Unravelling cereal aphid interactions with ancestral wheat *Triticum monococcum*

by

Amma Larissa Simon B.Sc. (Hons), M.Sc.

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DECLARATION

I hereby declare that I have composed this thesis. The work of this thesis is a record of my own work; any collaborative work has been specifically acknowledged.

Amma Larissa Simon

This thesis is dedicated to my late Grand-father “Dada”

ABSTRACT

Aphids are sucking insects that feed on phloem sap, damaging the plant through nutrient removal. Aphids indirectly damage plants by transmitting viruses and secreting honeydew onto leaves, which attracts saprophytic fungi that reduces the plant's photosynthetic ability.

In Europe the most econcomically important aphid species are *Rhopalosiphum padi* (L.), *Sitobion avenae* (F.) and *Metopolophium dirhodum* (Wlk.), these are pest of many crops, including wheat. *Rhopalosiphum padi* causes up to 30% yield loss through feeding and transmission of viruses including the Barley Yellow Dwarf Virus (BYDV). Historically, aphid populations have been controlled using inseciticides; however, with aphids developing insecticide resistance and bans on certain insecticidies, there are fewer chemical option for aphid control. There has been increased interest in Integrated Pest Magaement strategies including the use of host-plant resistance to control the aphid populations.

Host plants can prevent aphid damage from occurring through the expression of biophysical resistance traits such preventing access to the host phloem and/or biochemical resistance traits such as the production of volatile organic compounds (VOC's) which affect aphid behaviour, preventing them from alighting onto the plant. These traits result in reduced aphid populations and population growth on resistant plants.

Some genotypes of the ancestral Einkorn wheat species *Triticum monococcum* (L.) confer some resistance to *R. padi* including seedling genotypes MDR045 and MDR049, resulting in reduced aphid development and fecundity. The presence of this resistance in MDR045 and MDR049 at later growth stages was investigated in this thesis. I confirmed that seedling (growth stage (GS) 11, 12-13) MDR045 and MDR049 conferred resistance to *R. padi*. In older plants, MDR045 did not confer resistance at GS 51-55; however, MDR049 had resistance at GS 37-41 and 51-55 and a dramatically reduced nymph production at GS 51-55.

To uncover candidate genes involved in *T. monococcum* seedling resistance, RNA sequencing was carried out on *R. padi* infested and un-infested susceptible *T. monococcum* MDR037 and resistant *T. monococcum* MDR049. The reads generated could not be aligned with the *T. monococcum* genome, but alignment a close relative, *Triticum urartu*, identified candidate genes involved in translation regulation, glutathione synthase and callose synthase.

It was unknown whether VOC's from MDR045 and MDR049 would affect winged aphid behaviour. Volatiles collected from *R. padi* infested and un-infested susceptible (*Triticum aestivum* (L.) variety Sosltice, *T. monococcum* MDR037) and resistant (*T. monococcum* MDR045 and MDR049) plants were exposed to winged *R. padi* along with a control in a 4-arm olfactometer to determine aphid behaviour. Winged aphids spent more time in the un-infested Sosltice, and MDR037 area than the control. Winged aphids spent less time in un-infested and infested MDR049 volatile area. The compounds that underpin these aphid behaviours in MDR037 and MDR049 were identified.

To determine whether resistance observed under controlled conditions was present in the field, three years of field trials were conducted to observe aphid (*R. padi*, *S. avenae* and *M. dirhodum*) and natural enemy (parasitised aphids, lacewing eggs and larvae and ladybird larvae and adults) populations on susceptible Solstice and MDR037, and the resistant MDR0657, MDR045 and MDR049. Across all field trials, MDR045 and MDR049 had lower aphid populations than Solstice. During the 2018 heatwave, dramatic reduced aphid populations on MDR037 were observed, indicating an interaction between genotype and drought. Natural enemy populations were highest on Solstice and there was a positive correlation between natural enemy and aphid populations.

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CHAPTER 1

Introduction

1.1 PLANT PESTS

Many species of insects and mites are highly successful plant pests, which can cause damage and dramatic yield reductions to crops. Damage may be caused directly by feeding or indirectly by the transmission of plant pathogens. Some of the most important pests of horticulture and agriculture are plant hoppers, whiteflies and aphids, which all have large populations, short life cycles and vector crop viruses (Alford, 2011).

1.1.1 Aphids

Aphids belong to the superfamily Aphidoidea which are believed to have evolved 280 million years ago. Most of the ~6,000 extant species are small soft-bodied insects that feed by piercing the phloem of plants and taking up the sap (Dinant et al., 2010).

Some aphids are holocyclic, alternating between sexual and asexual reproduction. For most of the year, holocyclic aphids reproduce asexually (parthenogenesis). In the autumn, sexupara aphids are produced, which are able to produce males and oviparorous females that can mate and produce frost-resistant eggs to over-winter. In the spring, a fundatrix hatches from the egg and produces offspring which go on to reproduce parthenogenetically (Fig 1.1). This allows the population to grow exponentially during the warmer months while the over-wintering sexual reproduction enables survival over the colder months and creates genetic diversity (Balloux et al., 2003; Bengtsson, 2003; Duan et al., 2017). Some aphid species are anholocyclic and reproduce asexually all year round. In both types of life-cycles, there is a high reproductive rate and generation overlap which causes aphid populations to increase rapidly (Van Emden and Harrington, 2007).

Some aphids are host alternating (heteroecious), during the autumn sexual morphs migrate to their primary host (tree species) where sexual reproduction occurs. In the spring heterocious aphids migrate to their secondary host (gramineceous plants) (Leather and Dixon, 1982).

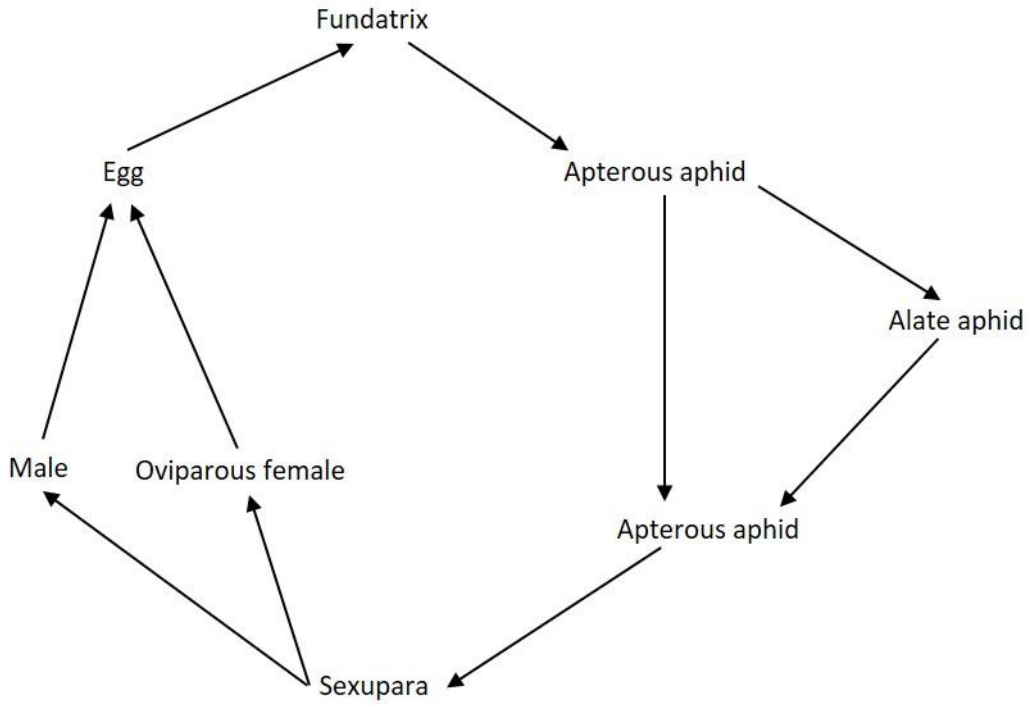


Figure 1.1. Schematic diagram of an holocyclic aphid life cycle

All aphids are dimorphic, with alate (winged) and apterous (wingless) offspring produced under different conditions (Duan et al., 2017). For example, when colonies get crowded on a host plant or the quality of the host decreases, nymphs which will develop into alate aphids are produced (Dixon and Glen, 1971; Wratten, 1977). These alate aphids can leave to find a new host, where they will produce apterous offspring and thus spread their populations. The lifecycles and dimorphism of aphids have made many species very effective pests of crops (Alford, 2011).

1.2 APHID FEEDING

Aphids have developed methods of bypassing plant defences so that they can remain feeding from the phloem for days (Tagu et al., 2008). To do this they must reach the phloem and xylem to drink, by navigating their stylets through the epidermis and parenchyma. The stylets are made up of two mandibular parts and two maxillary parts which are able to penetrate the leaf surface and epidermis to move intercellularly through the apoplast due to their flexibility (Will et al., 2013). As the stylets moves through the apoplast they may penetrate a mesophyll cell, this can alert the plant to the aphid, but it also gives the aphid the opportunity to acquire biochemical information, such as whether the plant is a suitable host (Jaouannet et al., 2014). Stylets secrete two different types of saliva; gelling and watery. Gelling saliva aids the movement of the stylets through the apoplast, and watery saliva is secreted into sieve elements to suppress sieve element occlusions and aid feeding (Fisher and Frame, 1984; Tjallingii and Hogen Esch, 1993). As the stylets moves through the apoplast, gelling saliva hardens and forms a sheath around the stylets so that it can move forward; this happens repeatedly until it reaches the vascular bundle forming a barrier between the stylets and parenchyma cells (Miles, 1965; Kimmins, 1986). To reach the phloem sap, the stylets must penetrate the sieve elements and then secrete watery saliva prior to feeding to aid sustained phloem sap ingestion (Tjallingii, 1985; Kimmins, 1986; Dinant et al., 2010). Drinking from the xylem requires active sucking, whereas, due to the turgour difference between the phloem sieve tube sources and sinks, feeding from the phloem is passive (Daudet et al., 2002; Gould et al., 2005).

The phloem transports photoassimilates from the source tissues (such as leaf mesophyll) to sink tissue (such as roots and shoots) through the sieve tube, made up of sieve elements and companion cells (Bel and Rijen, 1994; van Bel, 1996). The phloem is made up of three

distinct areas; collection phloem, transport phloem and release phloem (van Bel, 2003). Photoassimilates from the source tissues are loaded into the collection phloem, located in the minor veins of the leaves, and released into the transport phloem in veins, branches, stems, petioles and roots (Daudet et al., 2002; Gould et al., 2005). The transport phloem then passes the photoassimilates to the release phloem, where they are released from the sieve tube into sink tissue. (Fig 1.2.). The transport of photoassimilates and water from the source tissue to sink tissue via the collection, transport and release phloem is vital for new growth and plant development (Bel and Rijen, 1994; Daudet et al., 2002; van Bel, 2003).

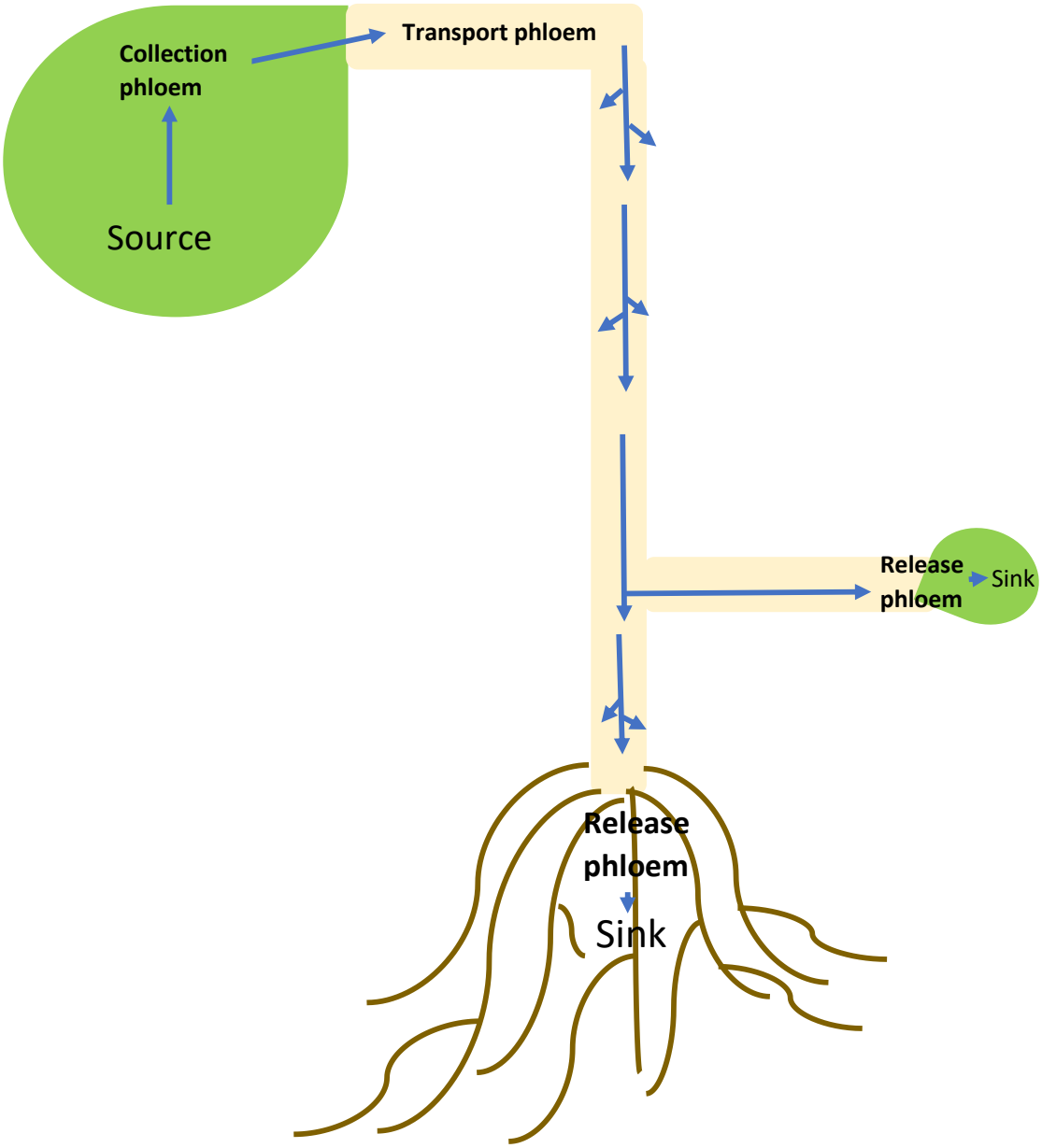


Figure 1.2. Depiction of photoassimilate (blue arrows) translocation via the phloem from source to sink. The collection phloem takes photoassimilates from the source tissue and releases them into the transport phloem which both holds and transports photoassimilates to the release phloem where photoassimilates are released into the sink tissue.

The presence of photoassimilates in the collection phloem creates high pressure, and conversely there is low pressure at the release phloem due to the lack of photoassimilates. This difference creates turgour pressure between the phloem sieve tube sources and sinks. It

is this pressure which means that when aphid stylets enter the phloem the aphid can feed passively (Patrick, 1997).

The xylem consists of vessel elements and tracheids, the latter holds water against gravity and vessel elements are open ended, dead, conducting cells that carry water and nutrients from the roots upwards to stems and leaves. This passive action occurs due to both root pressure and transpiration. Aphids drinking from the xylem must actively suck to acquire water and ions, this behaviour is rarer than phloem feeding and is often associated with dehydration (Spiller et al., 1990; Daniels et al., 2009). The importance of xylem feeding was highlighted by Daniels et al., (2000) who showed that sub-lethal doses of the neonicotinoid thiamethoxam can prevent xylem feeding and give a reduction in reproductive success and development of nymphs.

Phloem sap does not fulfil the dietary needs of aphids; mostly due to the lack of digestible nitrogen (Mittler, 1953). This deficit is overcome by the intracellular symbiotic bacteria *Buchnera* sp. which provides the missing nutrients (Mittler, 1953; Sandström and Moran, 1999). It has been shown that the nutritional profile of the sap differs between plant species, and aphid dependence on the symbiotic bacteria varies (Sandström and Moran, 1999).

1.3 CEREAL APHIDS AND THEIR HOSTS

Many aphid species infest cereal crops, and are therefore of high agricultural importance. Some cereal aphids are host alternating (heteroecious), overwintering on their primary host,

however, others only have Graminaceous plants as their host (autoecious) and overwinter on these.

In Europe the most prominent cereal aphids are *Rhopalosiphum padi* (L.), *Sitobion avenae* (F.) and *Metopolophium dirhodum* (Wlk.). All three species are virus vectors including acting as a vector for the Barley Yellow Dwarf Virus (BYDV) (Leather et al., 1989; Halbert et al., 1992; Chapin et al., 2001; Tanguy and Dedryver, 2009; Yu et al., 2013) which can cause large scale crop damage (Leather et al., 1989; Voss et al., 1997).

1.3.1 *Rhopalosiphum padi*

The bird cherry-oat aphid (*Rhopalosipuhm padi* (L.)) nymphs are light green in colour, whereas apterous adults are dark-green with red-brown towards the end of the abdomen near the cornicles, the legs are dark green with distinctive black joints and tips. Antennae and cornicles are black. Alate adults are slimmer and darker than apterous aphids (Fig 1.3). *Rhopalosiphum padi* are holocyclic; in the spring-summer months they are often found feeding on the stems and underside of cereal leaves and other graminaceous hosts (Leather and Dixon, 1982), they over-winter on the bird-cherry tree (*Prunus padus* (L.)) where sexual reproduction occurs (Leather and Bland, 1999). However, *R. padi* populations can also overwinter on graminaceous, and are found on crops particularly during warm autumns (Morgan, 2000). Despite not being the most abundant cereal aphid species in Europe, this aphid species causes extensive damage to cereals through the transmission of viruses including BYDV, causing up to 30% yield loss (Plumb, 1976; Leather et al., 1989). Autumn populations of *R. padi* on autumn sown crops can cause high levels of BYDV and other virus transmissions making this aphid species a major pest of cereal crops.

Figure 1.3. Image of alate, apterous and nymphal *Rhopalosiphum padi* (Image courtesy of the Rothamsted VCU database).

1.3.2 *Sitobion avenae*

The English grain aphid (*Sitobion avenae* (F.)) has colour polymorphisms, with nymphs being light green or red. Apterous aphids are mid-green, brown or shades in between these, alate aphids are slimmer and darker with dark marks on the upper side of the abdomen (Fig 1.4). Both forms have distinctive black leg joints and tips with black antennae and cornicles. They are holocyclic but they only have grass species (Poaceae) as their host, feeding on the ears or flowers of cereals in spring/summer and overwintering as eggs or as anholocyclic aphids (Hand and Wratten, 1985; Zhou et al., 1995).

Figure 1.4. Image of alate, apterous and nymphal *Sitobion avenae.* (Image courtesy of the Rothamsted VCU database).

