^{-1})		**c**). High (240 kg N ha^{-1})	
Variables	Aphid density	Peak aphid density	Aphid density	Peak aphid density	Aphid density	Peak aphid density
Aphid density tiller^{-1} (all weeks)	1		1		1	
Peak aphid density tiller $^{-1}$ (week 2)	-	1	-	1	-	1
Plant nitrogen content (g N kg BM^{-1})	-0.22	-0.28	-0.01	-0.01	-0.23	0.07
Nitrogen uptake efficiency	-0.22	-0.28	-0.01	-0.01	-0.23	0.07
Nitrogen utilisation efficiency	0.20	0.25	-0.08	0.01	0.25	0.08
Grain yield (kg m^{-2})	0.08	0.14	-0.01	0.16	0.08	0.21
Grain nitrogen content (%)	-0.06	-0.19	0.03	-0.08	-0.05	-0.10
Nitrogen use efficiency (kg DM kg N^{-1})	0.08	0.14	-0.01	0.16	0.08	0.21

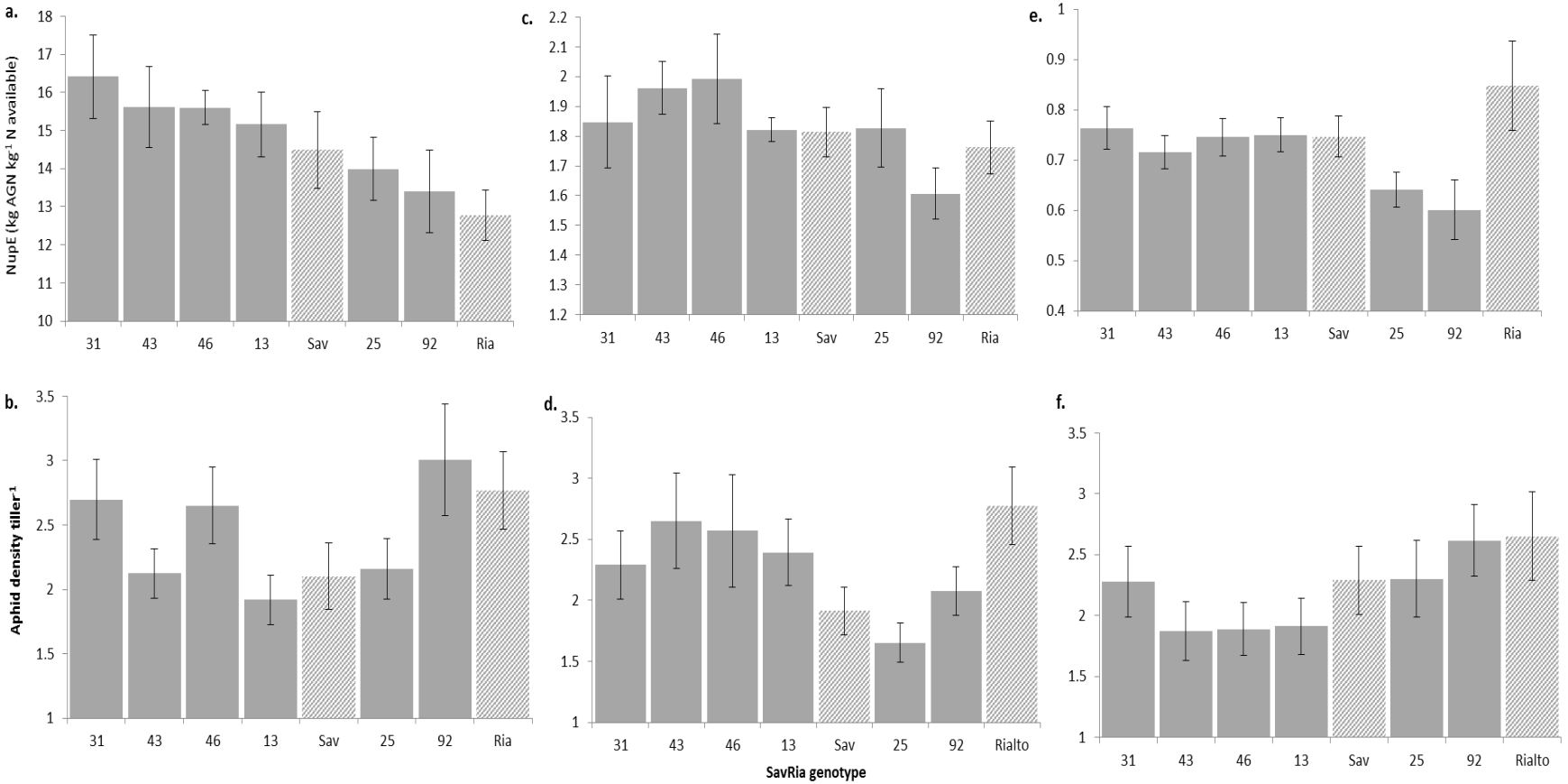

Figure 6.5. A comparison of the average nitrogen uptake efficiency of each SavRia genotype in relation to seasonal aphid densities tiller^{-1} under each nitrogen application rate (**a**, **b**: 0 kg N ha^{-1}; **c**, **d**: 120 kg N ha^{-1}; **e**, **f**: 240 kg N ha^{-1}). The SavRia genotypes are arranged by increasing nitrogen uptake efficiency in the zero nitrogen application rate Figure 6.5, **a**. (X axis).