1.3.3 *Metopolophium dirhodum*

The rose grain aphids (*Metopolophium dirhodum* (Wlk.)) are light yellow-green with dark brown-black leg joints and tips. Alate aphids have darker brown antenna than the apterous form (Fig 1.5). They are holocyclic with cereals as the spring-summer host and rose (*Rosa*) as their winter host where sexual reproduction occurs and eggs are laid.

Figure 1.5. Image of alate, apterous and nymphal *Metopolophium dirhodum.* (Image courtesy of the Rothamsted VCU database).

1.4 APHID DAMAGE

Direct damage to plants can be caused by aphids feeding on phloem sap, removing nutrients such as nitrogen, promoting negative nitrogen deposition (Dixon, 1998) and causing a reduction in assimilate allocation in sink tissue (Dixon, 1998).

Aphids also inflict damage indirectly by secreting honeydew and transmitting viruses. Honeydew excretion onto leaves attracts saprophytic fungi which covers the leaf surface, reducing the photosynthetic ability of the plant (Rabbinge et al., 1981).

Aphids transmit viruses through their saliva during feeding (Gildow and Rochow, 1983) and the viruses are classified persistent, non-persistent or semi-persistent depending on their route through the aphid. Persistent viruses go into the digestive system, and propagate within the host, viruses are transmitted vertically to the next generation. Semi-persistent viruses can survive for long periods within their host but are unable to propagate and be vertically transmitted. Non-persistent viruses are only able to survive for a limited amount of time within the host. Viruses are transmitted to and from aphids as they feed on the plant. One example is the Barley Yellow Dwarf Virus (BYDV) which is a persistent virus within the *Luteoviridae* family, which causes root and shoot tissues to die (Esau, 1957; Erion and Riedell, 2012). BYDV solely uses aphids as vectors (Miller et al., 2002) to aid transmission from one plant to another. It is known that BYDV must circulate within the aphid before being transmitted to a different plant, however, it is unable to replicate within the host (Miller et al., 2002). Once within the plant, BYDV is located in the phloem, making aphids an ideal vector. Aphids as a vector enable the virus to be transported long distances by alate aphids.

1.4.1 Aphid damage in an agricultural context

It is estimated that there are 2500 domesticated crops grown worldwide (Meyer et al., 2012) and that domestication of plants originated at several different times in different regions, probably linked to several factors including climate, human population increase and religious and cultural practices (Harlan, 1979). Crop domestication involved humans selecting desirable traits that increased the quality and quantity of the crop (Meyer et al., 2012) with less focus on traits that defend against biotic stressors. As a result many modern crops are less resistant to herbivory (Turcotte et al., 2014)

About 25% of plant species are hosts to aphids (Dixon et al., 1987) including many important crop species (Blackmann et al. 2007). Some aphid species can cause up to 30% yield

reduction (Leather et al., 1989; Voss et al., 1997) and this effect on the agricultural economy is predicted to increase due to climate change (Bezemer et al., 1998; Van Der Putten et al., 2010).

1.5 APHID-HOST INTERACTIONS

Aphids can extract nutrients from plants for days and cause extensive plant damage (Tagu et al., 2008), however, the plant can combat and/or prevent damage through various defence mechanisms. Aphids must first find their host, to do this they use olfactory and visual cues from the plant. Some plants are able to manipulate these cues to prevent aphids alighting onto the plant (Kogan and Ortman, 1978). Once on a host plant, aphids must extract nutrients from their host for development, reproductive success and survival. Plants can prevent feeding through biophysical or biochemical traits that either limit access to phloem sap or have a detrimental effect on the aphids (Painter, 1951).

1.5.1 Evolution of aphid-host interactions

Plant defence systems are a complex mix of different traits and mechanisms (Agrawal, 2007; Rasmann and Agrawal, 2009). Herbivores overcoming plant defences puts selective pressure on plants to develop different or more complex defence strategies and resistance mechanisms (Thaler, 1999; Agrawal, 2007; Rasmann et al., 2015; Turley and Johnson, 2015; Züst and Agrawal, 2016). Once these strategies and mechanisms have evolved, this again puts selective pressure onto herbivores to overcome or bypass the defence and/or resistance, creating an evolutionary arms race between the plant and herbivore (Czesak et al., 2008). For aphids and their hosts, this led to rapid diversification in both angiosperms and aphids during the early Cretaceous (Dixon, 1998). Studies have shown that resistance traits are under

selection from herbivores, and the plant defence theory and plant–herbivore coevolutionary theory both assume that resistance traits have evolved as adaptations to reduce herbivory (Thaler, 1999; Agrawal, 2007; Rasmann et al., 2015; Turley and Johnson, 2015; Züst and Agrawal, 2016).

1.5.2 Host Responses to Aphid feeding

Aphid feeding and damage can be detected by pattern recognition receptor (PRR) proteins that have been associated with detecting conserved Pathogen Associated Molecular Patterns (PAMPs) and Damage Associated Molecular Patterns (DAMPs) (Lam et al., 2001) in response to pathogens. It is hypothesised that insect-plant interactions may be similar and that plant PRRs use conserved herbivore associated molecular patterns (HAMPs) to initiate defence responses (Hogenhout and Bos, 2011). PRR recognition of aphid infestation causes a cascade of molecular changes that increases reactive oxygen species (ROS) in the plant cell (Ni et al., 2001). This induces the jasmonic acid (JA), salicylic acid (SA) and/or ethylene (ET) phytohormone signalling pathways in the host plant (Kessler and Baldwin, 2002; Walling, 2008).

One of the early stages of JA synthesis involves the lipoxygenase (LOX) enzyme. *Sitobion avenae* infestation has been shown to increase LOX enzyme activity (Zhao et al., 2009), leading to increased JA biosynthesis (He et al., 2002). Increased JA has been shown to increase proteinase inhibitor enzyme activity (Thaler et al., 2001). Proteinase inhibitors from potato (*Solanum tuberosum* (L.)) and lima bean (*Phaseolus lunatus* (L.)) have been shown to reduce the survival of the Russian wheat aphid (*Diuraphis noxia* (K.)), the greenbug (*Schizaphis graminum* (R.)) and *R. padi* (Tran et al., 1997).

Salicylic acid is antagonistic to JA, however, this pathway is also involved in aphid-plant interactions. Salicylic acid has been shown to induce ROS hydrogen peroxide (H_2O_2) in tobacco (*Nicotiana tabacum* (L.)) plants (Kawano and Muto, 2000). Hydrogen peroxide is a defence response in itself as it strengthens cell walls in plant interaction with powdery mildew (Hückelhoven et al., 1999), and is toxic to herbivores (Bi and Felton, 1995). The SA activity is dependent on peroxidases and catalases in plants (Kawano and Muto, 2000; Vasiukova and Ozeretskovskaia, 2007). *Diuraphis noxia* infestation has been shown to reduce catalase activity in *Triticum aestivum* (L.)) (Mohase and Van Der Westhuizen, 2002) which would suppress SA activity and the production of ROS. It has been shown that honeydew also causes accumulation of salicylic acid (SA) in leaf tissue (Schwartzberg and Tumlinson, 2014), however, it is unknown to what extent this affects plant defence.

Aphid infestation has also been linked with ET biosynthesis (Anstead et al., 2010). Aphid feeding induces the expression of ET-dependent *mir-1* defence in maize (*Zea mays* (L.)) - corn aphid (*Rhopalosiphum maidis* (F.)) interactions (Louis et al., 2015), and in cotton aphid (*Aphis gossypii* (G.)) interactions with resistant melon (Cucurbitaceae (J.)), a gene that helps integrate ET and JA pathways was upregulated (Anstead et al., 2010). This suggests that the ET pathway is important in aphid interactions with resistant plants.

The activation of these pathways can lead to defence mechanisms, such as the production of herbivore induced plant volatiles (HIPV) that prevent aphids from alighting onto the plant (Ozawa et al., 2000; Girling et al., 2008), the production of toxins (Goffreda et al., 1989; Kim et al., 2008) and/or structural defence mechanisms in the phloem which prevent aphid feeding (Will and Van Bel, 2006).

Phloem sieve element are very sensitive to damage. In response to damage to phloem cells, mechanosensitive calcium channels open, resulting in an influx of Ca^{2+} which relieves pressure and allows turgor-dependent Ca^{2+} influx (Will and Van Bel, 2006). This influx leads to occlusions such as protein plugging and callose sealing to prevent phloem sap loss (Will and Van Bel, 2006). Protein plugging has been shown to prevent aphid phloem feeding (Medina-Ortega and Walker, 2015) but it has been suggested that the when the stylets punctures the sieve tube, gel and watery saliva can aid feeding by quickly filling sieve elements with saliva. Thus the Ca^{2+} influx is prevented or limited, and the turgor pressure is elevated through the stylets allowing sustained aphid feeding (Will and Van Bel, 2006; Jaouannet et al., 2014).

1.5.3 Aphid-host interactions affecting allelochemical production

Semiochemicals are singular compounds or compound mixtures that allow communication between organisms of the same species (pheromones) or different species (allelochemicals). They are volatile organic compounds (VOCs) that are transported through the air (Pickett et al., 2013).

Semiochemicals are classified by: green leaf volatiles, terpenoids and aromatic volatiles. Green leaf volatiles (GLV) are C6 aldehydes, alcohols, and their esters (Matsui, 2006). Terpenoids include hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), and a few diterpenes (C20) (Chen et al., 2011). Aromatic volatiles have one or more rings with alternating covalent bonds as part of their chemical structure.

Allelochemicals can affect aphid behaviour towards a plant. Alate *R. padi* have been shown to be attracted to certain compounds such as (E)-2-hexenyl acetate, benzaldehyde, and linalool (Quiroz and Niemeyer, 1998; Schröder et al., 2015) and repelled by other compounds

such as α-farnesene, (E)-2-hexenal, indole, and (3E,7E)-4,8, 12-trimethyltrideca-1,3,7,11-tetraene (TMTT) (Schröder et al., 2015). Many of the same compounds are common between different plant species, however, aphids have different responses to the volatiles from host and non-host species (Bruce et al., 2005; Bruce and Pickett, 2011), for example, the black bean aphid (*Aphis fabae* (S.)) moved away from volatiles from non-host species Tansy (*Tanacetum vulgare* (L.)) but towards volatiles from host species broad bean (*Vicia. faba* (L.)) (Nottingham et al., 1991). It has been suggested that this is due to the composition and ratio of compounds within the volatile profile enabling the aphids to discriminate between host and non-host species (Bruce et al. 2005; Webster et al. 2010; Webster 2012).

Allelochemical production can change in response to herbivory. Initially there is a baseline level of VOC production including monoterpenes and sesquiterpenes, but herbivory induces changes in secondary metabolites leading to changes in VOCs emission (Mumm and Dicke, 2010; War et al., 2011). Compounds that are only produced in response to herbivory are called herbivore induced volatiles (HIPV) (Dicke and Baldwin, 2010). These VOCs are in some cases also emitted from neighbouring plants in lower amounts (Dicke and Van Loon, 2000; Dicke and Baldwin, 2010). Herbivore induced plant volatiles have been shown to affect aphid behaviour with many aphids moving away from plants producing HIPVs or preferring non-HIPV producing plants (Dicke, 1994; Pare and Tumlinson, 1999; Dicke and Baldwin, 2010; Catola et al., 2018).

1.6 WHEAT

Modern wheat (*Triticum aestivum* (L.)) is one of the major staple foods worldwide making it an economically important crop (Ortiz et al., 2008). It is mostly grown in monocultures, where the lack of genetic diversity makes crops more susceptible to biotic stressors as there is

less opportunity for plants to evolve resistance mechanisms. The main biotic stressors to crops, including wheat, are pathogens, pests and competitive plants (Pickett et al., 2014).

1.6.1 Evolution of wheat

Triticum aestivum is a hexaploid (A^uA^u BB DD genome) plant species that is part of the Poaceae family which evolved approximately 70 million years ago (Kellogg, 2001). Modern wheat evolved through various hybridisation events, wild diploid wheat *Triticum urartu* (T.) (A^uA^u genome) hybridised with the B genome ancestor to create the wild emmer wheat (*Triticum tugidum* (L.) subsp *dicoccoides*) (A^uA^u BB genome) (Huang et al., 2002; Peng et al., 2011; Dvorak et al., 2012). It is unknown which plant species the B genome was derived from, but its closest ancestor is the goat grass species (*Aegilops speltoides* (T.)) (SS genome) (Huang et al., 2002; Petersen et al., 2006). Wild *T. tugidum* was cultivated in the fertile crescent and the areas south of the Casbian Sea, and evolved into cultivated *T. tugidum* (Salamini et al., 2002). Cultivated *T. tugidum* hybridised with another goat grass species (*Aegilops tauschii* (C.)) to create spelt wheat (*Triticum spelta* (L.)) (A^uA^u BB DD) genome) (McFadden and Sears, 1946). *Triticum spelta* was cultivated and through breeding, evolved into the easy threshing *T. aestivum* (McFadden and Sears, 1946; Peng et al., 2011; Dvorak et al., 2012).

1.6.2 Aphid-wheat interactions in an agricultural context

Cereal aphids are adapted to feeding on graminaceous plants (Leather and Dixon, 1982) and can do so for days, quickly causing widespread infestations. Cereal aphid populations are currently controlled predominantly by insecticides (Tanguy and Dedryver, 2009), however,

there are increased reports of resistance towards insecticide classes in cereal aphids (Foster et al., 2014). These, coupled with restrictions on insecticidal classes that farmers can use, such as the recent neonicotinoid ban in the EU, leave fewer options to curb aphid damage to the wheat crop, ultimately affecting food security of wheat (Carvalho, 2006; Pickett, 2013).

1.7 INTEGRATED PEST MANAGEMENT

The 1962 publication of "Silent Spring" by Rachel Carson raised the issue of pesticide toxicity to non-targets and catalysed the development of alternatives in the modern era. Integrated Pest Management (IPM) as a concept was first mentioned by Benjamin Dann Walsh in 1866 (Sheppard, 2004) and continued to be developed in the 1950's (Stern et al., 1959). Integrated Pest Management is defined as "an ecosystem approach to crop production and protection that combines different management strategies and practices to grow healthy crops and minimise the use of pesticides" (Dedryver et al., 2010). IPM should result in sustainable food production, pest damage below that which is economically detrimental and insecticide use having little effect on the environment (Thacker, 2002). For example, the economic injury threshold for *S. avenae* is 7 aphids per wheat tiller (Larsson, 2005). To assist IPM implementation, accurate monitoring practices are needed to establish the economic injury level (population density that will cause economic damage to the plant) and the economic threshold (population density where control methods need to be implemented to prevent the density reaching the economic injury level) of pest species (Stern et al., 1959). IPM has many facets including the use of resistant plants, trap crops, trapping apparatus, insect quarantine, genetically modified organisms, biological control, beneficial insects, conservation, pheromones and pesticides (Thacker, 2002).

1.7.1 Natural enemies of aphids

Natural enemies of aphids include predators, parasitoids and entomopathogenic fungi which are all able to reduce aphid populations (Van Emden and Harrington, 2007). Natural enemies can be used for biocontrol as part of IPM strategies. In glasshouses, use of natural enemies as biocontrol (Zehnder et al., 2006; Boivin et al., 2012), resistant varieties and physical barriers (Zehnder et al., 2006) have been successfully used to reduce reliance on insecticides. Within a field setting natural enemies can help reduce aphid populations and are an important part of integrated pest management strategies. However, the presence of natural enemies is harder to control in the field than in glasshouses or polytunnels (Cook et al., 2007).

1.7.1.1 Parasitoid wasps

All parasitoid wasps are in the order Hymenoptera and can be ectoparasitoids, which lay their eggs on their host or endoparasitoids which lay their eggs within their host (Harvey et al., 2012). Endoparasitoids such as *Aphidius ervi* (H.) are commonly used in the biocontrol of aphid populations in glasshouses (Boivin et al., 2012) due to a high host specificity (Snyder and Ives, 2003). The parasitoids use plant odours to locate aphid populations, then at a closer range visual cues and cornicle secretions for host recognition (Powell et al., 1998). The females use an ovipositor to lay an egg within an aphid and when the egg hatches, the larva feeds on the aphid from the inside, during pupation, the aphid becomes mummified and sticks to the plant (Boivin et al., 2012). It has been shown, however, that aphids can overcome parasitism through symbiosis with endo-bacteria, for example the black bean aphid (*Aphis fabae* (S.)) with the endosymbiont *Hamiltonella defensa* (M.) resulted in reduced parasitism and therefore higher reproductive success (Vorburger et al., 2013). Aphid-parasitoid interactions display strong selection pressures in both directions leading to co-evolution of aphids and parasitoids (Henter, 1995).

1.7.1.2 Ladybirds

Ladybirds (*Coccinellidae* (L.)) are beetles that lay fertilised and unfertilised eggs. The ladybird larvae initially feed on the unfertilised eggs before switching to an aphid diet. The larvae then go through four instars, pupate and emerge as an adult, which also feeds on aphids. Thus ladybirds can be important for control of aphid populations (Triltsch, 1999). In glasshouses, the harlequin ladybird (*Harmonia axyridis* (P.)) predation complemented parasitic wasp *Aphelinus asychis* (Wlk.) parasitism of the poptato aphid (*Macrosiphum euphorbiae* (T.)) resulting in reduced peak aphid populations on *Rosa* plants (Snyder et al., 2004). Ladybird development is approximately parallel to aphid development, and their reproduction is influenced by the density of aphid populations, with lower populations reducing ladybird reproduction rates (Obrycki and Tauber, 1981). Ladybirds must also avoid self-competition between adults and larvae for food resources (Agarwala and Bhattacharya, 1995).

1.7.1.3 Lacewings

Lacewing (*Crysopidae*) are voracious predators, characterised by their green appearance and their stalked and oval shaped eggs. The larvae feed on aphids by dissolving and sucking their organs. Some *Crysopidae* are used as biological control agents of aphids in horticultural systems (Zehnder et al., 2006; Boivin et al., 2012). These include *Chrysoperla carnea* (Stelzl and Devetak, 1999) and *Chrysoperla externa* (Albuquerque et al., 1994) which have a fast reproduction rate with up to 12 generations per year in lab conditions (Stelzl and Devetak, 1999).

1.7.2 Population dynamics between aphids and predators or parasitoids

Population dynamics in herbivore-predator or parasitoid interactions are dependent on intrinsic and extrinsic factors. Intrinsic factors are genetic and influence survival, including rate of population increase, the ability to overcome host/prey defences and ability to evade predation. Extrinsic factors that affect the population size include size of predator populations, and host and habitat availability. Extrinsic factors can affect intrinsic factors and vice versa, for example, extrinsic predator population size can put a selective pressure on the intrinsic factor to evade predation which in turn can affect the predator populations. Over time this creates an evolutionary arms race in herbivore-predator/parasitoid interactions and this can lead to coevolution. An example of this is the secretions of alarm pheromones emitted from the cornicles of aphids which alert other aphids of a threat. (Bowers et al., 1972; Kunert et al., 2005). Other aphids on a plant respond to the alarm pheromone by walking away or falling to the ground (Montgomery and Nault, 1977).