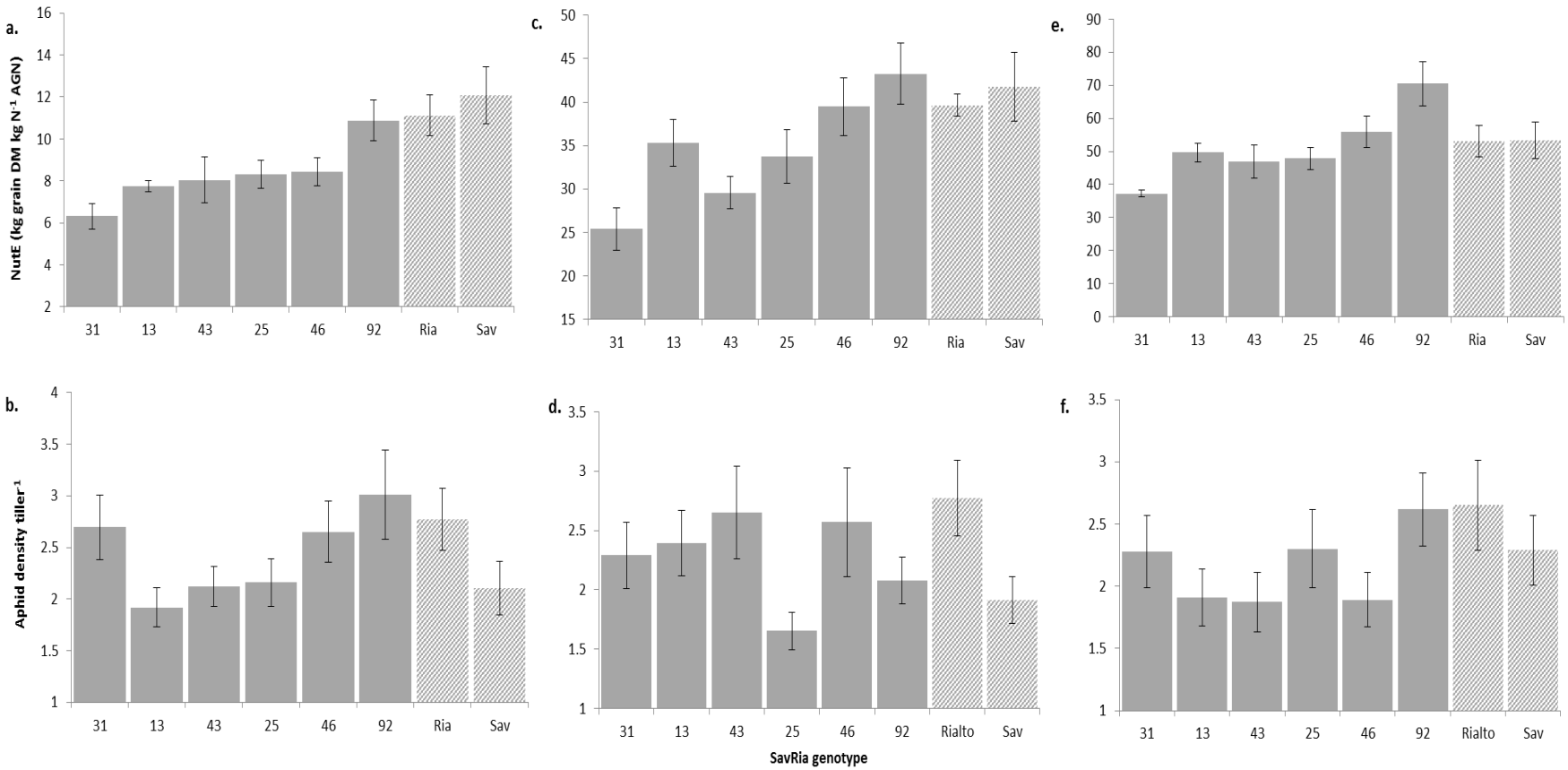

Figure 6.6. A comparison of the average nitrogen utilisation efficiency for each SavRia genotype in relation to seasonal aphid densities tiller^{-1} under each nitrogen application rate (**a**, **b**: 0 kg N ha^{-1}; **c**, **d**: 120 kg N ha^{-1}; **e**, **f**: 240 kg N ha^{-1}). The SavRia genotypes are arranged by increasing nitrogen utilisation efficiency in the zero nitrogen application rate Figure 6.6, **a**. (X axis).

6.4 Discussion

The experimental wheat plots were inoculated with a laboratory reared population of *S. avenae* as natural infestation had not occurred. Natural populations of *S. avenae* in England usually migrate on to winter wheat between March and June after overwintering as eggs or reproducing viviparously on Poaceae *sp* (Ankersmit & Carter 1981). Following inoculation the population growth and decline of the grain aphid *S. avenae* infesting the experimental wheat plots (2013) appeared to follow a typical pattern of grain aphid infestations on winter wheat (Figure 6.2, Table 6.3). However, the timing of colonisation, peak densities and subsequent infestations can differ greatly between years (Watt *et al.* 1984, Duffield *et al.* 1997, Jarosik *et al.* 2003).

During June *S. avenae* populations on winter wheat plots can grow rapidly during crop development as they move onto the wheat ears following grain set (Zadoks scale GS 59-61). It is on the ears that *S. avenae* continues to feed as grain fill progresses (GS 69-71) (Zadoks *et al.* 1974, Watt 1979, Jarosik *et al.* 2003). Alatae forms migrate from wheat plots as crop senescence increases, or aphid density becomes very high causing overcrowding (Watt 1979, Watt & Dixon 1981). Population density of *S. avenae* then declines rapidly as mortality increases, due to declining host plant quality, following the doughy stage of grain ripening (GS-80) (Ankersmit & Carter 1981). The developmental stage of the crop and therefore the rate of aphid mortality can differ between nitrogen application rates (Gash 2012).