Predators and parasitoids use olfactory and visual cues to find prey. It has been shown that plants change their volatile profiles in response to aphid infestation and that predators and parasitic wasps can use these to locate aphid populations (Pare & Tumlinson 1999; Dicke 1994). Some VOCs, such as α-farnesene and methyl salicylate, although repellent to herbivores, act as attractants to predators (Pare and Tumlinson, 1999; Mallinger et al., 2011). Olfactory cues can also come from the prey itself, for example aphid honeydew excretion has been shown to be attractive to *Chrysoperla carnea* (S.) (Dean and Satasook, 1983) and the alarm pheromone E-β-Farnesene has been shown to attract parasitoid *Aphidius ervi* (H.) (Du et al., 1998) and adult seven-spot ladybirds (*Coccinella septempunctata* (L.)) (Abassi et al., 2000).

1.7.3 Host plant resistance to insects

There are three different defence mechanisms plants use against insect herbivory; direct, indirect and tolerance (Strauss and Agrawal, 1999; Stamp, 2003; Boege and Marquis, 2005), all leading to the expression of resistance and tolerance traits (Agrawal, 2007; Czesak et al., 2008; Rasmann and Agrawal, 2009) which evolved as adaptations to reduce herbivory (Czesak et al., 2008).

Direct defence mechanisms are used by plants after the insect has alighted (antibiosis) and involves reducing the ability of the insect to access the food source, reducing the quality of the food, or making the food source toxic by the production of phytochemicals. This leads to a decrease in insect development and/or reproduction(Painter, 1951).

Indirect defence mechanisms are used by plants to prevent insects alighting (antixenosis), using visual warning cues and/or the production of VOCs that are emitted from the plant and affect insect behaviour, for example by causing the herbivores to express non-host behavioural responses and/or attracting predators and parasites of the herbivore (Kogan and Ortman, 1978; Mumm and Dicke, 2010).

Tolerance reduces the adverse effects of herbivory, so despite infestation, the growth, reproduction and yield of the plant are not affected (Strauss and Agrawal, 1999), for example by compensatory growth, rapid healing and/or changes in photosynthesis (Thacker, 2002).

1.7.3.1 Plant antixenosis to aphids

Antixenosis results from olfactory and visual cues from the plants that elicit a non-host behavioural response in the herbivore (Kogan and Ortman, 1978). Visual cues, including colour, can affect aphid behaviour towards plants, it has been shown that alate *R. padi* are

more likely to alight onto lime (wavelength 600-610 nm) and yellow (wavelength 525-531 nm) (Schröder et al., 2014).

It has been shown that aphids are able to distinguish between volatiles from resistant and susceptible genotypes of maize, a study showed that the corn leaf aphid (*Rhopalosiphum maidis* (F.)) were less attracted to a resistant genotype (Mp708) than a susceptible genotype (Louis et al., 2015). The aphid behaviour towards a single volatile compound can be different from the behaviour elicited by the same compound in a blend (Webster et al., 2010). It has been suggested that the composition of multiple compounds, and the ratios between these compounds are important to host- a non-host responses in aphids (Bruce et al. 2005; Webster et al. 2010; Webster 2012).

1.7.3.2 Plant antibiosis to aphids

Antibiosis occurs after aphids have alighted onto the plant and comes from a multitude of biophysical and/or biochemical traits (Painter, 1951). The biophysical obstacles include increased trichome size and density (Bahlmann et al., 2003) and the presence of waxy surfaces (Wojcicka, 2013), although for some plant species there is no link between waxy surfaces and reduced aphid development and reproductive success (Bahlmann et al., 2003; Costa et al., 2014). Biophysical antibiosis towards aphids can also occur at the feeding site in the phloem. Electrical Penetration Graph (EPG) studies have shown that aphids on resistant genotypes have fewer feeding events and increased salivation (Tjallingii and Hogen Esch, 1993; Prado and Tjallingii, 1994; Caillaud et al., 1995; Greenslade et al., 2016; Simon et al., 2017). Prior to feeding, watery saliva is secreted into sieve elements to prevent occlusions within sieve elements; hence increased salivation indicates that there could be occlusions in resistant genotypes (Medina-Ortega and Walker, 2015).

Biochemical traits include the production of toxins that reduce aphid development, reproduction and survival. An example of such toxins are indole-3-carbinol which reduced reproduction in the peach-potato aphid (*Myzus persicae* (S.)) (Kim et al., 2008) and 2-tridecanone in wild tomato (*Lycopersicon hirsutum* f. *glabratum*) which increased mortality of potato aphid (*Macrosiphum euphorbiae* (T.)) (Musetti and Neal, 1997).

Biophysical and biochemical traits can be combined, as the toxin compounds 2,3,4-tri-O-acylglucoses were produced from trichomes on tomato (*Lycopersicon pennellii* (C.)). These toxins reduced settlement and increased the mortality of the potato aphid (*Macrosiphum euphorbiae* (T.)) (Goffreda et al., 1989).

1.8 *TRITICUM MONOCOCCUM*

Einkorn wheat (*Triticum monococcum* (L.)) is a diploid wheat (A^m A^m genome) species and was one of the earliest wheat species used in agriculture. It was domesticated from the wild einkorn wheat (*Triticum boeoticum* (B.)) and grown throughout the Neolithic age and up to the Bronze age (Salamini et al., 2002). *Triticum monococcum* shares a common ancestor with *Triticum uratu* (A^u A^u genome), a progenitor of the modern *T. aestivum* (Dizkirici et al., 2016)..

1.8.1 *Triticum monococcum* natural resistance to aphids

No resistance has been identified towards cereal aphids in commercial modern wheat *T. aestivum* varieties in the UK, however, different levels of susceptibility have been shown in *T. monococcum* (Sotherton and Lee, 1988; Hu et al., 2016), although resistance varies between genotypes. Some genotypes have been shown to have partial antibiosis to aphids,

including *S. avenae* (Sotherton and Lee, 1988; di Pietro et al., 1998; Simon et al., 2017), the wheat aphid (S*chizaphis graminum* (R.)) (Migui and Lamb, 2003), *M. dirhodum* (Sotherton and Lee, 1988) and *R. padi* (Migui and Lamb, 2003; Greenslade et al., 2016). Recent work has shown that *T. monococcum* lines MDR045, MDR657 and MDR049 confer partial antibiosis resistance towards *R. padi* (Greenslade et al., 2016; Aradottir pers comms.) and MDR045 and MDR049 towards *S. avenae* (Simon et al., 2017). Aphid feeding behaviour studies have linked antibiosis in *T. monococcum* to aphids inability to find the phloem and/or carry out feeding and sustained feeding events characterised by increased time within the mesophyll, increased salivation and reduced number of feeding and sustained feeding events (Tjallingii and Hogen Esch, 1993; Prado and Tjallingii, 1994; Caillaud et al., 1995; Greenslade et al., 2016; Simon et al., 2017).

1.9 CONCLUSION

Rhopalosiphum padi can cause up to 30% yield loss in wheat, through both direct feeding damage and the transmission of BYDV (Leather et al., 1989; Voss et al., 1997). Restrictions on pesticide usage and increased insecticide resistance, results is an increased need to find alternative methods for control, such as IPM and the use of resistant genotypes to control cereal aphid populations including *R. padi* (Smith, 2005).

Triticum monococcum lines MDR657, MDR045 and MDR049, have been shown to confer some antibiosis to *R. padi* at the seedling stage (Greenslade et al., 2016; Aradottir pers comms.). It is now important to develop a deeper understanding of the cereal aphid – wheat interactions, so that any resistance traits may be available for incorporating into other wheat varieties.

1.9.1 Aims of the thesis

The overall aim of the work presented in this thesis was to determine whether ancestral wheat *Tritium monococcum* (L.) lines MDR045 and MDR049 confer resistance to the bird cherry-oat aphid (*Rhopalosiphum padi* (F.)) and to then unravel the determinants of this resistance.

The objectives were:

1) To establish if *Triticum monococcum* genotypes that confer antibiosis to *Rhopalosiphum padi*, emit volatile organic compounds that prevent aphid alighting (pre-alighting resistance) and if so, to identify which compounds are involved (Chapter 2).

2) To study whether antibiosis shown by *T. monococcum* genotypes at the seedling stage is also present at later plant growth stages (Chapter 3).

3) To identify differences in gene expression in resistant and susceptible *T. monococcum* that occurs in response to *R. padi* infestation and identify candidate genes that underpin resistance traits (Chapter 4).

4) To determine whether *T. monococcum* genotypes that confer resistance under controlled conditions are resistant in field conditions and whether these genotypes attract more aphid natural enemies than susceptible genotypes. (Chapter 5).

CHAPTER 2

Unravelling pre-alighting resistance to the bird cherry-oat aphid (*Rhopalosiphum padi*) in ancestral wheat (*Triticum monococcum*).

2.1 INTRODUCTION

Plants produce secondary metabolites called semiochemicals, some of these signal and facilitate interactions with other organisms (allelochemicals) such as insects. Semiochemicals, and therefore allelochemicals are small (low molecular weight), volatile organic compounds (VOCs) which are transported through the air (Pickett et al., 2013).

Plant semiochemicals are usually emitted from cells in the epidermis in vegetative and root tissue (Knudsen et al., 1993; Dudareva et al., 2004; Chen et al., 2011) and are classified as: terpenoids, green leaf volatiles, and aromatic volatiles. Terpenoids include hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), and a diterpenes (C20) and are synthesised by terpene synthases (Chen et al., 2011). Green leaf volatiles (GLV) are C6 aldehydes, alcohols, and their esters, which are synthesised through the hydroperoxide lyase pathway within oxylipin metabolism (Matsui, 2006). Aromatic volatiles have one or more rings with alternating covalent bonds as part of their chemical structure

Allelochemical production differs between plant species (Schröder et al., 2015) and even on a genotype or cultivar level (Dong et al., 2015; Schröder et al., 2015). These differences can be qualitative, with different species or genotypes producing different compounds, an example being GLV (Z)-3-Hexen-1-ol which is emitted by faba bean (*Vicia faba* (L.)), maize (*Zea mays* (L.)) and wheat (*Triticum aestivum* (L.)) but not potato (*Solanum tuberosum* (L.)) (Du et al., 1998; Webster, Bruce, Dufour, et al., 2008; Schröder et al., 2015). In other cases different plant species show quantitative differences in the production of the same

compound(s), as shown by *T. aestivum* and oat (*Avena sativa* (L.)) producing different concentrations of the terpenoid compound linalool (Quiroz and Niemeyer, 1998).

Insects can use the qualitative and quantitative differences in allelochemical production to discriminate between plants and show either host plant attraction or non-host avoidance (Bruce and Pickett, 2011). For example, the black bean aphid (*Aphis fabae* (Scop.)) moved away from volatiles from non-host species Tansy (*Tanacetum vulgare* (L.)) and summer savory (*Satureja hortensis* (L.)) but moved towards volatiles from host species *V. faba* and Brussels sprouts (*Brassica oleracea* (L.)) (Nottingham et al., 1991).

Olfactory receptors on insect antennae contain sensilla that detect the allelochemicals (Bromley et al., 1979, 1980; Fan et al., 2011) and this can lead to different behaviours including aggregation, dispersion, orientation towards or away from the VOCs, or elicit no behaviour (J. A. Miller et al., 2009). Insect movement in response to a stimulus from a certain direction is called taxis (J. A. Miller et al., 2009).

For aphids, it has been shown that both qualitative differences and quantitative differences in compound concentrations, as well as the ratios between compounds within a mixture, can affect behaviour and discrimination between plant species (Bruce et al., 2005; Webster, Bruce, Pickett, et al., 2008; Webster et al., 2010). Qualitatively different compounds elicit different behavioural responses in aphids, for example, the bird cherry-oat aphid (*Rhopalosiphum padi* (L.)) moves towards (*E*)-2-hexenyl acetate but has a neutral response to (*E*)-3-hexen-1-ol (Quiroz and Niemeyer, 1998). The amount of a certain compound can also affect aphid behaviour, *A. fabae* moved away from octanal at 0.01ng/µl but had a neutral response to octanal at higher concentrations (Webster et al., 2010), and *R. padi* has been shown to only move away from α-farnesene at 1000ng/µl (Schröder et al., 2015) and not at higher concentrations. Mixtures of compound and ratios between compounds within a

mixture can affect aphid behaviour as whilst *A. fabae* had moved away from individual compounds identified from broad bean (*Vica faba* (L.)) plants, an artificial blend of these compounds caused *A. fabae* to move towards these volatiles (Webster, Bruce, Pickett, et al., 2008).

Generally, the antennae of cereal aphids *Metopolophium dirhodum* (W.), *Sitobion avenae* (F.), and *R. padi* detect green leaf volatiles such as (E)-2-hexenal and (Z)-3-hexenyl (Visser and Yan, 1995; Visser et al., 1996; Quiroz and Niemeyer, 1998; Schröder et al., 2015) and the aromatic compound benzaldehyde (Visser and Yan, 1995; Visser et al., 1996; Quiroz and Niemeyer, 1998). Detection can however, vary between aphid species, with *M. dirhodum* antennae responding to decanol, but *S. avenae* not responding (Visser and Yan, 1995). Behavioural responses can also vary between morphs, apterous *R. padi* moved towards to 11 individual allelochemicals from *T. aestivum* and *A. sativa* (hexanal, (*Z*)-3-hexenyl acetate, (*E*)-2-hexenyl acetate, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, heptanal, octanal, nonanal, decanal, benzaldehyde and linalool), but alate *R. padi* only moved towards to three of these compounds ((*E*)-2-hexenyl acetate, (*E*)-2-hexen-1-ol and benzaldehyde) and a compound apterous *R. padi* did not respond to ((*Z*)-3-hexen-1-ol) (Quiroz and Niemeyer, 1998). This does not necessarily mean that the antennae of the alates did not detect the compounds, but that the behaviours exhibited differed. Alate and apterous *R. padi* behaviour towards blends of these 11 and four compounds were not tested but despite the behavioural differences to individual compounds, both alate and apterous *R. padi* had positive taxis to entrained *T. aestivum* and *A. sativa* volatiles (Quiroz and Niemeyer, 1998).

Volatile organic compound production can also vary within plant genotypes/cultivars, for example within cereals, where different barley (*Hordeum vulgare* (L.)) cultivars produced

different semiochemicals (Dong et al., 2015). Quantitative differences can also be present at a cultivar level, such as in *T. aestivum* cultivars Conan and Reeder, which produced varying amounts of (Z)-3-hexenyl acetate (Weaver et al., 2009) and durum wheat (*Triticum durum* (D.)) cultivars that produced varying amounts of ketones and aldehydes (Mattiolo et al., 2017). Plants use these semiochemicals to interact with other organisms. For example, (Z)-3-hexenyl acetate has been shown to mediate plant-plant interactions to warn of a herbivore attack (Arimura et al., 2001). As different aphid species detect different volatile compounds, and aphid behaviour can be dependent on the amounts of volatile compounds. Qualitative and quantitative differences between cultivars semiochemistry can determine what behaviour is elicited by an insect. For example, different semiochemistry between maize (*Zea mays* (L.)) cultivars meant that *R. padi* movement towards *Z. mays* volatiles was cultivar dependant (Schröder et al., 2015).

Some plants emit allelochemicals that elicit negative behaviour in insects, these plants have pre-alighting resistance (antixenosis) (Kogan and Ortman, 1978). The allelochemicals cause aphids to move away form or avoid the source of these odours. This response is similar to behaviour exhibited towards non-host species (Kogan and Ortman, 1978; Nottingham et al., 1991). A study has shown that ancestral wheat *Triticum monococcum* (L.) plants confer antixenosis to *S. avenae* under both controlled environment and field conditions however, the genotype of *T. monococcum* used in this study was not provided (Hu et al., 2016). It has been shown that semiochemical production can vary between genotypes level and that aphids are able to discriminate between genotypes. Other resistance traits vary between *Triticum monococcum* genotypes, for example, genotype MDR049 has post-alighting resistance traits that reduces aphid development and reproduction, but genotype MDR037 is susceptible to aphid infestation (Greenslade et al., 2016; Simon et al., 2017)

Semiochemical production is not static and can change when a plant experiences abiotic stress including flooding, (Velikova et al., 2012) and drought (Catola et al., 2016; Campbell et al., 2019) and/or biotic stress including herbivory (Hegde et al., 2011; Piesik et al., 2011; Mccormick et al., 2014; Catola et al., 2018) and pathogen infection (Piesik et al., 2007, 2011). Volatiles that are emitted in response to herbivory are called herbivore-induced plant volatiles (HIPV), these are specific compounds that are only synthesised in response to herbivory (Dicke and Baldwin, 2010). Herbivory can also cause quantitative changes in the amounts of compounds produced (Dicke and Baldwin, 2010). The onset of insect feeding or detection of salivary proteins instigates an antagonistic relationship between salicylic acid (SA) and jasmonic acid (JA), which regulates HIPV synthesis (Ozawa et al., 2000; Girling et al., 2008). HIPVs can be locally synthesised at the site of damage or systemically produced (Dicke, 1994) and can affect the responses of other insects to the infested plant.

Increased GLV production has been associated with drought stress (Catola et al., 2016), herbivory (Piesik et al., 2011; Mccormick et al., 2014) and pathogen infection (Piesik et al., 2011). Green leaf volatiles also are involved in plant-plant interactions, and can initiate plant defence responses in healthy plants, for example healthy lima bean (*Phaseolus lunatus* (L.)) plants exposed to GLVs (*E*)-2- hexenal, and (*Z*)-3-hexenyl acetate for 3hours expressed lipoxygenase (LOX) gene which is associated with JA mediated defence responses (Arimura et al., 2001). *Z. mays* plants exposed to GLV (*Z*)-3-hexen-1-ol for 14hours produced HIPVs (Ruther and Kleier, 2005). The production of HPIVs can affect insect aphid behaviour (Dicke, 1994; Pare and Tumlinson, 1999; Dicke and Baldwin, 2010; Catola et al., 2018). Cereal aphids, such as *R. padi*, the peach-potato aphid (*Myzus persicae* (S.)) and the corn aphid (*Rhopalosiphum maidis* (F.)), move towards odours of healthy host crops when tested against HIPV odours, or move away from HIPV odours when tested against a control (Quiroz

et al., 1997; Bernasconi et al., 1998; Verheggen et al., 2013). *Rhopalosiphum padi* are also affected by HIPVs from their winter host species the bird cherry tree (*Prunus padus* (L.)), as spring migrants had moved away from *R. padi* infested *P. padus* volatiles but had a neutral response to un-infested *P. padus* volatiles (Glinwood and Pettersson, 2000).