6.4.1 S. avenae density in relation to nitrogen application rate

The results of this field study do not support the hypothesis that wheat plants receiving higher nitrogen application rates will have higher aphid densities tiller^{-1}. The peak densities of *S. avenae* tiller^{-1} were not significantly different between nitrogen application rates (Table 6.2). Nitrogen application rate was not a significant predictor of aphid densities tiller^{-1} (Table 6.4). The aphid densities tiller^{-1} were highest on the zero nitrogen application rate relative to the low and high nitrogen application rates (Figure 6.3, Table 6.5). These results contrast with the results of Aqueel and Leather (2011), who

showed a positive correlation between aphid fecundity and nitrogen application rates in the laboratory.

Unlike experiments under laboratory conditions aphid populations in the field can by influenced by a wide range of processes that cannot be controlled. For example, a field study by Duffield *et al.* (1997) showed that *S. avenae* populations were larger on wheat plants receiving higher nitrogen application rates over two seasons of a three year study. In the final season of their study, larger populations of *S. avenae* were, however, recorded on plants that received no nitrogen (Duffield *et al.* 1997). Duffield *et al.* (1997) concluded that this might have been due to entomopathogenic fungi that reduced aphid numbers where there were higher nitrogen application rates, which had denser canopies that were more favourable conditions for the fungi, and less favourable for *S. avenae* that were further reduced by fungal infection.

Wheat plots with less dense canopies have been associated with higher peak densities of *S. avenae* in other studies (Honek & Martinkova 1999). Canopy densities were significantly higher under the higher nitrogen application rates (Chapter 5: Figure 5.2.b). However, canopy density was not a significant predictor of aphid density tiller^{-1} which was tested in the generalised linear model; this non-significant predictor without interaction effects was removed from the model following Zuur *et al.* (2010). Therefore, in a similar way to Duffield *et al.* (1997) *S. avenae* densities tiller^{-1} may have been lower under higher nitrogen application rates due to an interaction between meteorological factors and aphid densities, but not due to entomopathogenic fungi interacting with canopy density.

6.4.2 Sitobion avenae density in relation to SavRia genotype

SavRia genotype was a significant predictor of aphid densities (Table 6.4). There was significant variation in aphid densities tiller^{-1} between the SavRia genotypes over time (Figure 6.2, Table 6.4 & 6.5). SavRia genotype 92 had the highest aphid densities tiller^{-1} under the zero nitrogen application rate, while SavRia genotypes 13, 25 and 43 had significantly lower aphid densities relative to genotype 92 in the zero nitrogen application rate (Table 6.5). The nitrogen uptake efficiency of SavRia genotypes 13 and 43 were both in the higher end of the spectrum (Figure 6.6, a). Aqueel and Leather (2011)

demonstrated that aphid fecundity increased with nitrogen application rate on four different wheat varieties, although the increase in *S. avenae* fecundity was not consistently different between the four wheat varieties.

Aphid fecundity on SavRia genotype 43 and SavRia GT 25 was the highest and lowest respectively in the laboratory experiment reported in chapter 4, which suggested that these genotypes had different levels of susceptibility or resistance to aphid infestation, as the differences were not related to plant nitrogen content. The low aphid densities recorded on SavRia GT 43 under the zero and high nitrogen application rates during the field experiment suggests this genotype is not more susceptible to aphid infestation. However, these differences between the laboratory and field experiment may be due to the growth stage of the plant at which they were recorded, which can significantly influence cereal aphid reproduction (Leather & Dixon 1981).

SavRia genotype 25 had consistently low seasonal aphid densities in the zero and low nitrogen application rates (Figure 6.4.a, b), consistent with the results of the laboratory experiment presented in chapter 4. However, under the high nitrogen application rate, seasonal aphid densities on SavRia GT 25 were in the higher range relative to other genotypes (Figure 6.4.c). High nitrogen application rates can make resistant plants more susceptible to aphid infestation (Li *et al.* 2014), which could explain the higher aphid densities on genotype 25 under the high nitrogen application rate. There was a significant interaction between SavRia genotype and nitrogen application rate (Table 6.4). The seasonal aphid densities responded differently on the SavRia genotypes between the nitrogen application rates (Figure 6.4).

In this experiment plant senescence was a significant predictor of aphid densities tiller^{-1} (Table 6.4). Plant senescence in wheat includes the process of nitrogen in plant tissues being remobilised as amino acids and remobilised to the developing grain (Distenfeld *et al.* 2014). This coincides with the leaves turning yellow as chlorophyll breaks down (Hörtensteiner 2009). The senescence of SavRia genotypes was associated with a decline in aphid densities tiller^{-1} (Table 6.4). This relationship was also consistent with the laboratory experiment presented in chapter 4, in which the fecundity of *S. avenae* increased on SavRia genotypes with a higher concentration of chlorophyll in the leaves, i.e. the converse of senescence.

There was a significant interaction between SavRia genotype and nitrogen application rate on plant senescence (Table 6.4). Senescence of SavRia genotypes was significantly higher in the low nitrogen application rate relative to the control treatment, particularly between weeks 4 and 5 (Chapter 5: Table 5.5, Figure 5.5). The higher level of plant senescence under the low nitrogen application rate was associated with an increase in aphid densities tiller^{-1} relative to the zero nitrogen application rate (Table 6.5). *S. avenae* populations were predominantly feeding on the developing ears by week 4 (17 July) of the field trial (Figure 6.2, Table 6.3). The higher level of plant senescence in the low nitrogen application rate between weeks 4 and 5 suggests that a higher amount of nitrogen may have been remobilised from somatic plant tissues to the developing grain (Hörtensteiner 2009, Distenfeld *et al.* 2014). This may explain the interaction between SavRia genotype and nitrogen application rate on plant senescence having a positive influence on aphid densities tiller^{-1} under the low nitrogen application rate (Table 6.5). Moreover, week four of the field trial was the only week higher aphid densities tiller^{-1} were recorded on plants under the low nitrogen application rate (Figure 6.3).