In the same way that different crop species and cultivars produce qualitatively and quantitatively different allelochemicals, there are a number of examples which show how plant volatile changes in response to herbivory can also vary between cultivars. These variations in response to herbivory can affect aphid behaviour. In the case of the cotton aphid (*Aphis gossypii* (G.)), aphids moved towards volatiles from un-infested pepper (*Caspium* (L.) spp) but had no behavioural response to infested *Caspium.* This change in behaviour was cultivar dependant (da Costa et al., 2011)

It has previously been shown that *R. padi* developmental and reproductive success as well as its feeding behaviour, varies on different *T. monococcum* genotypes. *Triticum monococcum* genotype MDR037 has been shown to be susceptible to aphid feeding and infestation, but *R. padi* on MDR045 and MDR049 had reduced developmental and reproductive success (Greenslade et al., 2016; Chapter 3). As discussed, aphid responses to allelochemicals from plants can vary between species. Therefore the work presented in this Chapter explores whether *T. monococcum* has antixenosis towards *R. padi* and if this depends on the *T. monococcum* genotype. Specifically, experiments were done to establish whether *T. aestivum* Solstice and *T. monococcum* genotypes MDR037, MDR045 and/or MDR049 confer pre-alighting resistance to *R. padi,* as well as the effects of *R. padi* infestation on semiochemical production and aphid responses.

2.1.1 Chapter hypotheses

The chapter tests the following hypotheses:

1) *Rhopalosiphum padi* behavioural responses to semiochemicals from *Triticum aestivum* Solstice, *Triticum monococcum* MDR037, MDR045 and MDR049 will vary between genotypes
2) *Rhopalosiphum padi* infestation will change *R. padi* behavioural responses to semiochemicals from *T. aestivum* Solstice, *T. monococcum* MDR037, MDR045 and MDR049.
3) Any behavioural change detected due to genotype or *R. padi* infestation will result from qualitative and quantitative differences in plant semiochemistry

2.2 METHODS

2.2.1 *Rhopalosiphum padi* (L.) cultures

Cultures of non-viruliferous bird cherry-oat aphid (*Rhopalosiphum padi* (L.)) were reared in ventilated Perspex cages on barley (*Hordeum vulgare* (L.)) variety Saffron with a light source coming from the rear of the cage. Cultures were maintained by transferring approximately 30 aphids from the culture to new plants in a new cage. Alate aphids were produced by allowing the populations to become over-crowded.

2.2.2 Plant material

Triticum monococcum (L.) genotypes MDR037, MDR657, MDR045 and MDR049 seeds were provided by the Wheat Genetic Improvement Network and *Triticum aestivum* var. Solstice seeds were provided by Rothamsted Research. All seeds were stored at 4^{o}C.

2.2.3 Soil material

In all laboratory experiments, plants were sown in standard Rothamsted soil (from Petersfield Products, Cosby, Leicester). The soil was made up of 75% medium grade peat, 12% screened sterilised loam, 3% medium grade vermiculite and 10% grit with an N content of 14% and a P_2O_5 content of 16%.

2.2.4 Plant growth

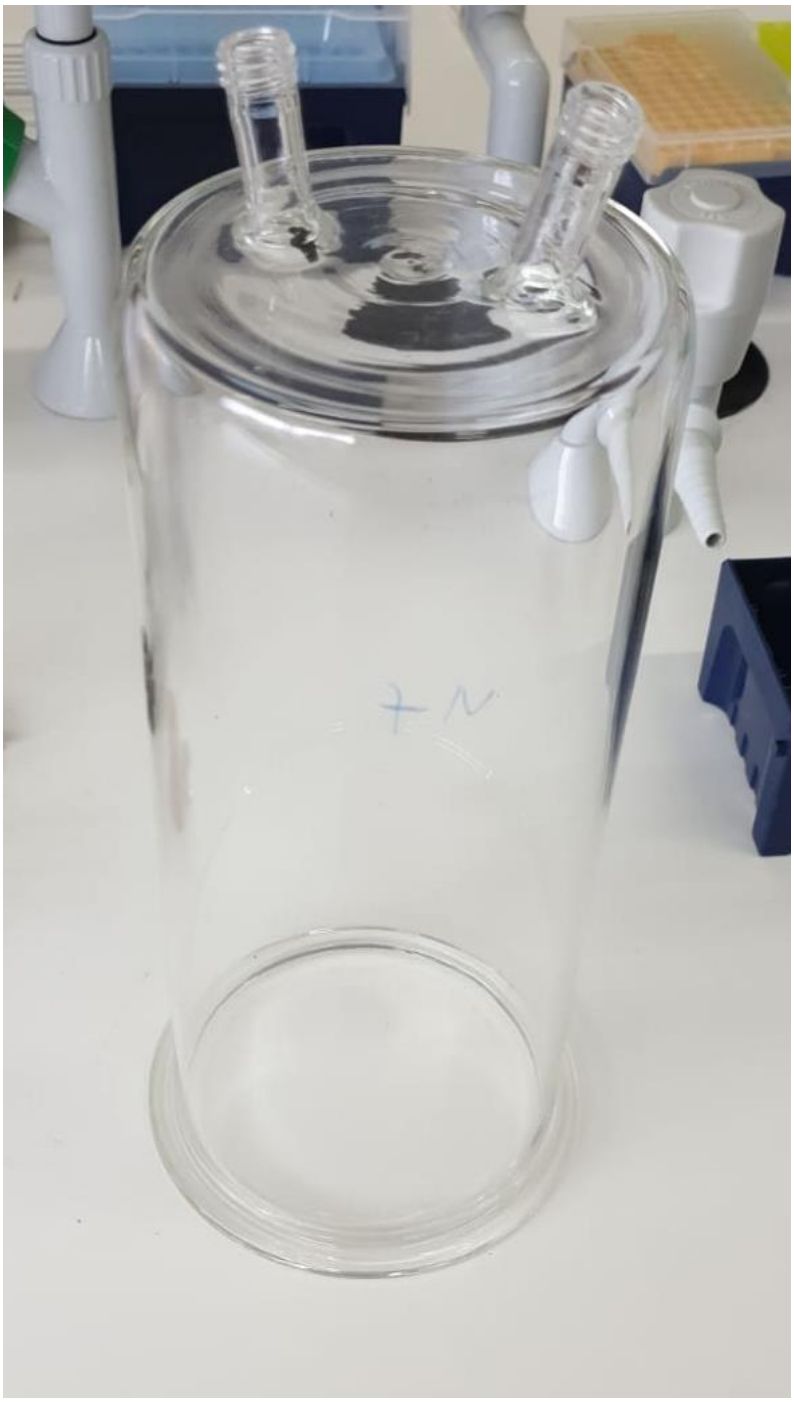
Seeds of *T. aestivum* var. Solstice, *T. monococcum* MDR037, MDR045 and MDR049 were germinated by placing 25 seeds onto three damp filter papers within a 9cm diameter petri dish and kept in the dark for three days under a tray in a controlled environment room at 21^{o}C (± 1^{o}C). Metal plates with 25 holes were placed on top of 5inch diameter pots filled with soil. Seedlings were sown into moistened soil through the holes in the metal plate, one seedling per hole. Pots were placed under a propagator with no ventilation for one day and with ventilation for two days, after which the propagator was removed. Pots were placed in a randomised order, in a controlled environment room at 21^{o}C (± 1^{o}C), with 200$_{(\mu mol.m^2_sec^{-1})}$ light for 16:8 h light: dark photoperiod and were watered daily. Due to limited availability of the entrainment apparatus, four pots (100 plants) were entrained at any one time.

2.2.5 Entrainment

Volatiles were collected by air entrainment, from plants with and without *R. padi* infestation 14 days post sowing at growth stage 13 (Zadoks et al., 1974). For the aphid treatment, 50 *R. padi* were placed on plants four hours prior to entrainment.

Specially designed entrainment glassware (Fig 2.1a), was washed with teepol detergent, rinsed with distilled water and acetone, and baked at 140^{o}C for six hours prior to use. Carbon filters were conditioned by running a flow of nitrogen through the filter whilst being baked at 172^{o}C for two hours prior to the first experiment and after every three uses of the filter to prevent any oxidisation. Porapak Q (50 mg, 60/80 mesh; Supelco, Bellefonte, PA, USA) absorbent tubes were cleaned with 2ml of redistilled diethyl ether and heated at 132^{o}C for two hours under a flow of nitrogen after each use. The specially designed glassware was used to contain the plants; the glassware was clipped to the metal plates which the plants had grown through. Charcoal filtered air was pushed into the glassware at 500 ml air/minute and pulled out through a Porapak adsorbent tube inserted into the collection ports on top of the vessels at 400 ml air/minute (Fig 2.1b). A positive pressure was created to avoid drawing in air from outside the experimental unit. Volatiles were collected over 72 hours, after which the Porapak tubes were eluted with 750µl of redistilled diethyl ether and stored at -20^{o}C. A minimum of four biological replicates were done for each wheat genotype and treatment.

(a)



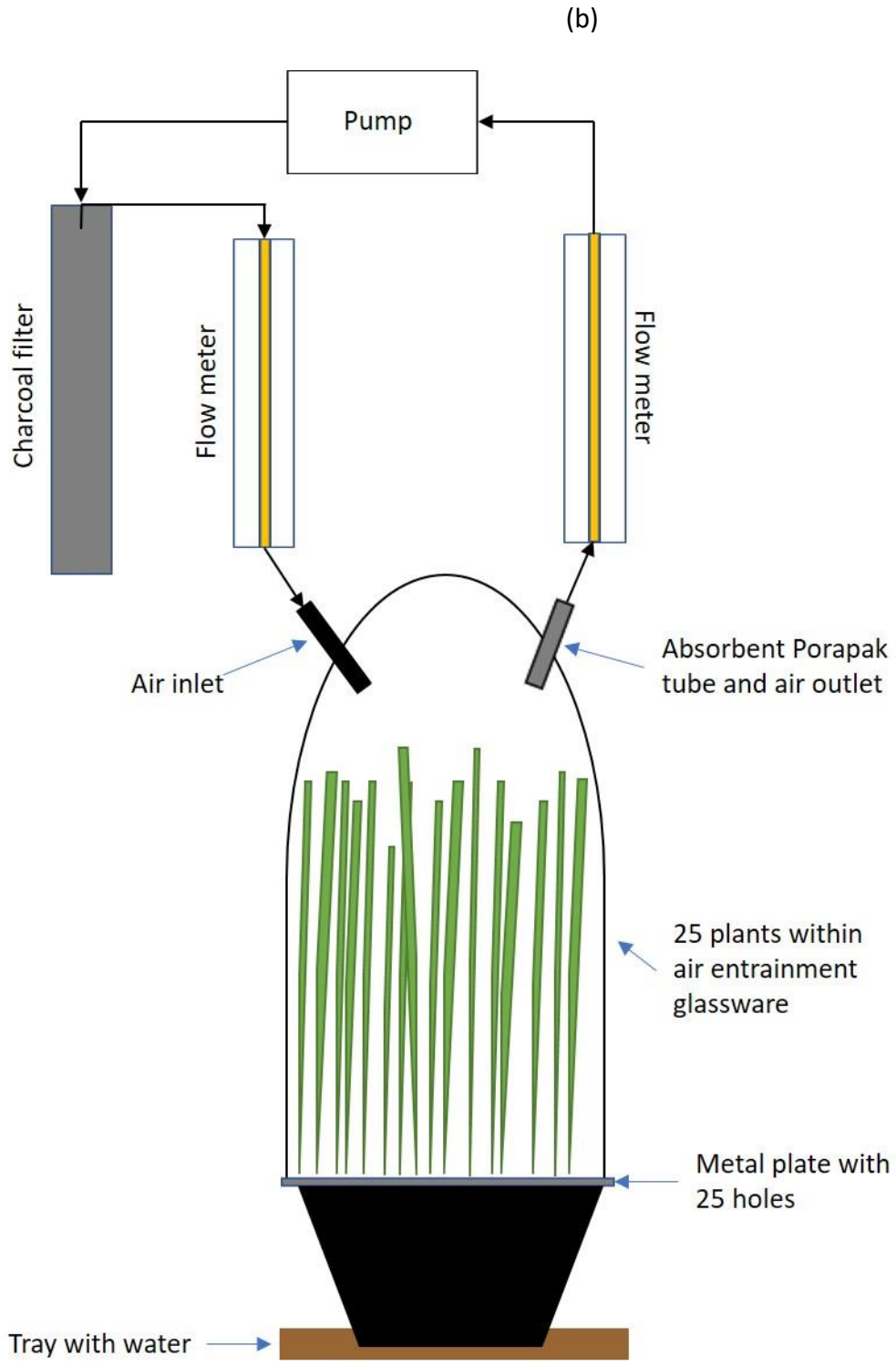


Figure 2.1. Air entrainment **a)** glassware **b)** experimental set up. Black arrows demonstrate the direction of air flow.

2.2.6 Chemical analysis of plant volatiles

Volatiles collected by air entrainment, were analysed using Gas Chromatography (GC), 4µl aliquots of each sample was injected into Agilent 6890A GC (fitted with a non-polar HP1 column 50m length x 0.32mm inner diameter x 0.52µm film thickness, (J&W Scientific)), equipped with a cool-on-column injector and a flame ionisation detector. The GC oven temperature was maintained at 30^{o}C for 5minutes and then programmed to rise 5^{o}C min^{-1} to 150^{o}C and then rise 10^{o}C min^{-1} to 230^{o}C. The run time was 60minutes and hydrogen was used as the carrier gas. Compounds with a lower boiling point will elute through the GC column quicker than compounds with a higher boiling points. The FID ionises each compound as it is eluted from the column, registering time taken for each compound to pass through the GC column (retention time), the ions generated per unit time is relative to the amount of each compound (peak area).

Three technical replicates were done for each sample. Four of the biological replicates, showing consistent GC profiles across technical and biological replicates, were combined using 250µl of each replicate, the combined sample was then condensed from 1ml to 500µl using a flow of nitrogen and analysed by GC using the same method to determine the number of individual compounds and the total peak area of plant volatiles. These combined samples were used in all further experiments.

2.2.6.1 Statistical analysis of plant volatile profiles

The differences in the total number of individual compounds and the total peak area in the volatile profiles of un-infested and infested Solstice, MDR037, MDR045 and MDR049 were subjected to a two-factor analysis of variance (ANOVA) with Tukey's HSD post hoc in which the wheat genotype and the presence of *R. padi* infestation were factors. Interactions between factors were also included in analysis. The total number of individual compounds

was subjected to square root transformation, and the total peak area subjected to $\log_{10}$ transformation to comply with assumptions of normality and equal variance. GenStat® (2016, 18th Edition, © VSN International Ltd, Hemel Hempstead, UK) statistical software was used for the analyses.

2.2.7 Behavioural bioassays

The behavioural responses of *R. padi* to volatiles were studied using 'four-arm' olfactometers (Pettersson, 1970). Before use, the Perspex equipment was washed with teepol detergent and rinsed in distilled water, after which the equipment was rinsed in 70% ethanol followed by distilled water and allowed to air dry.

At the end of each arm of the olfactometer was a 5ml plastic syringe, with gauze to prevent aphids from entering the syringe. For each experiment, 10µl of volatile sample was put on a strip of filter paper and placed within syringe number 1 connected to olfactometer arm 1, and 10µl of redistilled diethyl ether (solvent used to elute volatiles off Porapak) was used as a control in the remaining three arms (Fig 2.2). Air was pulled through the central chamber at 200 ml per minute. The olfactometry set-up was replaced after each replicate.

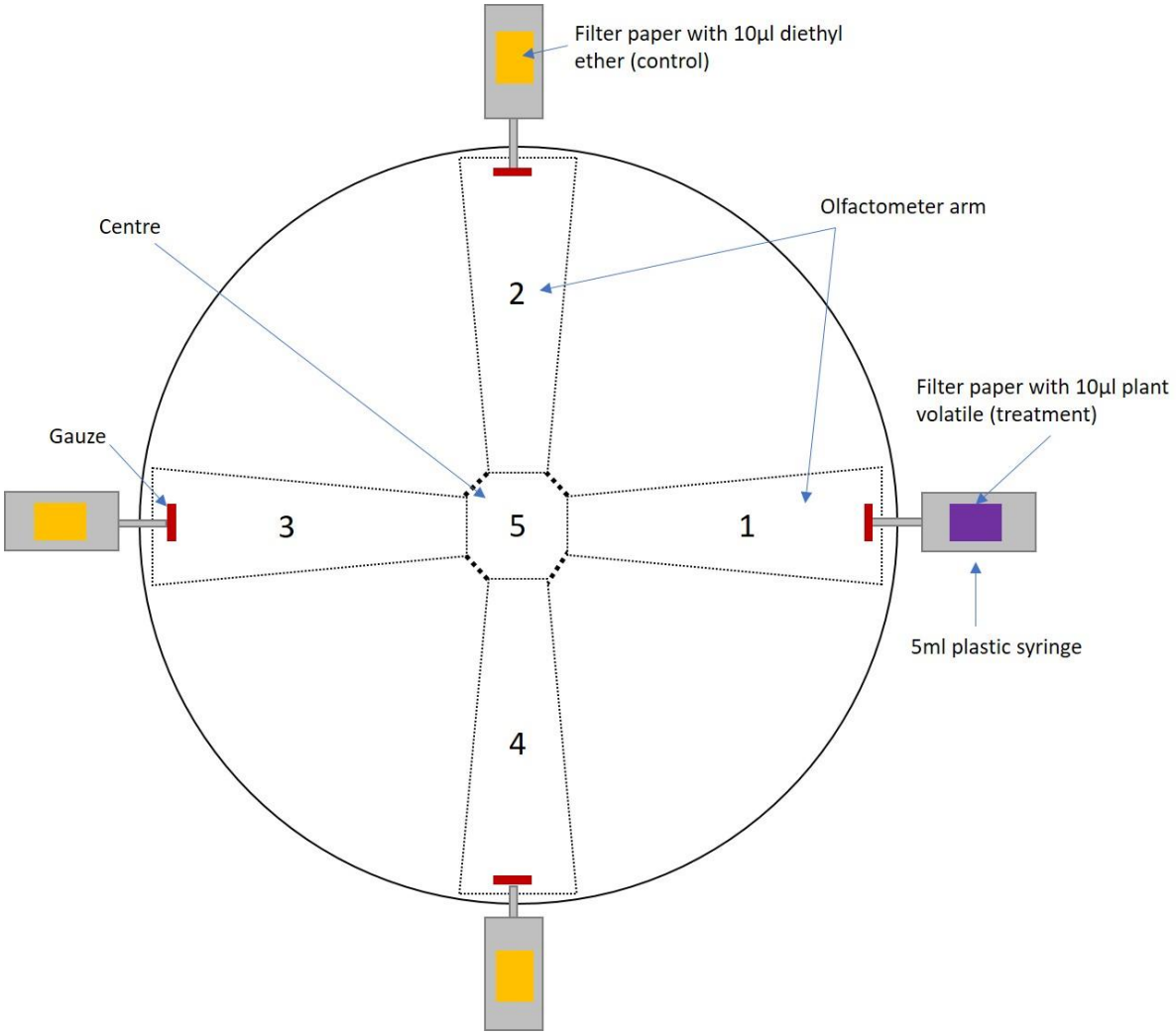


Figure 2.2. A schematic diagram of four-arm olfactometry bioassay set-up. Alate *R. padi* were free to move within the olfactometer arms and centre areas.