The highest seasonal aphid densities were recorded on SavRia GT 92, which had the second lowest nitrogen uptake efficiency under the zero nitrogen application rate (Figure 6.5, a, b). The second highest seasonal aphid densities were recorded on SavRia genotype 31, which had the highest nitrogen uptake efficiency under the zero nitrogen application rate (Figure 6.5, a, b). Aphid densities tiller^{-1} did not to respond consistently to SavRia genotypes with high and low nitrogen uptake efficiencies between each nitrogen application rate (Figure 6.5).

6.4.3 Sitobion avenae density in relation to nitrogen uptake efficiency

There was no correlation between nitrogen uptake efficiency and either seasonal or peak aphid densities under the three nitrogen application rates (Table 6.6). These results do not support the hypothesis that aphid densities will be higher on SavRia genotypes that have higher nitrogen uptake efficiencies. Consistent with the laboratory experiment presented in chapter 4, *S. avenae* population performance is not higher on plants with higher nitrogen uptake efficiency. These results are comparable with those of Khan

and Port (2008), who showed that *S. avenae* did not consistently have higher fecundity on wheat plants with higher nitrogen content.

6.4.4 Sitobion avenae density in relation to nitrogen utilisation efficiency

The results do not support the hypothesis that aphid densities will be higher on SavRia genotypes that have higher nitrogen utilisation efficiencies. The differences observed in seasonal and peak aphid densities tiller^{-1} between SavRia genotypes were not correlated with the differences in the nitrogen utilisation efficiency of the SavRia genotypes (Figure 6.5). SavRia GT 92 had high nitrogen utilisation efficiencies under both the zero and high nitrogen application rates (Figure 6.5). Aphid densities tiller^{-1} were the highest and second highest on SavRia genotype 92 under the zero and high nitrogen application rate respectively (Figure 6.5). In contrast, when grown in the zero nitrogen application rate SavRia GT 31 had the lowest nitrogen utilisation efficiency but high aphid densities tiller^{-1} (Figure 6.5). Despite the relationship between genotype 92 and aphid densities tiller^{-1}, no correlation between aphid densities and nitrogen utilisation efficiency was identified (Table 6.6, Figure 6.5).

Wheat plants with higher nitrogen utilisation efficiency are likely to have a higher concentration of amino acids in the plant phloem during grain fill (Hörtensteiner 2009, Distenfeld *et al.* 2014, Karrou & Nachit 2015). During this critical growth stage of wheat *S. avenae* feeds from plant phloem being transported to the grain (Ankersmit & Carter 1981). However, aphids are not just influenced by the concentration of nitrogenous compounds in the phloem, but by the composition of essential amino acids and other macromolecules in plant phloem (Hale *et al.* 2003, Nowak & Komor 2010). There may be a higher concentration of amino acids in the plant phloem of SavRia genotypes with higher nitrogen utilisation efficiencies. However, there may not be a higher concentration of the essential amino acids, which has been shown to be more important for aphid performance (Karley *et al.* 2002).

6.4.5 The correlation between Sitobion avenae density and the components of nitrogen use efficiency under the different nitrogen application rates.

The most important variables determining nitrogen use efficiency in a wheat crop are total nitrogen uptake, nitrogen utilisation efficiency, grain yield, percentage grain nitrogen and nitrogen availability from the soil and fertiliser (Barraclough *et al.* 2009). The grain yield and grain nitrogen content of wheat can be contributed to in different ways by the nitrogen uptake and nitrogen utilisation efficiencies, which differ between wheat genotypes and different nitrogen application rates (Gaju *et al.* 2011). Therefore, these important agronomic plant traits were tested for a correlation with aphid densities under each nitrogen application rate.

There were no correlations between either grain yields or grain nitrogen content with seasonal or peak aphid densities under the different nitrogen application rates (Table 6.6). The hypothesis that aphid densities on the SavRia genotypes will be positively correlated to components of their nitrogen use efficiency under the different nitrogen application rates is therefore not supported by these data. The results of this study suggest that the grain aphid *S. avenae* did not respond to wheat genotypes with higher nitrogen use efficiencies during this growing season.

6.4.6 Summary

In summary aphid density tiller^{-1} varied in relation to SavRia genotype. The aphid densities responded differently to the SavRia genotypes under the different nitrogen application rates. The plant traits monitored in chapter 5, including the nitrogen use efficiency and its components varied between the nitrogen application rates and also between SavRia genotypes. However, the variation in aphid densities tiller^{-1} on the SavRia genotypes, were not correlated to variation in the components of nitrogen use efficiency under the different nitrogen application rates. There was a significant interaction between SavRia genotype and nitrogen application rate on nitrogen utilisation and nitrogen use efficiency identified in chapter 5. There was also a significant interaction between SavRia genotype and nitrogen application rate on aphid density tiller^{-1}. Nevertheless, there was no correlation between seasonal aphid densities nor peak aphid densities with the nitrogen uptake,

nitrogen utilisation nor nitrogen use efficiencies of SavRia genotypes under the three nitrogen application rates.