The bioassays were carried out at 22^{o}C, in a chamber with direct light from above, and the olfactometer was turned 90 degrees every two minutes to prevent environmental bias. Aphids were starved for one hour prior to experiments and one alate aphid was introduced into the centre of the olfactometer. Behaviour was observed for 16 minutes (Bruce et al., 2015), recording the time spent within each of the four arms or at the centre, using the Olfa computer program (Italy, 1995). If the aphid did not enter one of the four arms within two minutes, the aphid was determined as being unresponsive and the replicate was discarded. Ten replicates were done for each wheat genotype and treatment.

2.2.7.1 Behavioural bioassay statistical analysis

The aphid behavioural responses to wheat volatiles, was analysed by comparing the time aphids spent in the treatment arm with the average time spent in the control arms using a paired t-test. GenStat® (2016, 18th Edition, © VSN International Ltd, Hemel Hempstead, UK) statistical software was used for the analyses.

2.2.8 Coupled Gas-Chromatography - Electroantennography

Coupled gas chromatography-electroantennography (GC-EAG) was used to test antennal responses of alate *R. padi* to infested and un-infested MDR037 and MDR049 volatile samples. The aphid head was removed, and the ends of the antennae cut off to ensure good conductivity. The aphid head was inserted into a AgAgCl glass electrode filled with a saline solution (as described in Maddrell, (1969) but without glucose) and the antennae into the other glass electrode ensuring good baseline signal. The effluent from the GC column was split so the effluent was simultaneously delivered to the antenna via a purified airflow and to the GC detector (Fig 2.3). 4µl aliquots of each sample was injected into Agilent 6890A GC (fitted with a non-polar HP1 column 50m length x 0.32mm inner diameter x 0.52µm film thickness (J&W Scientific)), equipped with a cool-on-column injector and an FID. The GC oven temperature was maintained at 30^{o}C for 2minutes and then programmed to rise 10^{o}C min^{-1} to 250^{o}C. The run time was 30minutes and the helium was the carrier gas. The signals from the antenna were passed via an amplifier (UN- 06; Syntech, Hilversum, The Netherlands), the outputs from this and the FID were monitored and analysed simultaneously using Syntech software (Syntech, Hilversum, The Netherlands). FID peaks were assumed to be EAG active if they elicited responses on at least six replicates.

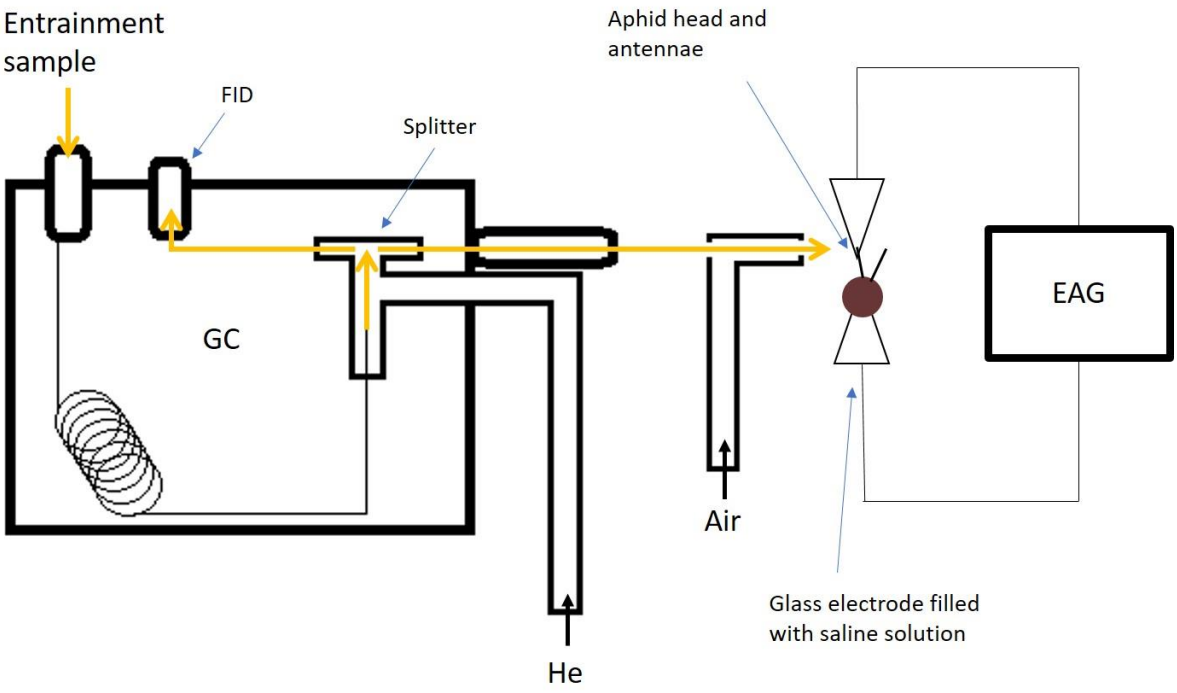


Figure 2.3. A schematic diagram of a coupled gas chromatography – electroantennography set-up.

2.2.9 Compound identification

Compounds that were active to the antennal responses of alate *R. padi* were identified using Gas Chromatography Mass Spectrometry (GC-MS), on a Micromass Autospec Ultima, magnetic sector mass spectrometer. 4µl aliquots of each sample was injected into Agilent 6890N GC (fitted with a non-polar HP1 column 50m length x 0.32mm inner diameter x 0.52µm film thickness, (J&W Scientific)), equipped with a cool-on-column injector and ionisation was by electron impact (70eV, 220^{o}C). The GC oven temperature was maintained at 30^{o}C for 5minutes and then programmed to rise 5^{o}C min^{-1} to 250^{o}C. The run time was 70minutes and helium was used as the carrier gas. Tentative identifications were obtained with the NIST mass spectral database (2011) or by spectral interpretation. Identification of compounds from GC-MS was confirmed by injecting an authentic compound standard (50ng/µl) into an Agilent 6890A GC with a cool-on-column injector. The Kovats retention index (KI) of the authentic compound, GC-MS and GC-EAG KI of the peak were compared.

2.2.10 Compound quantification

Entrainment samples were quantified by comparing their peak area with that of an authentic compound, at the estimated concentration of its respective chemical in the air entrainment sample. Co-injecting the synthetic compound with the entrainment sample aimed to double the peak area to prove identity and estimate compound concentration. Co-injections started at a concentration of 5ng/µl and were decreased until the peak height was double that from the semiochemical profile.

2.2.11 Tentative compound presence in GC-EAG tested samples

The tentative presence and absence of each physiologically active compound in all entrainment samples was identified by determining if the KI of the authentic compound used for compound identification (section 2.2.9) was present in all other entrainment samples.

2.2.11.1 Tentative compound presence statistical analysis

To analyse correlations between the presence and absence of physiologically active compounds and the entrainment samples, a principle component analysis was carried out using RStudio: Integrated Development Environment for R (2016, Edition 1.1.453, ©RStudio Inc, Boston, Massachusetts, USA) software.

2.3 RESULTS

2.3.1 Gas Chromatography

After the headspace volatiles of un-infested and infested *T. aestivum* Solstice, *T. monococcum* MDR037, MDR045 and MDR049 were collected, the volatile compounds were separated and analysed using gas chromatography (GC).

The GC traces from *T. aestivum* Solstice and *T. monococcum* MDR037, MDR045 and MDR049 with and without *R. padi* are provided in the appaendix (Fig A1) and the total number and peak areas of the compounds are given in Table 2.1.

Differences were observed in volatile profiles between on wheat genotype as Solstice and MDR037 volatile profiles produced the highest number of individual of compounds ($F_{3,23}$ = 6.22, P <0.001), MDR037 volatile profile also had the highest total peak area ($F_{3,23}$ = 1.28, P <0.001). The presence of *R. padi* infestation affected the volatile profiles as overall infested plants had a fewer compounds ($F_{3,23}$ = 2.93, P <0.001). There was an interaction between the genotype and *R. padi* infestation for the number of compounds ($F_{3,23}$ = 14.58, P <0.001) as infested MDR049 volatile profile had a higher number of compounds than un-infested MDR049 (Appendix Fig A1, Table 2.1). The volatile profiles of *R. padi* infestated had a higher total peak area ($F_{3,23}$ = 1.74, P <0.001) than un-infested plants. There was also an interaction between the genotype and infestation ($F_{3,23}$ = 1.78, P <0.001)as infested MDR049 volatiles had lower total peak area than un-infested plants (Appendix Fig A1, Table 2.1).

Table 2.1. Total number and peak area of compounds in a 4µl aliquot of entrainment samples from *Triticum aestivum* Solstice, *Triticum monococcum* MDR037, MDR045 and MDR049, with and without *Rhopalosiphum padi* infestation. The least significant difference G represents the genotype; T represents the *R. padi* infestation treatment.

	Total number of compounds	**Total peak area**
Solstice un-infested	238 a	29263900 ab
Solstice infested	166 b	28284000 ab
MDR037 un-infested	240 a	29784800 b
MDR037 infested	209 c	30601600 c
MDR045 un-infested	168 b	29535700 ab
MDR045 infested	148 d	29832800 b
MDR049 un-infested	128 e	30724400 d
MDR049 infested	168 b	18815000 e
LSD (G x T)	8.72	124181.8
P value	< 0.001	< 0.001

2.3.2 Behavioural bioassays

4-arm olfactometer behavioural bioassays with alate *R. padi* were used to determine whether the VOCs produced by Solstice, MDR037, MDR045 and MDR049 with and without *R. padi* infestation elicited different behavioural responses. Results are shown in Figure 2.4.

For the un-infested plants, *R. padi* spent longer in the arm containing VOCs of Solstice ($t_{1,9}$ = 4.28, $P < 0.05$) and MDR037 ($t_{1,9}$ = 4.75, $P < 0.05$), whereas they spent less time in the arm containing the VOCs from MDR049 compared with the control arms. ($t_{1,9}$ =4.30, $P < 0.01$) (Fig 2.4a.). There was no difference in aphid behaviour when aphids were exposed to VOCs from MDR045 ($t_{1,9}$ =0.24, $P = 0.41$).

For the infested plants, *R. padi* spent less time in the arm containing VOCs from MDR049 than in the control arms ($t_{1,9}$ = 3.53, P <0.001) (Fig 2.4b). In contrast to what was observed with VOCs from un-infested plants, there was no difference in aphid behaviour to plant volatiles collected from *R. padi* infested Solstice, MDR037 or MDR045.

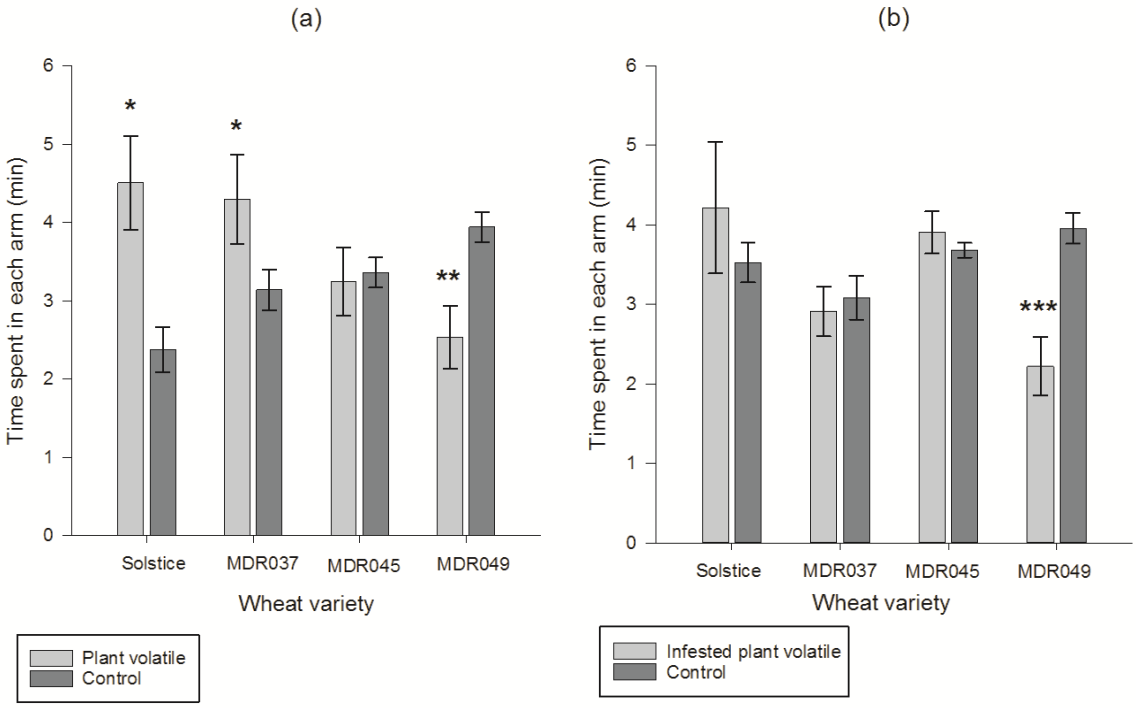


Figure 2.4 Average time alate *Rhopalosiphum padi* aphids spent in the arm containing volatile organic compounds from **(a)** un-infested *Triticum aestivum* Solstice, *Triticum monococcum* MDR037, MDR045 and MDR049 **(b)** *R. padi* infested *T. aestivum* Solstice, *T. monococcum* MDR037, MDR045 and MDR049 compared with average time alate *R. padi* spent in the control (re-distilled diethyl ether) arms in a four-arm olfactometer. Error bars represent ± SEM. * <0.05, **<0.01, ***<0.001.

2.3.3 Coupled Gas Chromatography Electroantennography (GC-EAG) and compound identification

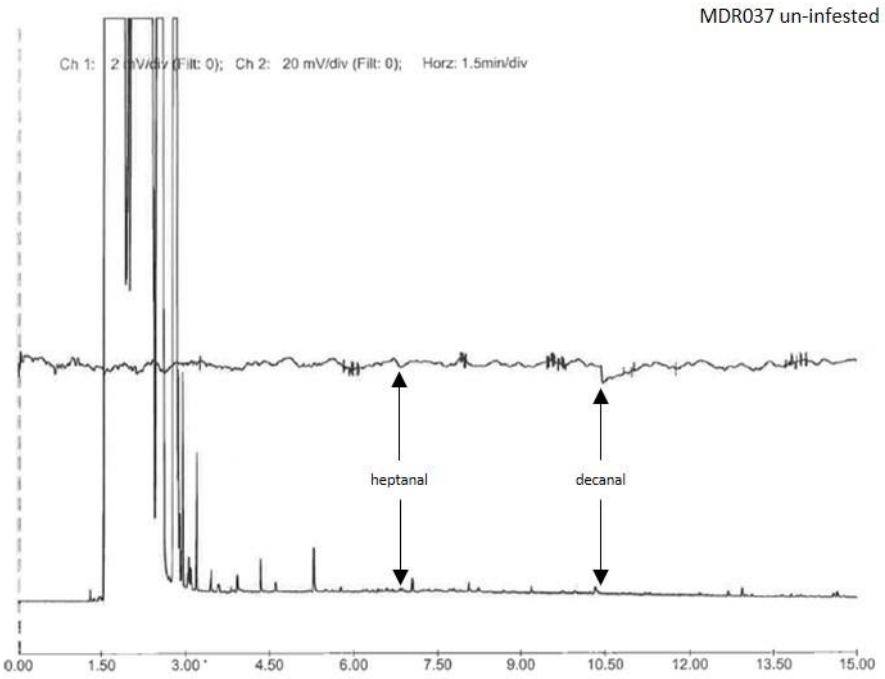
To identify the compounds within a VOC mix that elicit behavioural responses in *R. padi*, GC-EAG was carried out on the entrainment samples from MDR037 and MDR049 with and without *R. padi* infestation. The resulting traces showing the responses of alate aphid antennae to VOCs from Solstice and *T. monococcum* MDR037 and MDR049 infested and

un-infested with *R. padi* are shown in Figure 2.5. Alate *R. padi* moved away from MDR049 un-infested and infested VOCs but moved towards VOCs from un-infested MDR037 and there was no behavioural response to infested MDR037.

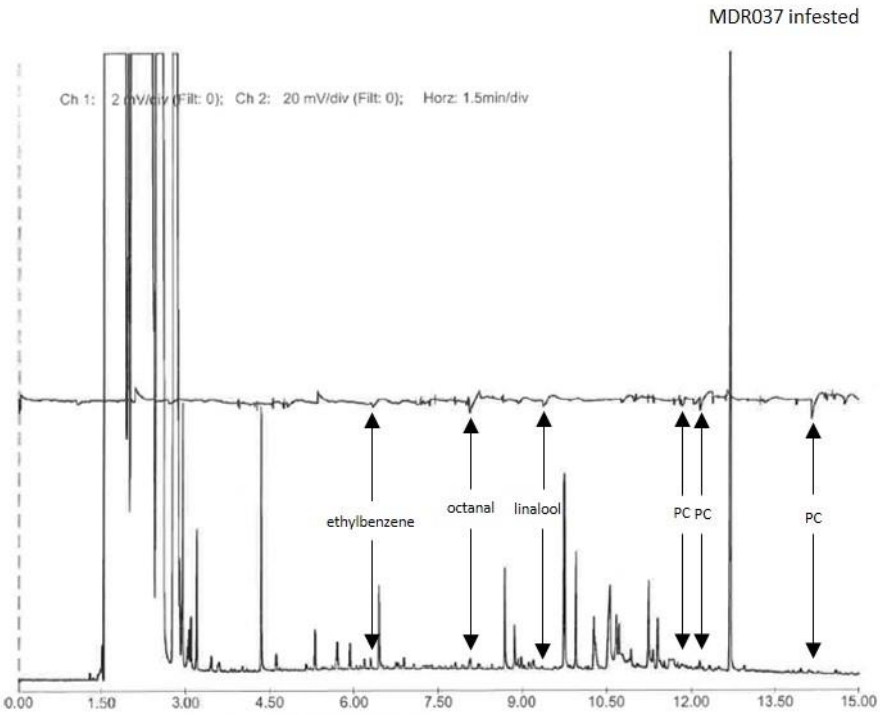
Responses were observed from alate *R. padi* antennae to two compounds from the VOCs of un-infested MDR037, six compounds from *R. padi* infested MDR037, five compounds from un-infested MDR049 and five compounds from *R. padi* infested MDR049. (Fig 2.5 & Table 2.2).

Compound identity was then determined by gas-chromatography mass-spectrometry (GC-MS) (Fig 2.5 & Table 2.2). A methyl siloxane from infested MDR049 elicited response from the antennae, however this is associated with industrial and consumer product waste (Alaee et al., 2013) and was considered a contaminant and not investigated further. Compound identifications were confirmed by comparing the KI of individual electrophysiologically active compound standards (50ng/µl) to the respective KI from the GC-MS and EAG.