I conclude that these studies have not supported the hypothesis that breeding wheat varieties with higher nitrogen use efficiencies influences aphid densities, therefore altering the potential for infestation by aphids. I recommend however that the hypotheses tested in this study be tested over different growing seasons at different locations, due to the way in which abiotic factors can influence grain aphid populations in the field (Duffield *et al.* 1997). Grain aphid populations can change significantly between seasons, particularly in sites with less favourable growing conditions (Duffield *et al.* 1997, Brabec *et al.* 2014). It would also be prudent to include other species of cereal aphids in future studies, in particularly species of aphid shown to be consistently responsive to increased nitrogen application rates. The results of this study show that under the conditions used in these experiments wheat genotypes with higher nitrogen use efficiencies were not more susceptible to grain aphid infestations.

7 Discussion

Future food security will be reliant on substantial increases in wheat yields. Whilst increasing the grain yields of wheat could contribute towards feeding the population that is predicted by 2050 (Challinor 2011, Meyers *et al.* 2015), insect pests have the potential to prevent possible increases in wheat yield being achieved. The aims of this research were to contribute to an understanding of how wheat production can be improved through breeding and agronomy in relation to the risk of cereal aphid infestations. This broad aim was achieved using a combination of laboratory, greenhouse and field experiments that monitored cereal aphid performance on a population of winter wheat with different nitrogen use efficiencies. Cereal aphids can be serious pests on crops of winter wheat in the temperate regions of the world (Ankersmit & Carter 1981, Foster *et al. 2014*).

As crop pests, cereal aphids can cause significant decreases in grain yield through removing nutrients from the plant phloem during feeding and as vectors for plant viruses' (Kieckhefer & Gellner 1992, Foster *et al. 2014*). Aphids are particularly sensitive to changes in the nitrogen dynamics of their host plants, which includes cereals (Dixon 1998, Ponder *et al* 2000). Improving the nitrogen use efficiency of wheat is part of breeding strategies, intended to increase wheat yields, in combination with reducing nitrogen application rates (Barraclough *et al.* 2010, Karrou & Nachit 2015).

The doubled haploid SavRia winter wheat population was produced with the intention of contributing towards wheat improvement (Wilkinson *et al.* 2012). The SavRia population contains novel wheat genotypes with different nitrogen use efficiencies and the component traits of nitrogen uptake and nitrogen utilisation efficiencies; there was ample variation in these traits between genotypes to investigate them in relation to cereal aphid performance. The specific objectives of this research were to identify if genetic variation within the SavRia population was associated with cereal aphid performance and if SavRia wheat genotypes with higher nitrogen use efficiencies alter the potential for infestation by *Sitobion avenae*.

7.1.1 Genetic variation in the SavRia population in relation to cereal aphid performance

Genetic variation associated with cereal aphid performance (Chapter 3) was identified within the SavRia population. Genetic markers were identified and located on a genetic map of the SavRia population; these genetic markers were associated with either an increase or decrease in the performance traits of *Sitobion avenae* and *Metopolophium dirhodum*. There were twelve genetic

markers located that were associated with the performance traits of these cereal aphids. Five of these genetic markers were associated with an increase and seven of them with a decrease in cereal aphid performance traits.

The QTL associated with the component traits of nitrogen use efficiency have not all been identified (Coque *et al.* 2008, Bernard & Habash 2009). In the future if these QTL are identified and characterised, then they could be analysed to test if they coincide with QTL that are associated with an increase in cereal aphid performance traits. The genetic markers identified in this study that were associated with a change in cereal aphid performance could be used in such analyses. This approach will identify if improving the component traits of nitrogen use efficiency increases the susceptibility of wheat to cereal aphids.

The new information on the location of seven genetic markers associated with a reduction in aphid performance could be used for improving aphid resistance in wheat. The seven genetic markers, which were associated with a reduction in cereal aphid performance, are flanked by genes that infer a negative influence on the performance measures of *S. avenae* and *M. dirhodum* in different environments (Chapter 3). The putative genes that flank these genetic markers can now be discovered and characterised in future studies. After being characterised, putative genes in this wheat population that infer aphid resistance could be used in marker assisted breeding programs (Yadawad *et al.* 2015). To produce wheat populations with durable resistance to aphids gene pyramiding techniques should be used (Joshi & Nayak 2010). Genes with minor and major effects, which infer aphid resistance through different modes of action, will play a pivotal role in increasing aphid resistance in wheat (Joshi & Nayak 2010). Improving aphid resistance in wheat can reduce yield loss associated with aphid infestations and hence an increase in nitrogen use efficiency (Ladha *et al.* 2005). It is, therefore, an important wheat improvement strategy (Cao *et al.* 2015).

A limitation of this study, was that the subset of ninety four SavRia genotypes monitored for aphid performance for subsequent use in the QTL analysis were not the same subset of ninety four genotypes that Wilkinson *et al.* (2012) uploaded to the genetic database available from CerealsDB. To overcome this limitation the aphid performance data used in the current QTL analysis could be reanalysed in combination with the genetic data for the entire SavRia population once they become available.

7.1.2 The nitrogen use efficiency of wheat in relation to infestation by Sitobion avenae

A key question that was addressed in this thesis was: are wheat genotypes with higher nitrogen use efficiencies more susceptible to cereal aphid infestations? The desirable agronomic traits nitrogen uptake and nitrogen utilisation efficiencies, which are components of nitrogen use efficiency were investigated in relation to aphid performance. Data on aphid performance indices of development time, fecundity and population densities on wheat genotypes can be used to infer the potential for infestation by aphids on these genotypes (Jarosik *et al.* 2003, Powell *et al.* 2006, Dahlin & Ninkovic 2013). Wheat improvement strategies have targeted agronomic traits that can contribute to higher yields by improving the component traits of nitrogen use efficiency (Karrou & Nachit 2015).

Laboratory and field studies were used to identify if improving desirable agronomic traits in wheat increases the potential for infestation by *S. avenae*. A subset of nine SavRia genotypes with different nitrogen uptake efficiencies were monitored for *S. avenae* performance measures in the laboratory (Chapter 4). Nitrogen use efficiency and its components were also quantified for a subset of six SavRia genotypes under different nitrogen application rates in the field (Chapter 5), *S. avenae* population densities on these genotypes were monitored and compared with the components of their nitrogen use efficiencies under each nitrogen application rate (Chapter 6).

The prediction underlying the investigation presented in chapter 4 was that plants with higher nitrogen uptake efficiencies could influence *S. avenae* performance in a similar way to that caused by increasing nitrogen fertiliser application for their host plants. The fecundity and longevity of *S. avenae* are positively correlated with rates of nitrogen fertiliser application (Aqueel & Leather 2011). Consequently increasing nitrogen fertiliser application rates can increase the potential for infestation of wheat by *S. avenae* (Aqueel & Leather 2011, Wang *et al.* 2015). A subset of nine SavRia genotypes which captured a range of variation in nitrogen uptake efficiencies was grown in the laboratory and inoculated with *S. avenae* at growth stage 25. The performance measures of development time form nymph to reproduction, fecundity and the intrinsic rate of increase were recorded on the SavRia genotypes.

The performance measures of *S. avenae* were not significantly higher on genotypes with higher nitrogen content in the plant tissue (Chapter 4). The results of this study showed aphid performance was not closely related to the nitrogen uptake of wheat plants at this growth stage. There was no reduction

in the development time of *S. avenae* and neither were there increases in their fecundity on SavRia genotypes with higher nitrogen uptake efficiencies. These results show that wheat genotypes with higher nitrogen uptake efficiency do not increase the potential for infestation of wheat crops by *S. avenae* at this growth stage. These results are reasonably consistent with those of Khan and Port (2008), who showed that different populations of *S. avenae* do not consistently have higher fecundity on wheat plants (GS-13) with higher nitrogen content in the plant tissue.

Aphid populations reared under standard laboratory conditions for many generations have been shown to adapt their feeding behaviour and other biological characteristics, such as host plant selection and alarm pheromone detection to laboratory conditions (Thieme & Dixon 2015). It is, therefore, also important to investigate the relationship between cereal aphids on their host plants using naturally occurring aphid populations. The performance of cereal aphids can be influenced by the growth stage of cereals on which they are feeding (Watt 1979, Leather & Dixon 1981, Watt & Dixon 1981). *Sitobion avenae* can feed from young leaves of wheat plants during early growth stages but infestations are usually more severe at later growth stages when *S. avenae* feed from the ears (Watt 1979, Ankersmit & Carter 1981). Due to the intrinsic limitations of laboratory experiments restricted to specific growth stages, it is advantageous to combine the rigour of controlled laboratory experiments with the greater realism of the external environment in carefully designed field experiments.

A field trial was used to monitor the population densities of *Sitobion avenae* over a prolonged sequence of growth stages on six SavRia genotypes and parental varieties, under realistic edaphic conditions where wheat genotypes completed flowering and grain fill (Chapters 5 & 6). A number of the phenotypic traits of SavRia genotypes that were measured increased with nitrogen fertiliser application, including grain nitrogen concentration and nitrogen utilisation efficiency (Chapter 5). The SavRia genotypes also had different nitrogen uptake, nitrogen utilisation and nitrogen use efficiencies. The nitrogen utilisation and nitrogen use efficiencies of SavRia genotypes interacted with nitrogen application rate.

The densities of *S. avenae* did not increase on the SavRia genotypes with increasing nitrogen application rates (Chapter 6). The *S. avenae* population densities responded differently to the SavRia genotypes between nitrogen application rates. There was no correlation between aphid population densities on SavRia genotypes and the component traits of nitrogen use efficiency under the different nitrogen application rates. *S. avenae* can feed

from wheat plants during most of their developmental cycle, including during flowering and grain fill (Ankersmit & Carter 1981). However it is on the ears of wheat, during grain set and filling, that *S. avenae* are most fecund (Watt 1979). *Sitobion avenae* was, therefore, an ideal species of cereal aphid to investigate performance in relation to components of nitrogen use efficiency. It was also particularly appropriate for examining nitrogen utilisation efficiency which functions during flowering when *S. avenae* are feeding from the grain (Watt 1979, Ankersmit & Carter 1981).

It is appropriate to repeat field experiments over several growing seasons in different soil types at different locations due to environmental differences and stochastic changes between seasons. The field experiment in this study was over one growing season in only one location, which was inoculated with a laboratory reared population of *S. avenae*. In this study there may, therefore, have been seasonal factors, which were not accounted for, that influenced the population densities of *S. avenae* on SavRia genotypes with different nitrogen use efficiencies under the different nitrogen application rates. However, the phenotypic plant traits of SavRia genotypes did increase with nitrogen fertiliser application but the aphid densities on these same SavRia genotypes did not increase under higher nitrogen fertiliser application rates. In other field studies, when conditions were favourable for aphid populations, wheat plots receiving higher nitrogen fertiliser applications had higher population densities of *S. avenae* (Duffield *et al.* 1997). I conclude, therefore, that there is no clear evidence from this study to support the hypothesis that increasing nitrogen use efficiency and its component efficiencies increases the risk of increased aphid infestation rates.