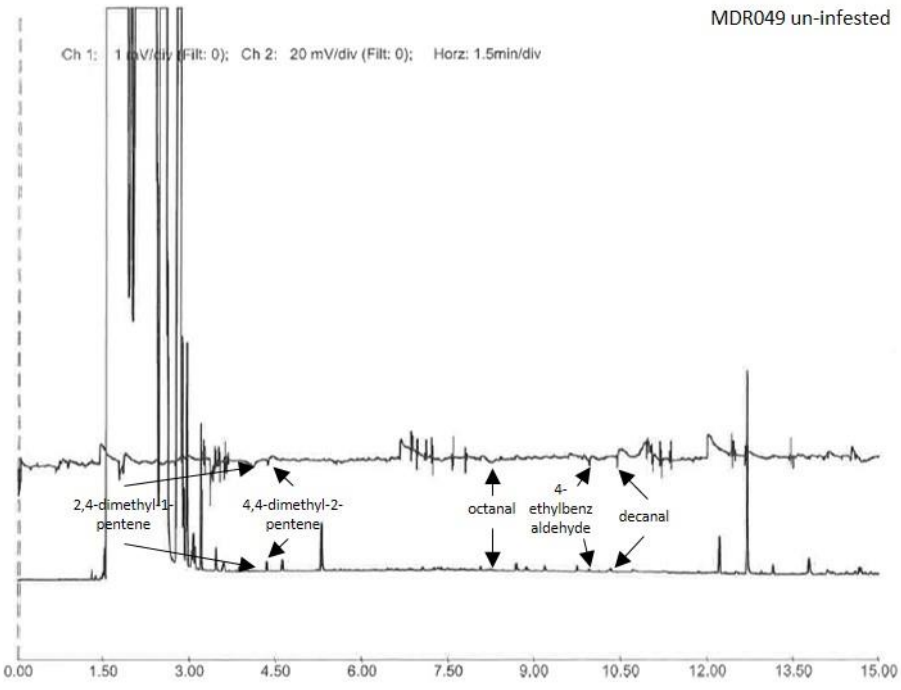
(a)



(b)


MDR037 infested
Ch 1: 2 mV/div (Filt: 0); Ch 2: 20 mV/div (Filt: 0); Horz: 1.5min/div
ethylbenzene
octanal
linalool
PC PC
PC
0.00 1.50 3.00 4.50 6.00 7.50 9.00 10.50 12.00 13.50 15.00

(c)


MDR049 un-infested
Ch 1: 1 mV/div (Filt: 0); Ch 2: 20 mV/div (Filt: 0); Horz: 1.5min/div
2,4-dimethyl-1-pentene
4,4-dimethyl-2-pentene
octanal
4-ethylbenz aldehyde
decanal
0.00 1.50 3.00 4.50 6.00 7.50 9.00 10.50 12.00 13.50 15.00

(d)

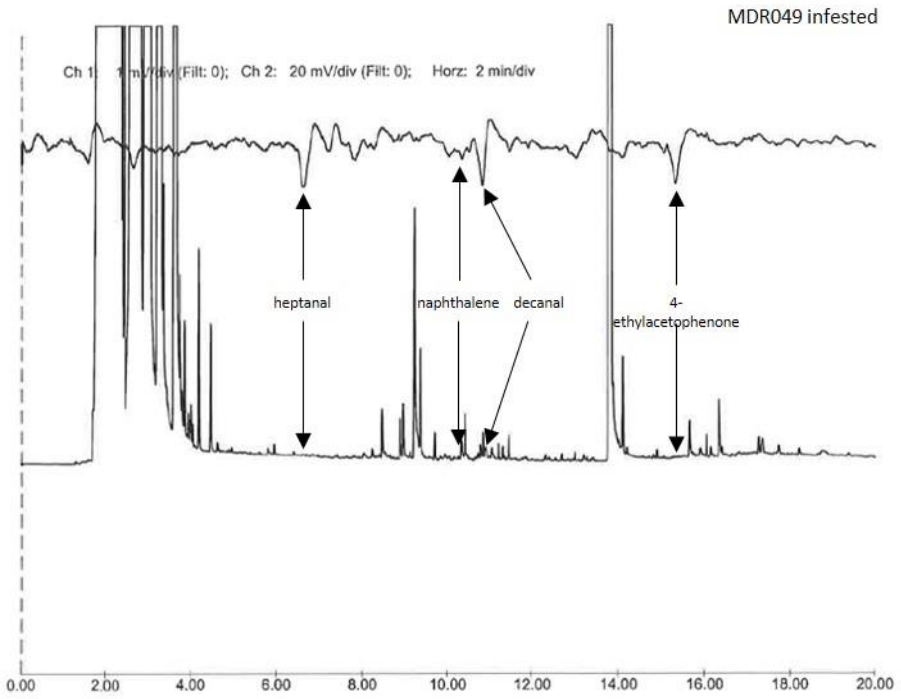
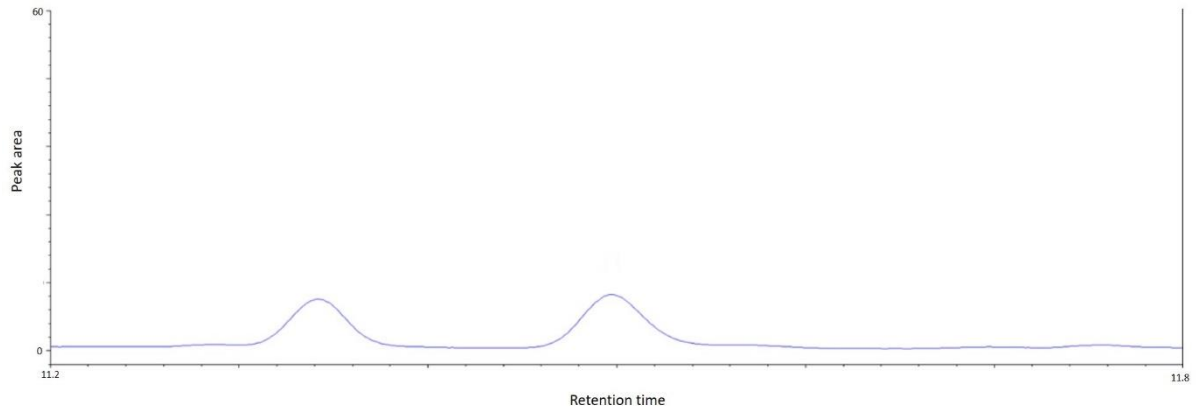


Figure 2.5. Coupled Gas Chromatography electroantennography (GC-EAG) traces of alate *Rhopalosiphum padi* antenna showing the responses to volatile organic compounds from *Triticum monococcum* **(a)** MDR037 un-infested **(b)** MDR037 infested with *R. padi* **(c)** MDR049 un-infested **(d)** MDR049 infested with *R. padi.*

3.3.4 Compound quantification

It is known that quantitative changes in VOCs can affect aphid attraction, so the quantity of each physiologically active compound in the VOC profile was identified by co-injections with standards of known concentration, where a doubling in peak height is indicative of the compound being tested having the same concentration as the standard. The GC traces of the co-injections for heptanal and decanal (physiologically active from un-infested MDR037) are shown in Figure 2.6 and 2.7. The traces for other physiologically active compounds from the infested MDR037 and un-infested and infested MDR049 are shown in the appendix (Fig A2-A13). Amounts of all physiologically active compounds and their ratios to one another are shown in Table 2.2.

(a)



(b)

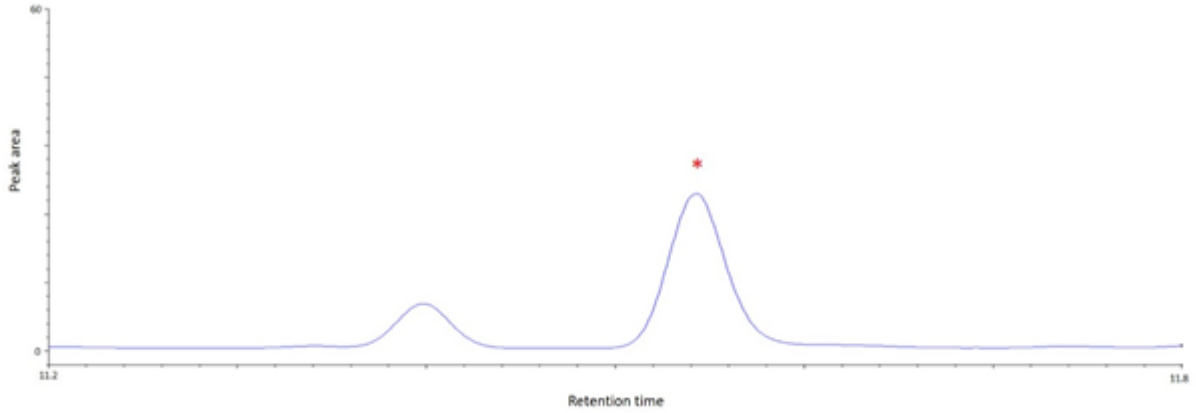
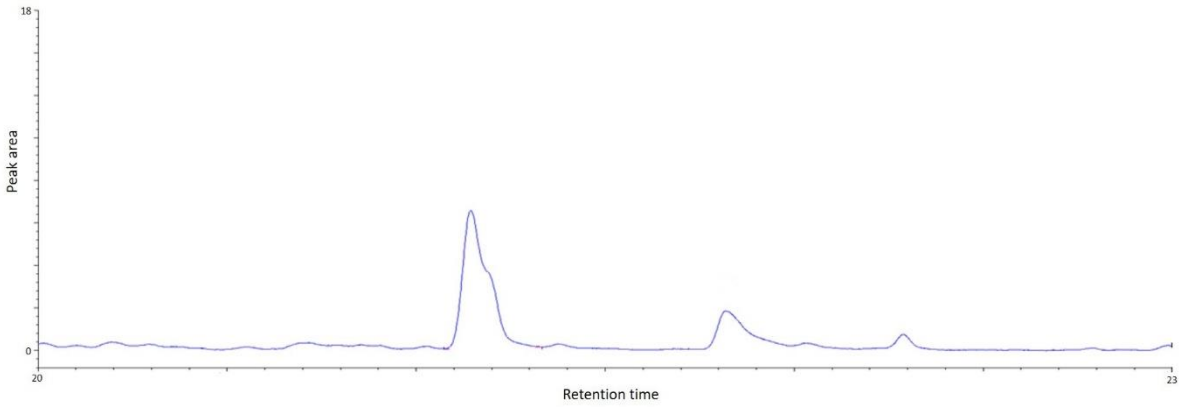


Figure 2.6. Gas Chromatography (GC) trace on a non-polar GC (HP-1) of **a)** air entrainment sample of *Triticum monococcum* MDR037 **b)** air entrainment sample of *T. monococcum* MDR037 co-injected with heptanal (1ng/µl). * denotes peak enhancement

(a)



(b)

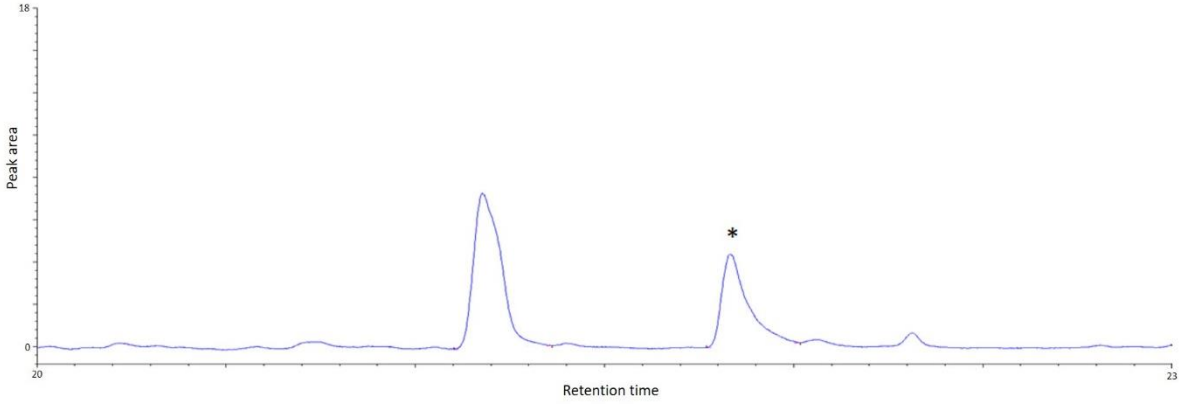


Figure 2.7. Gas Chromatography (GC) trace on a non-polar GC (HP-1) of **a)** air entrainment sample of *Triticum monococcum* MDR037 **b)** air entrainment sample of *T. monococcum* MDR037 co-injected with decanal (425ng/µl). * denotes peak enhancement

In general, physiologically active compounds in un-infested MDR037 were not physiologically active in infested MDR037. Decanal was physiologically active in both un-infested and infested MDR049, and un-infested MDR037, but was present at different concentrations. In un-infested MDR049 there was 500pg/µl of decanal produced, in un-infested MDR037, 425pg/µl and in infested MDR049 200pg/µl. Apart from decanal all other physiologically active compounds in un-infested MDR049 were not physiologically active in

infested MDR049. Heptanal was physiologically active in un-infested MDR037 and infested MDR049, heptanal was present at a higher concentration in un-infested MDR037 (1ng/µl) than in infested MDR049 (325pg/µl). Heptanal and decanal were present in different ratios in un-infested MDR037 (0.43:1) and infested MDR049 (1:1.54). Octanal was physiologically active in infested MDR037 and un-infested MDR049 but was present at a higher concentration in infested MDR037 (125pg/µl) than in un-infested MDR049 (40pg/µl). The results are summarised in Table 2.2

Table 2.2. Coupled Gas Chromatography electroantennography. Kovats Index (KI) and identification, quantification and ratio of compounds in entrainment samples from un-infested and *R. padi* infested *Triticum monococcum* MDR037 and MDR049, that gave electrophysiological responses in *Rhopalosiphum padi* antennae

	GC-EAG KI	**Compound identification**	**Compound quantification (pg/µl)**	**Ratio**
MDR037 un-infested	885	Heptanal	1000	2.35
	1188	Decanal	425	1
MDR037 infested	847	Ethyl benzene	100	1
	979	Octanal	125	1.25
	1088	Linalool	275	2.5
	1270	Porapak contaminant		
	1359	Porapak contaminant		
	1586	Porapak contaminant		
MDR049 un-infested		2,4-dimethyl-1-pentene	25	1
	712	4,4-dimethyl-2-pentene	40	1.6
	993	Octanal	40	1.6
	1143	4-ethylbenzaldehyde	125	5
	1187	Decanal	200	8
MDR049 infested	875	Heptanal	325	4.33
	1161	Naphthalene	75	1
	1188	Decanal	500	6.67
	1238	4-ethylacetophenone	725	9.66
	1761	Porapak contaminant		

2.3.5 Presence of physiologically active compounds in volatile profiles

Physiologically active compounds were qualitatively different between volatile samples for example none of the physiologically active compounds in un-infested MDR037 were physiologically active in infested MDR037. To examine whether this was due to the absence of those compounds from the other samples, the KI of physiologically active compounds (Table 2.2) were searched for in VOC samples from all other treatments (Figure 2.4). However, since the standard injections and co-injections were not carried out, the identification of these compounds in other samples is only tentative.

The tentative and confirmed physiologically active compounds in the VOC profiles of un-infested and infested *T. monococcum* Solstice and *T. monococcum* MDR037, MDR045 and MDR049 are shown in Table 2.3. As infested and un-infested Solstice and MDR045 volatiles were not tested in GC-EAG studies, there are no confirmed physiologically active compounds for these samples.

Tentative identification of physiologically active compounds in Solstice volatile profiles showed that more compounds were identified in infested Solstice volatile profile (heptanal, ethylbenzene, linalool, octanal, 4-ethylbenzaldehyde, 2,4-dimethyl-1-pentene, 4-ethylacetophenone and naphthalene) than in un-infested Solstice (decanal, heptanal, ethylbenzene, octanal, 4,4-dimethyl-2-pentene). The same number of compounds were tentatively identified in un-infested and infested MDR045, with most identified compound being present in both profiles (decanal, linalool, octanal, 4-ethylbenzaldehyde and 2,4-dimethyl-1-pentene) (Table 2.3). Interestingly, decanal and octanal were present in un-infested and infested MDR037 and MDR049 but *R. padi* antenna only detected decanal in un-infested MDR037, un-infested and infested MDR049. Octanal was only detected in infested

MDR037 and un-infested MDR049, despite being present in all GC-EAG tested semiochemical profiles (Table 2.2, 2.3).

Table 2.3. Presence of tentative (black cross) and confirmed physiologically active (red cross) compounds in the semiochemical profiles of un-infested and *Rhopalosiphum padi* infested *Triticum monococcum* Solstice, *Triticum monococcum* MDR037, MDR045 and MDR049

Compound	**Solstice**		**MDR037**		**MDR045**		**MDR049**	
	Un-infested	**Infested**	**Un-infested**	**Infested**	**Un-infested**	**Infested**	**Un-infested**	**Infested**
Decanal	X		X	X	X	X	X	X
Heptanal	X	X	X		X			X
Ethylbenzene	X	X		X		X		
Linalool		X		X	X	X	X	X
Octanal	X	X	X	X	X	X	X	X
4-ethylbenzaldehyde		X			X	X	X	
2,4-dimethyl-1-pentene		X			X	X	X	
4,4-dimethyl-2-penetene	X						X	
4-ethylacetophenone		X						X
Naphthalene		X			X	X	X	X

To determine if there were any correlations between the presence and absence of physiologically active compounds, the *T. monococcum* genotype, and the presence or absence of herbivory, a principle components analysis was done (Figure 2.8). This showed that tentatively identified compounds from Solstice plant volatile profiles were most dissimilar to the compounds identified from *T. monococcum* volatile profiles. There were also differences within the *T. moncococum* genotypes, confirmed and teneatively identified compounds from MDR037 profiles were distinct from tentatively identified compounds from MDR045 volatile profiles. *Rhopalosiphum padi* infestation had the largest effect on confirmed and tentatively

identified compounds from Solstice and MDR049 (Table 2.3, Figure 2.8). Compounds identified from un-infested MDR049 volatile profile were more similar to compounds from infested MDR037 volatile profile and the compounds from un-infested MDR049 profile was most similar to un-infested and infested MDR045 profile (Fig 2.8). *Rhopalosiphum padi* infestation affected the presence of compounds in the MDR037 volatile profile but had little effect on presence of compounds in the MDR045 volatile profile (Table 2.3, Fig 2.8).

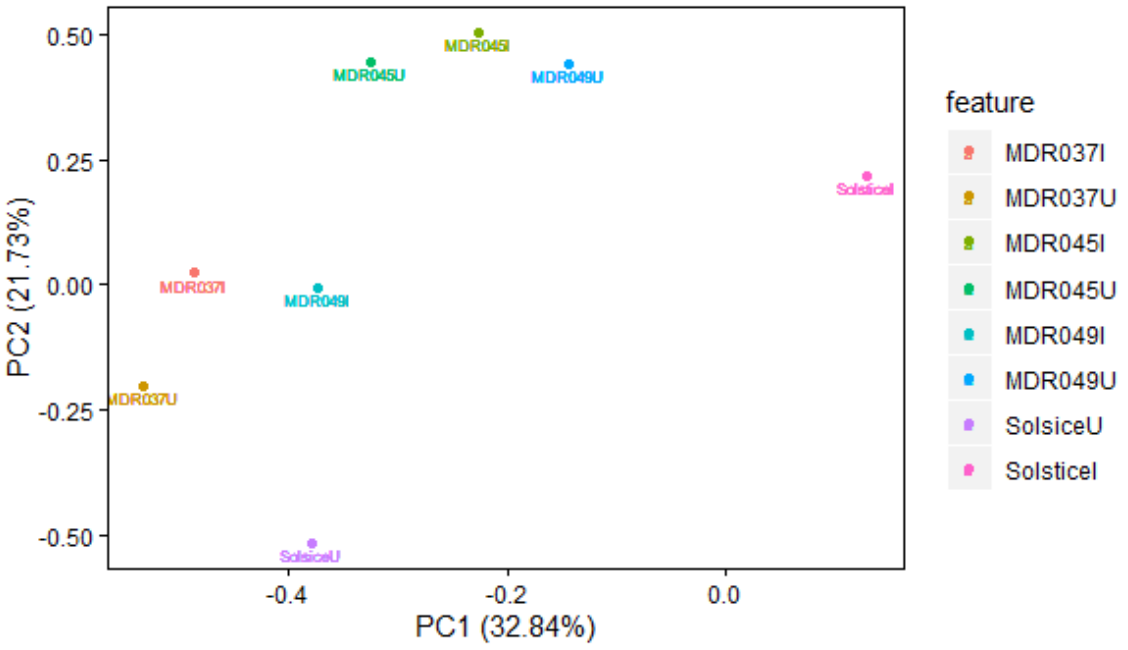


Figure 2.8. Principle component analysis of the tentative presence and absence of physiologically active compounds to alate *Rhopalosiphum padi* antenna in the plant volatile profiles of un-infested (U) and *Rhopalosiphum padi* infested (I) *Triticum aestivum* Solstice and *Triticum monococcum* genotypes MDR037, MDR045 and MDR049.

2.4 DISCUSSION

This work has shown that *T. monococcum* genotype MDR049 confers antixenosis to *R. padi,* and that *R. padi* infestation causes changes in the volatile profiles of all genotypes. These changes in *T. aestivum* Solstice and *T. mococccum* MDR037 altered aphid responses to these volatiles. The compounds (and their relative amounts) that underpin changes in aphid attraction to MDR037 and MDR049 in the presence and absence of *R. padi* herbivory were identified.

Qualitative differences were observed in the volatile profiles of all genotypes. Overall Solstice and MDR037 produced a similar number of volatile compounds, but there were differences between the *T. monococcum* species, as MDR037 produced more compounds than MDR045 and MDR049. Differences in semiochemistry between cultivars has previously been shown in *T. aestivum* and *T. durum* (Weaver et al., 2009; Mattiolo et al., 2017), but in this chapter, such differences have been reported between *T. moncococum* genotypes.

In this discussion the terms attraction and repulsion are used for positive and negative taxis towards the volatile organic compounds (VOC's) from the plant entrainment samples.

Behavioural responses to volatiles varied between the genotypes tested, with aphids moving towards volatiles from Solstice and MDR037, but showing no behavioural response to MDR045 volatile and moved away from MDR049 volatiles. Cultivar differences affecting alate *R. padi* behaviour have previously been shown in *Z. mays,* where aphids moved towards volatiles from cultivar 6Q-121 but had no behavioural response to cultivars 78-15B and CRN 3505 (Schröder et al., 2015). Aphids were repelled by MDR049 volatiles in the olfactometer experiments, demonstrating that MDR049 has antixenosis to alate *R. padi* (Kogan and Ortman, 1978). Greenslade et al., (2016) showed that *R. padi* have a higher reproduction and

development on Solstice and MDR037 and lower on MDR049. Thus there is a link between *R.padi* attraction (this work) and host suitability (Greenslade et al., 2016; Chapter 3) for Solstice, MDR037 and MDR049. Whilst MDR045 does not confer antixenosis to alate *R. padi*, growth stage 12-13 plants have been shown to confer antibiosis to *R. padi* (Chapter 3), making it a poor host. Aphids did not show a behavioural response to MDR045 volatiles, so aphids were only attracted to plants that were suitable hosts. It was previously shown that *T. monococcum* had antixenosis towards *S. avenae* (Hu et al., 2016), however, the genotype of this plant was not provided. The present study shows the importance of genotype, with *R. padi* showing different responses to the three *T. moncooccum* genotypes.

It has previously been shown that VOCs emitted by plants in response to herbivory change both qualitatively and quantitatively (Dicke, 1994; Röse et al., 1996; Pare and Tumlinson, 1999; Thaler, 1999; War et al., 2011) including HIPV production (Dicke and Baldwin, 2010), and that this can affect aphid behaviour (Quiroz et al., 1997; Webster, Bruce, Pickett, et al., 2008; Hegde et al., 2011). In the present study, alate *R. padi* moved towards volatiles from un-infested Solstice and MDR037, but there was a neutral or no behavioural response to *R. padi* infested Solstice and MDR037 volatiles. These results differ from those of Quiroz et al. (1997) who reported that infested *T. aestivum* variety Ciko volatiles were repellent to *R. padi*. The differences in results are likely due to the fact that in the present work, diethyl ether (used to elute the volatiles from the Porapak) was used as a control for both infested and un-infested behavioural bioassays, whereas in the work of Quiroz et al. (1997), aphids were given a direct choice between volatiles from infested Ciko as a treatment and un-infested Ciko volatiles as the control, showing that in this case aphids preferred un-infested wheat to infested wheat.

The change in aphid behaviour in response to volatiles from un-infested Solstice and MDR037 and infested Solstice and MDR037 suggests that alate *R. padi* are able to determine the suitability of the host from the plant volatiles. Alate aphids are colonisers of new host plants and are produced in response to overcrowding (Dixon and Glen, 1971; Wratten, 1977), so plants that are not colonised by aphids would be better as hosts, as there would be less competition for food resource. In support of this, the present work has also shown that aphids moved away from volatile from MDR049 but to a greater degree in response to infested MDR049 volatiles. As alate *R. padi* only moved towards plant volatiles from plants that were more favourable for aphid reproduction, it can be concluded that in this case, aphid behavioural response is indicative of the suitability of that host for colonisation.

Qualitative and quantitative similarities were identified in compounds which elicited physiological responses from alate *R. padi* antennae from un-infested and infested MDR037 and MDR049. Decanal was physiologically active in samples collected from un-infested MDR037 and un-infested and infested MDR049, and although not physiologically active, decanal was also tentatively identified in infested MDR037. Decanal is a GLV aldehyde that has been previously identified in un-infested and *R. padi*-infested *T. aestivum* volatile profile (Quiroz et al., 1997; Quiroz and Niemeyer, 1998), and is attractive to apterous *R. padi* (Quiroz and Niemeyer, 1998) . Whilst decanal was present in the volatile profiles of un-infested MDR037 and un-infested and infested MDR049, there were quantitative differences, where least amount of decanal was present in the volatile profile of un-infested MDR049. The amounts of decanal in un-infested and infested *T. aestivum* var. Ciko were not provided by Quiroz and Niemeyer (1998), and as decanal was only tentatively identified in infested MDR037, it is unknown whether *R. padi* infestation does affect the quantity of decanal emitted from *T. aestivum* var. Ciko and *T. moncococcum* MDR037. However, the current

work does establish that *R. padi* infestation of MDR049 causes an increase in the amount of decanal produced.

Heptanal was also physiologically active in more than one volatile profile, from un-infested MDR037 which the aphids were attracted to and infested MDR049 which the aphids were repelled by. Heptanal has been shown to be present in *H. vulgare* and *T. durum* (Dong et al., 2015; Mattiolo et al., 2017) and to be attractive to apterous *R. padi* (Quiroz and Niemeyer, 1998). In the current work, the quantities of heptanal differed between the *T. monococcum* genotypes and the presence of *R. padi*, with over three times the amount of heptanal emitted from un-infested MDR037 than infested MDR049. It has been shown that aphid behaviour can be affected by the amount of an individual compound and the ratios between compounds (Bruce et al., 2005; Webster et al., 2010; Webster, 2012; Schröder et al., 2015). The damson-hop aphid (*Phorodon humuli* (S.)) was attracted to (E)-2-hexenal and β-caryophyllene in its natural ratio of 39:1 but not to a 1:1 ratio (Campbell et al., 1993). The ratios between heptanal and decanal were very different between the two VOCs profiles, with heptanal:decanal being 0.43:1 in un-infested MDR037 and 1:1.54 in infested MDR049. Heptanal and decanal, as well as the other physiologically active compounds, are not specific to *T. moncococcum* and are produced by a variety of crops including *Hordeum vulgare* (L.) (Dong et al., 2015) *Trititcum durum* (Mattiolo et al., 2017). Thus if aphids are able to distinguish between ratios of commonly occurring semiochemicals (Bruce et al., 2005; Webster, 2012), a large range of plants can be recognised without increasing the number of olfactory receptors needed. Additionally, heptanal and decanal were not the only physiologically active compounds identified in infested MDR049 volatile profile. There was a larger blend of compounds, with aphid antennae also responding to naphthalene and 4-ethylacetophenone from the infested MDR049 volatile profile.

Naphthalene has been shown to present in *T.aestivum* var. Commander and Stettler (Cruz et al., 2012) volatile profiles and be electrpphysiologically active to *Busseola fusca* (F.) and the spotted stalk borer (*Chilo partellus* (S.)) (Birkett et al., 2006). *Rhopalosiphum padi* behaviour to this compound is unknown, however, *A. pisum* attraction has been negatively associated with increased levels of naphthalene in *V. faba* volatile profile (Babikova et al., 2014). 4-ethylacetophenone has been previously identified in the volatile profile of oranges (Hernández et al., 1996) and electrophysiologically active to *B. fusca* and *C. partellus* antennae (Birkett et al., 2006). Here it has been shown that both naphthalene and 4-ethylacetophenone are physiologically active to alate *R. padi* antennae.

Whilst individual compounds can elicit behavioural responses, blends of compounds as well as the ratios between compounds have been shown to affect aphid behaviour (Campbell et al., 1993; Bruce et al., 2005; Webster et al., 2010; Webster, 2012). Unfortunately alate *R. padi* behavioural towards ratio specific blends of physiologically acrtive compounds were not investigated due to time constraints.

Octanal is a green leaf volatile that has been shown to be present in Sorghum (*Sorghum bicolor* (L.)), *Z. mays* (L.) (Birkett et al., 2006), *H. vulgare* (L.) (Dong et al., 2015), *V. faba* (L.) (Webster, Bruce, Dufour, et al., 2008) and *T. aestivum* both un-infested and infested with *S. avenae* and *R. padi* (Quiroz et al., 1997; Liu et al., 2009) and is physiologically active in *A. fabae* (Webster, Bruce, Dufour, et al., 2008). Octanal was also physiologically active to alate *R. padi* in samples from infested MDR037 (125pg/µl) and un-infested MDR049 (40pg/µl) in the present study. There was more octanal present in the volatile profile of infested MDR037 than un-infested MDR049. The amount of octanal has been shown to be cultivar dependant in *H. vulgare* (Dong et al., 2015) and may be the case here also, although the cultivar difference

cannot be separated from the *R. padi* infestation treatment, as octanal was not physiologically active in the semiochemical profile of infested MDR037 or un-infested MDR049, but was only tentatively identified in the volatile profiles of these plants.

In the present study many compounds were present in volatile profiles of plants that were not detected by aphid antennae. This could be due to the amount of compound present, as the intensity of *B. fusca* and *C. partellus* antennae EAG responses have been shown to be dose dependant (Birkett et al., 2006). This is important as if there is a small response from *R. padi* antenna, it could be undetected in a noisy background signal (Figure 2.3). Interestingly linalool was mostly identified in infested plants but was only identified as physiologically active in infested MDR037 volatile profile. Linalool has also be associated with fungal and insect presence on plants (Maes and Debergh, 2003; Piesik et al., 2007), so this compound may be produced in response to general biotic stress.

The physiologically active compounds tentatively identified in un-infested Solstice (decanal heptanal, ethylbenzene, octanal, 4,4-dimethyl-2-pentene), MDR037 (decanal heptanal and octanal) MDR045 (decanal, heptanal, linalool, octanal, 4-ethylbenzaldehyde, 2,4-dimethyl-2-pentene and naphthalene), MDR049 (decanal, linalool, octanal, 4-ethylbenzaldehyde, 2,4-dimethyl-1-pentene, 4,4-dimethyl-2-pentene and naphthalene) and infested Solstice ((heptanal, ethylbenzene, linalool, octanal, 4-ethylbenzaldehyde, 2,4-dimethyl-1-pentene, 4-ethylacetophenone and naphthalene), MDR037 (decanal, ethylbenzene, linalool and octanal), MDR045 (decanal, heptanal, ethylbenzene, linalool, octanal, 4-ethylbenzaldehyde, 2,4-dimethyl-1-pentene and naphthalene) and MDR049 (decanal, heptanal, linalool, octanal, 4-ethylacetophenone and naphthalene) were different at a crop species and genotype level.

Tentatively identified physiologically active compounds that Solstice emitted was distinct those emitted from the *T. monococcum* genotypes. Although the total number and total peak area of un-infested Solstice and MDR037 volatile profiles were similar, there were qualitative differences in the tentatively identified physiologically active compounds produced. Despite these differences, alate *R. padi* were attracted to both un-infested Solstice and MDR037 volatiles. Alate *R. padi* also showed the same behaviour towards infested and un-infested MDR049 (repelled), although different physiologically active compounds were emitted. This indicates that different compounds, can elicit the same behavioural response from alate *R. padi*. It is unknown, however, whether these changes would also cause different behaviours in other cereal aphid species or in apterous *R. padi*, indeed it has been shown that different aphid species and morphs have different behaviours towards VOCs (Visser and Yan, 1995; Visser and Piron, 1997; Quiroz and Niemeyer, 1998)

There were large qualitative differences in the tentatively identified compounds from Solstice un-infested and infested volatiles where alate aphids were attracted to un-infested volatiles but had no behavioural response to infested volatiles. The qualitative differences could explain why the behavioural differences occurred. To support this, there was little qualitative difference in the compounds emitted from un-infested and infested MDR045 and there was no difference in *R. padi* behaviour to these volatiles. The quantities of all of these compounds are unknown, but it can be speculated that the qualitative and quantitative changes between un-infested and infested Solstice and MDR037 caused the observed change in aphid behaviour from attraction (to un-infested plants) to no behavioural response (to infested plants).

The confirmed and tentatively identified compounds from un-infested and infested MDR045 volatile profiles were similar to those from un-infested MDR049 but compounds in infested MDR049 volatile profiles were similar to those from infested MDR037.

Interestingly, alate *R. padi* were repelled by un-infested and infested MDR049 volatiles but showed neutral or no behavioural response to un-infested MDR045 and infested MDR045 and MDR037. Although there were qualitative similarities in tentatively identified compounds that elicited physiological responses, the quantity of compounds and ratio can also have an effect on aphid behaviour (Webster et al., 2010; Schröder et al., 2015), and as the compounds were only identified tentatively, it is unknown whether the amounts of physiologically active compounds differed between MDR045 and un-infested MDR049, an infested MDR037 and MDR049. These would need to be quantified and tested in behavioural bioassays.

2.4.1 Conclusion

The studies presented in this chapter have identified antixenosis to *R. padi* in *T. monococcum* genotype MDR049. Additionally, alate *R. padi* show different behavioural responses to volatiles from un-infested and infested susceptible genotypes, with aphids only showing attraction to volatiles un-infested susceptible plants. These differences in behaviour are likely because of qualitative differences between plant allelochemical profiles, compound concentrations and ratio between compounds.

CHAPTER 3

Temporal changes in post-alighting resistance to *Rhopalosiphum padi* (bird cherry-oat aphid) in ancestral wheats.

3.1 INTRODUCTION

Plants have limited light, water and nutrient resources for development, growth and reproduction. Herbivory can negatively affect this and even lead to plant death, some plants use defence mechanisms, also called resistance traits, to limit or prevent attack from herbivores (Agrawal, 2007; Czesak et al., 2008; Rasmann and Agrawal, 2009).

Plants with post-alighting resistance (antibiosis) to insects use direct defence mechanisms, either reducing the ability of the insect to access the food source, reducing the quality of the food, or making the food source toxic by the production of phytochemicals. This leads to a decrease in insect development and/or reproduction (Painter, 1951). These biochemical and/or biophysical mechanisms use resources that could otherwise be used for plant development and reproduction creating a trade-off between plant fitness and defence (Coley et al., 1985). As herbivory is not a constant threat to plants, continuous diversion of resources away from growth and reproduction to defence would be costly. Optimal defence theory (ODT) predicts the allocation of chemical defence against insects, within the context of plant fitness. It is assumed that changes in resource allocation will be determined by the rate of herbivorous attack in the absence of defence (Tiffin, 2002; Boege and Marquis, 2005), the importance of the organ under attack for development and reproduction (Boege and Marquis, 2005) and the cost of the defence allocation (Koricheva, 2002). ODT predicts that plant growth stage and genotype can affect resistance strategies (Koricheva, 2002; Tiffin, 2002; Farnsworth, 2004; Boege and Marquis, 2005). The ODT theorises that younger plants

will have better anti-herbivory defence (Boege and Marquis, 2005) as seedlings need more protection (Nicole M. van Dam et al., 1995; Iwasa et al., 1996), it has been shown that even younger leaves on the same gypsy flower (*Cynoglossum officinale* (L.)) plant have higher levels of defence compounds and lower herbivory (van Dam et al., 1996).

A contrasting theory is that herbivory may occur more on seedlings than adult plants (Fenner et al., 1999), because of the phenomenon of Adult Plant Resistance, which has been observed in plant-pathogens interactions (Dyck, 1979; Dyck and Samborski, 1979). These opposing theories show that whether younger or older plants have increased anti-herbivory defence, may be species dependent.

The ODT can also be applied to resistant plants, as resistance relies on plant defence mechanisms, the same trade-offs between resource allocation for plant growth and reproduction and defence mechanisms will still apply. The ODT theorises that younger leaves have more defence than older leaves has been shown in daisy (*Senecio*) hybrids resistant to Western flower thrips (*Frankliniella occidentalis* (P.)), where younger leaves had higher concentrations of pyrrolizidine alkaloids (PAs), which reduced *F. occidentalis* survival (Leiss et al., 2009). Similarly, ancestral einkorn wheat (*Triticum monococcum* (L.)) genotypes TM44 and TM46 only conferred antibiosis to *Sitobion avenae* (F.) at seedling stage and not as adult plants (di Pietro et al., 1998; Migui and Lamb, 2004). Other *T. monococcum* genotypes, MDR045 and MDR049, have also been shown to have antibiosis to *Rhopalosiphum padi* (L.) as seedlings (Greenslade et al., 2016; Aradottir pers comms.), but in this case, it is not known if the antibiosis is present at other growth stages.