7.1.3 Concluding remarks

The genetic variation in the SavRia population that was associated with a decrease in the performance measures of cereal aphids will add to the database of genetic markers that are associated with aphid resistance traits in wheat. Increasing aphid resistance in wheat can reduce inputs of chemical based insecticides, which have been shown to negatively impact the environment (Foster *et al.* 2014). Growing aphid resistant wheat varieties is a key component in integrated pest management programs (Nyaanga *et al.* 2014). Reducing chemical input to agricultural cropping systems can make agricultural production more sustainable (Fields 2004).

The performance measures of *Sitobion avenae* were not higher on SavRia genotypes with higher nitrogen uptake efficiencies in the laboratory experiments. Furthermore in a field trial, there was no correlation between *S.*

avenae population densities with nitrogen use efficiency or its component traits nitrogen uptake and nitrogen utilisation efficiency under different nitrogen application rates. The results of these studies suggest that the potential for infestations of wheat by *Sitobion avenae* does not increase on wheat genotypes with higher nitrogen use efficiencies.

Improving nitrogen use efficiency in wheat can contribute towards future food security (Barraclough *et al.* 2010, Karrou & Nachit 2015). The research presented in this thesis has increased our understanding of how breeding to improve nitrogen use efficiency as part of wheat improvement strategies is related to cereal aphid infestations. The results showed that under these experimental conditions using laboratory reared aphids, breeding wheat genotypes to increase the component traits of nitrogen use efficiency does not alter the potential for infestation by *Sitobion avenae* on wheat genotypes with higher nitrogen use efficiencies.

8 Glossary

8.1.1 Table of abbreviations used throughout the thesis, predominantly in chapter 3.

Abbreviations	
DH	Doubled Haploid
SNP	Single nucleotide polymorphism
SavRia	Savannah x Rialto DH population
QTL	Quantative trait loci
JIC	John Innes Centre
HGCA	Home grown cereals authority
JI.N.o 2	John Innes N.O2 compost
LabIND	Laboratory individual experiment
LabPOP	Laboratory population experiment
GH	Greenhouse experiment
LIDEvtime	Development time of aphids LabIND
LIN1Devtime	Nymphs produced in a time equal to development time LabIND
LIrM	Intrinsic rate of increase LabIND
startPOP	Starting population LabPOP
Devtime	Development time of aphids LabPOP
peakPOP	Peak population LabPOP
popGR	Population growth rate LabPOP
popGR/T	Population growth rate unit time^{-1} LabPOP
aphid/tiller	Aphids tiller^{-1} LabPOP
GHstartPOP	Starting population GH
GHpeakPOPMD	Peak population of *Metopolophium dirhodum* GH
GHpeakPOPSA	Peak population of *Sitobion avenae* GH
GHpeakPOPALL	Peak population of *M. dirhodum* & *S. avenae* GH
GHpopGR	Population growth rate GH
PM	Powdery mildew
P60	60 well tray
A1	An adult population of the same age class grown from nymphs
N1	The resulting progeny from a population of nymphs
GS	Plant growth stage on Zadoks plant growth scale
IPM	Integrated pest management
MAS	Marker assisted selection
FASTA	Fast All- sequence searching with any alphabet
BLAST	Basic local alignment search tool
LOD	Logarithm of odds
CIM	Composite interval mapping
cM	centi-Morgan theoretical unit of distance along chromosomes

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10 Appendices

10.1 Appendix I

The SavRia genetic map on supplementary CD.

The raw genetic map that was downloaded from CerealsDB (Wilkinson *et al.* 2012).

The formatted genetic map with the aphid performance traits for each genotype attached and ready for analysis with the Windows Cartographer QTL program.

A graphical representation of the SavRia genetic map built using SNP markers from the doubled haploid Savannah cross Rialto winter wheat population. There are 37 linkage groups displayed, 32 of which have been assigned to chromosomes and labelled with the correct chromosome identifier.

10.2 Appendix II

The frequency histograms for all plant traits that were analysed using ANOVA are displayed (Figure 4.10), and all aphid performance traits (Figure 4.11).

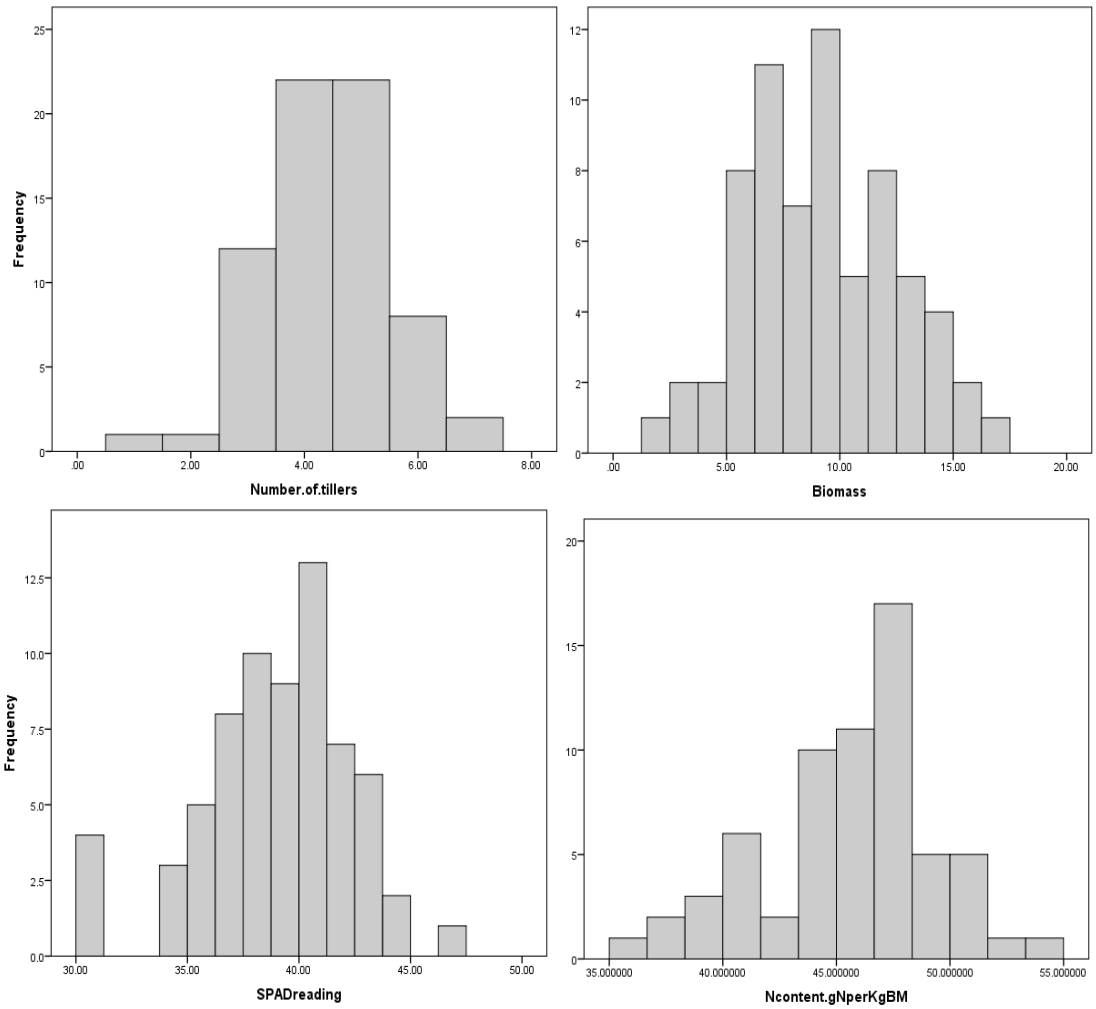

Figure 4.10 Frequency histograms for the plant traits that were analysed using one-way ANOVA. Frequency is the common Y-axis, the plant traits from left to right are the number of tillers, plant biomass (g), chlorophyll concentration (SPAD reading) and nitrogen content (gN kg BM^{-1}).

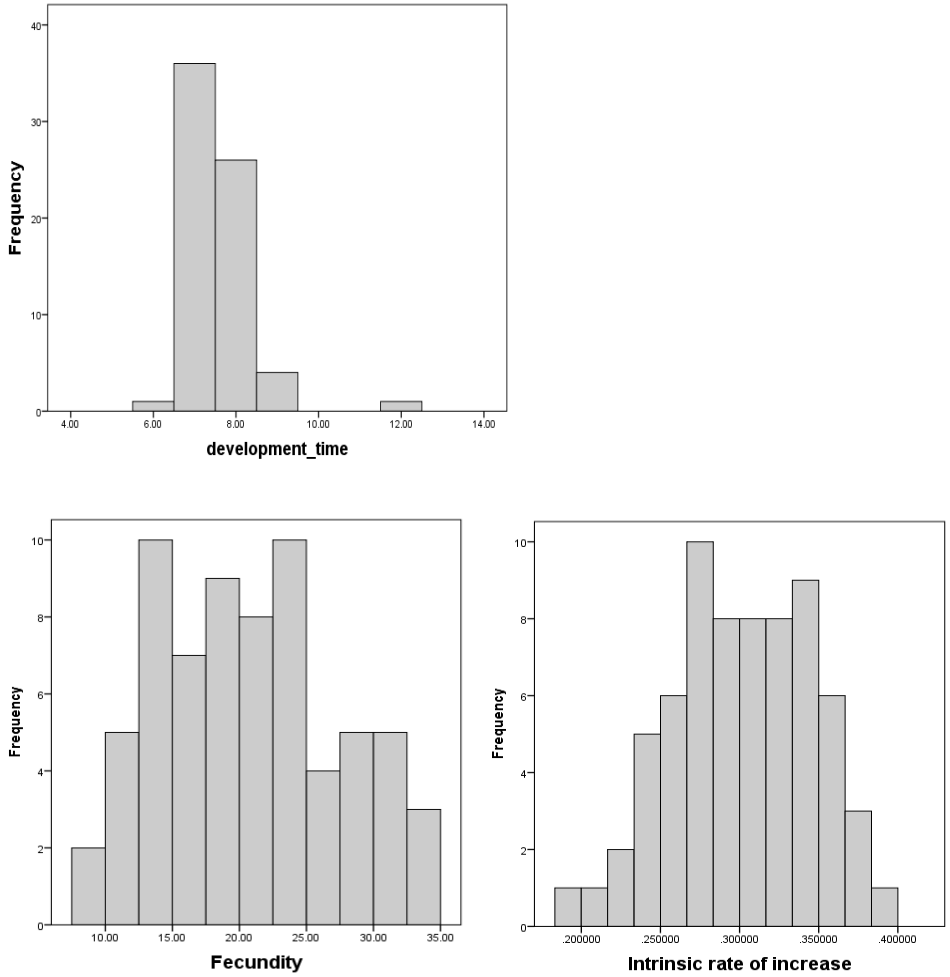

Figure 4.11. Frequency histograms for the aphid performance traits development time (X-axis), which was not normally distributed. fecundity $7d^{-1}$ and the intrinsic rate of increase (X-axes), which were both normally distributed. Frequency is the common Y-axis.