In this chapter I determine whether *T. moncoocccum* MDR045 and MDR049 antibiosis resistance to *R. padi* is dependent on plant growth stage, by investigating *R. padi* development, reproductive success and survival on the susceptible wheat (*Triticum aestivum*

(L.)) Solstice, *T. monococcum* MDR037 and the antibiosis *T. moncoccum* MDR045 and MDR049 at four plant growth stages; 9, 11-12, 37-41 and 51-55 (Zadoks et al., 1974)

3.1.1 Chapter hytpotheses

This chapter follows up on the preliminary data on *T. monococcum* MDR045 and MDR049 and tests the following hypotheses:

1) Plant growth stage affects *Rhopalosiphum padi* development, reproductive success and survival on *Triticum aestivum* Solstice and *Triticum monococcum* MDR037, MDR045 and MDR049.
2) The level of antibiosis towards *R. padi* will change with increased plant maturity.

3.2 METHODS

3.2.1 Plant growth

Triticum aestivum Solstice and *Triticum monococcum* MDR037, MDR045 and MDR049 were grown to growth stage (GS) 11 and 12-13 in standard Rothamsted soil (Section 2.2.2, 2.2.3) in 2cm diameter seed trays in a controlled environment room at 20^{o}C (± 1^{o}C),16:8 h light: dark photoperiod with 200$_{\mu mol.m^{2}.sec^{-1}}$ light intensity. Plants for testing at GS 37-41 and GS 51-55- were grown in soil in seed trays in the controlled environment for seven days, and then vernalised at -5^{o}C (± 1^{o}C), temperature for six weeks. After vernalisation, plants were transferred to 12.5cm diameter pots and grown in a glass house until the start of experimentation when plants were moved to a controlled environment room at 20^{o}C (± 1^{o}C), 200$_{(\mu mol.m^{2}_sec^{-1})}$ light for 16:8 h light: dark photoperiod. Seedlings were watered with approximately 8ml and older plants approximately 15ml daily.

3.2.2 Clip cages

Each clip cage (MacGillivray and Anderson, 1957) was made by gluing a 2.5cm foam disc to a 2cm diameter Perspex tubing of approximately 2cm height. A mesh was glued to the other side of the Perspex tube creating one side of the clip cage. The process was repeated for the other side of the clip cage. The two parts of the clip cage were connected by a metal hair clip, the ends of the clip were heated using a Bunsen burner and slowly pushed into the sides of each of the Perspex tubes.

(a)

(b)

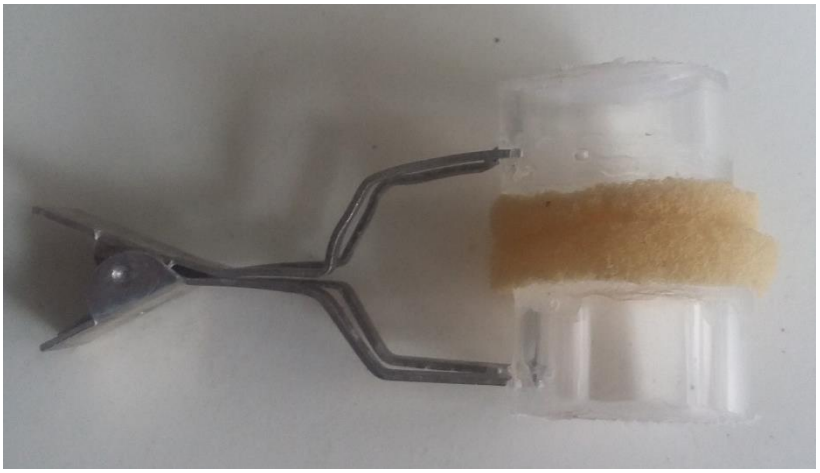


Figure 3.1. An assembled clip cage from **a)** above the clip cage, showing the mesh, 2.5cm diameter foam and metal hair clip **b)** the side of the clip cage showing the 2.5cm diameter foam, 2cm diameter perspex tubing and metal hair clip.

3.2.3 Experimental design

For seedling (GS 11 and GS 12-13) experiments, seed trays of 8 × 5, 2cm diameter cells were used, where every other row of cells had been covered with black pvc tape for the clip cage on the plant to rest on (Fig 3.2). For GS 37-41 and 51-55 plants, each pot was in an individual tray for watering. In each experiment, ten replicates of each wheat genotype were arranged in a randomised experimental design.

Figure 3.2. Image of experimental design of plants in clip cages resting on a pvc strip on a seed tray.

3.2.4 Assessment of aphid development, survival, reproductive success and life length

Bioassays for development, survival and reproductive success were carried out on all four wheat genotypes at the four different plant growth stages; 11, 12-13, 37-41 and 51-55. Experiments on each were done independently and in sequence due to limited space, however all experiments were carried out in the same controlled environment room.

Three to five mature alate aphids were placed on each plant in a 2cm clip cage and allowed to oviposit overnight. The following morning the mature aphids were removed with a fine-haired paintbrush and the neonate nymphs (<1 day old) were counted and weighed in Eppendorf tubes using a Microbalance (Cahn 33; Scientific and Medical Products Ltd, Manchester, UK). The average nymph weight was determined by:

$$\text{average nymph weight} = \frac{\text{Total nymph weight}}{\text{Number of nymphs}}$$

After weighing, nymphs were transferred back to their respective plant using a fine-haired paintbrush and left undisturbed for six days. After six days, the number of survivors was recorded, and they were re-weighed to determine the mean Relative Growth Rate (mRGR). This was calculated (Radford, 1967; Leather & Dixon, 1984) as:

$$\text{mRGR} = \left(\frac{\ln(\text{average seven day weight}) - \ln(\text{average birth weight})}{6}\right)$$

The % survival after six days was calculated as:

$$\%\text{survival} = \left(\frac{\text{number of nymphs at six days}}{\text{number of nymphs at birth}}\right) \times 100$$

After weighing, one of the nymphs, chosen at random, was transferred back to their original plant, left undisturbed to develop and monitored each day; all aphids developed into adult apterous aphids. The time taken to produce their first nymph and the number of nymphs they produced over their lifetime were recorded. Nymphs were removed to prevent overcrowding.

3.2.5 Statistical analysis

The differences in aphid mRGR, the percentage survival after six days, the number of days to produce a first nymph and the number of nymphs produced were subjected to a two-factor analysis of variance (ANOVA) with Tukey's HSD post hoc in which the plant growth stage and the plant genotype were factors, interactions between factors was also included in analysis. Data were subjected to transformation to comply with assumptions of normality and equal variance. The number of days to produce the first nymph was square root transformed, the number of nymphs log transformed and percentage survival logit transformed before statistical analysis. Confidence level of $P<0.05$ was considered statistically significant. GenStat® (2016, 18th Edition, © VSN International Ltd, Hemel Hempstead, UK) statistical software was used for the analyses. It was not possible to achieve normality for the parameter of 'length of life', hence Friedman's non-parametric 2-way ANOVA was used for the analysis, in which plant growth stage and the plant genotype were factors, interactions between factors was not included in the analysis. RStudio: Integrated Development Environment for R (2016, Edition 1.1.453, ©RStudio Inc, Boston, Massachusetts, USA) software was used for the analysis.

3.3 RESULTS

3.3.1 Aphid development

Overall, plant growth stage alone had no effect on mean relative growth rate (mRGR) of the aphids. However, the genotype of the wheat on which the aphids were feeding affected mRGR ($F_{3,134} = 2.93$, $P = 0.049$), with the aphids on MDR049 having the lowest mRGR.

This was true at all wheat stages and there was no interaction between growth stage and genotype. (Fig. 3.3).

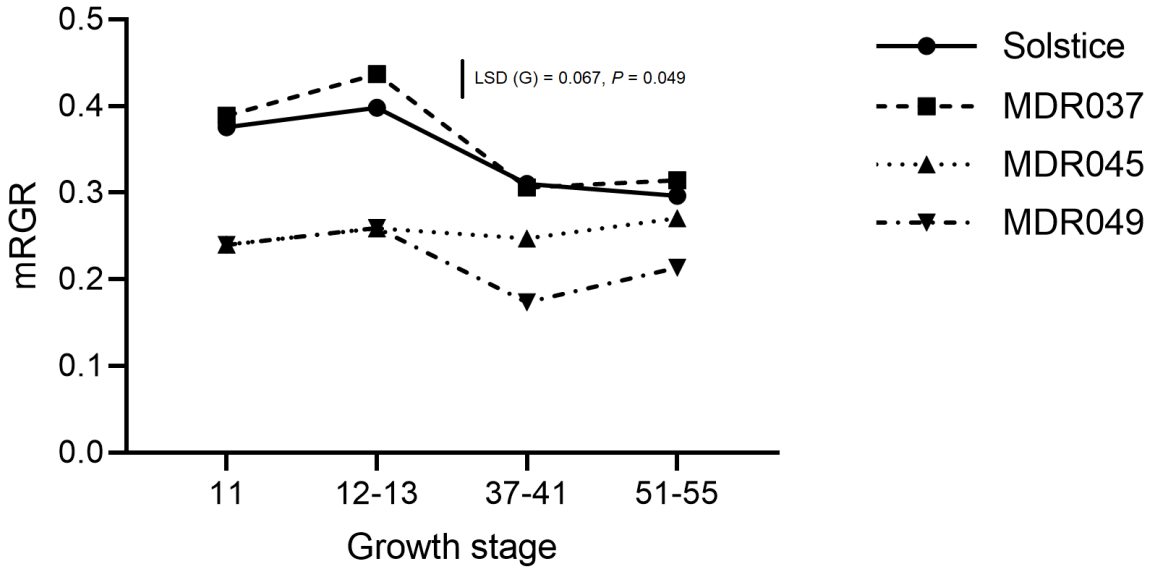


Figure 3.3. Mean Relative Growth Rate (mRGR) of *Rhopalosiphum padi* on *Triticum monococcum* MDR037, MDR045 and MDR049 and *Triticum aestivum* Solstice at four growth stages; initial growth stages were 11, 12-13, 37-41 and 51-55. The least significant difference G represents the genotype.

Plant growth stage did affect aphid survival rate ($F_{3,134}$ = 2.67, P = 0.044), but genotype alone had no effect. There was an interaction between genotype and plant growth stage ($F_{9,134}$ = 1.98, P = 0.047) (Fig. 3.4). Growth stage only affected aphids on MDR045 and MDR049, where aphids on 12 week-old plants had lowest survival rate (Fig 3.4.)

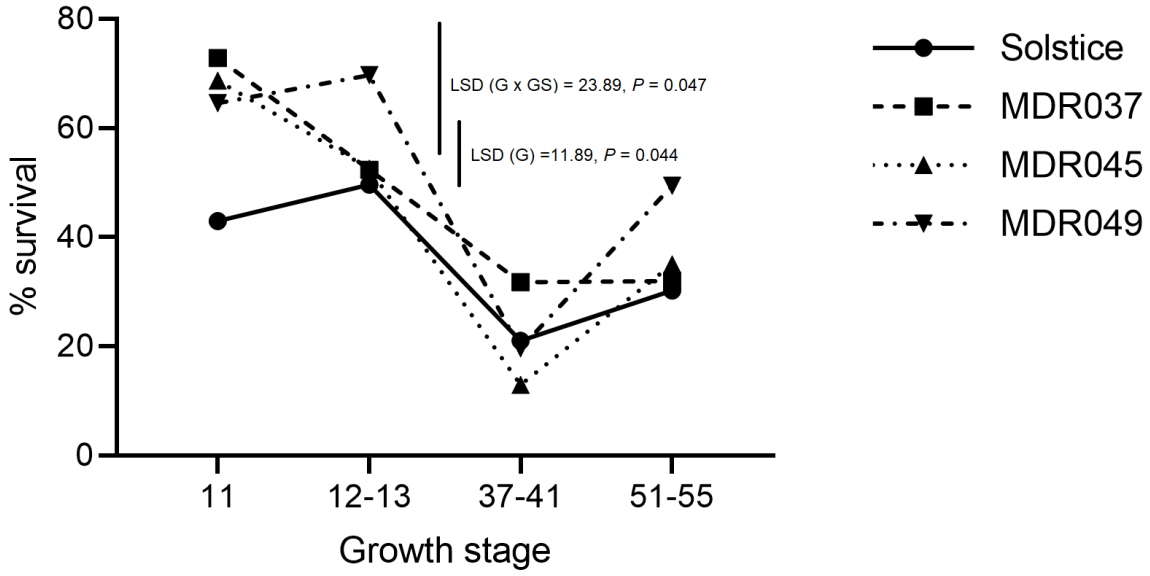


Figure 3.4. Percentage survival of *Rhopalosiphum padi* on *Triticum monococcum* MDR037, MDR045 and MDR049 and *Triticum aestivum* Solstice at four growth stages; initial growth stages were 11, 12-13, 37-41 and 51-55. The least significant difference G represents the genotype, GS represents the growth stage.

3.3.2 Aphid reproductive success

Aphids on older (GS 37-41 and GS 51-55) plants took longer to produce their first nymph ($F_{3,121}$ = 39.97, P < 0.001), but wheat genotype had no effect. However, there was an interaction between plant growth stage and genotype ($F_{9,121}$ = 2.32, P = 0.020) with aphids on GS 11 MDR049 and GS 51-55 MDR045 taking longer to produce their first nymph than those on their respective susceptible Solstice plants (Fig 3.5.).

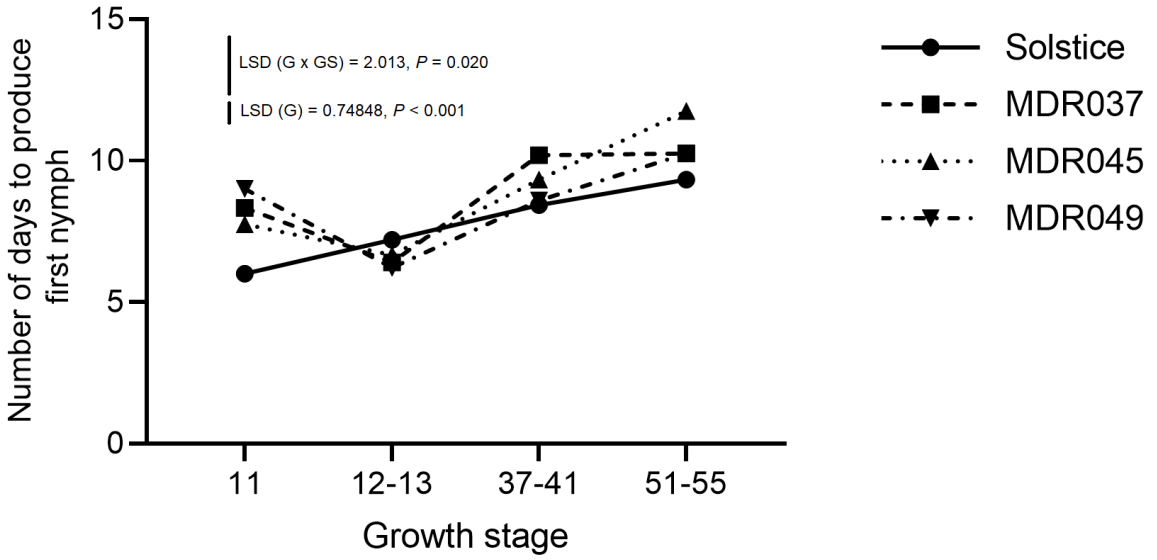


Figure 3.5. Average number of days *Rhopalosiphum padi* took to produce their first nymph on *Triticum monococcum* MDR037, MDR045 and MDR049 and *Triticum aestivum* Solstice at four growth stages; initial growth stages were 11, 12-13, 37-41 and 51-55. The least significant difference G represents the genotype, GS represents the growth stage.

Nymph production was dependant on the plant growth stage ($F_{3,112}$ = 17.15, P < 0.001), with aphids producing fewer nymphs on GS 37-41 and GS 51-55 plants. Plant genotype also affected nymph production ($F_{3,112}$ = 21.30, P < 0.001) with aphids on MDR045 and MDR049 producing fewer nymphs. There was no interaction between plant growth stage and genotype (Fig 3.6.).

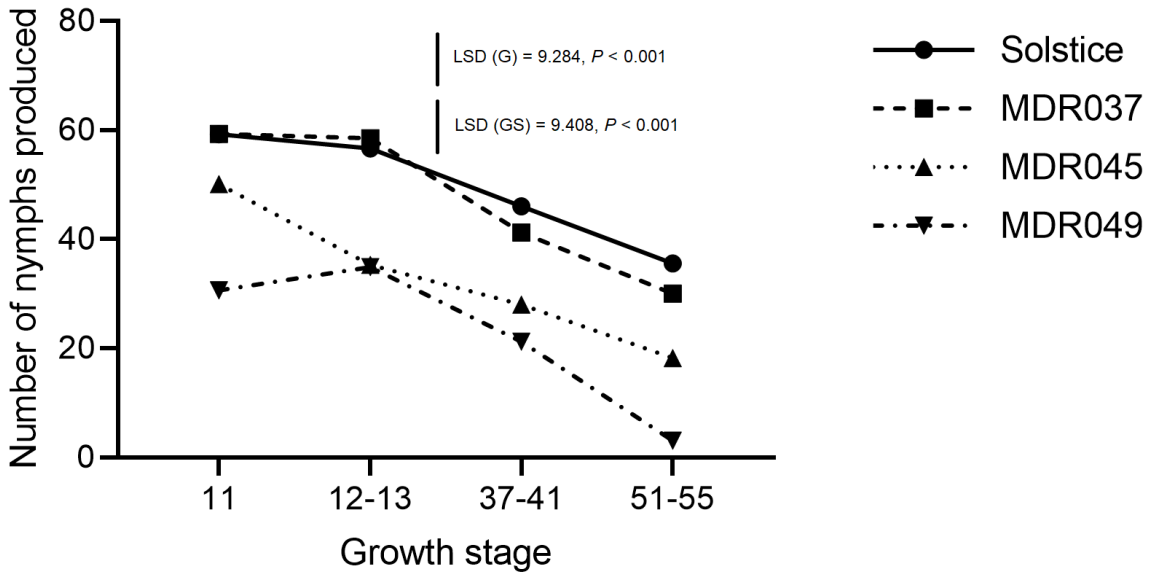


Figure 3.6. Number of nymphs *Rhopalosiphum padi* produced on *Triticum monococcum* MDR037, MDR045 and MDR049 and *Triticum aestivum* Solstice at four growth stages; initial growth stages were 11, 12-13, 37-41 and 51-55. The least significant difference G represents the genotype, GS represents the growth stage.

3.3.3 Aphid life length

Plant growth stage had no effect on life length, but genotype did ($F_{3,134}$ = 7.88, P = 0.040) with aphids on MDR049 dying earlier than those on susceptible plants. (Fig 3.7.).

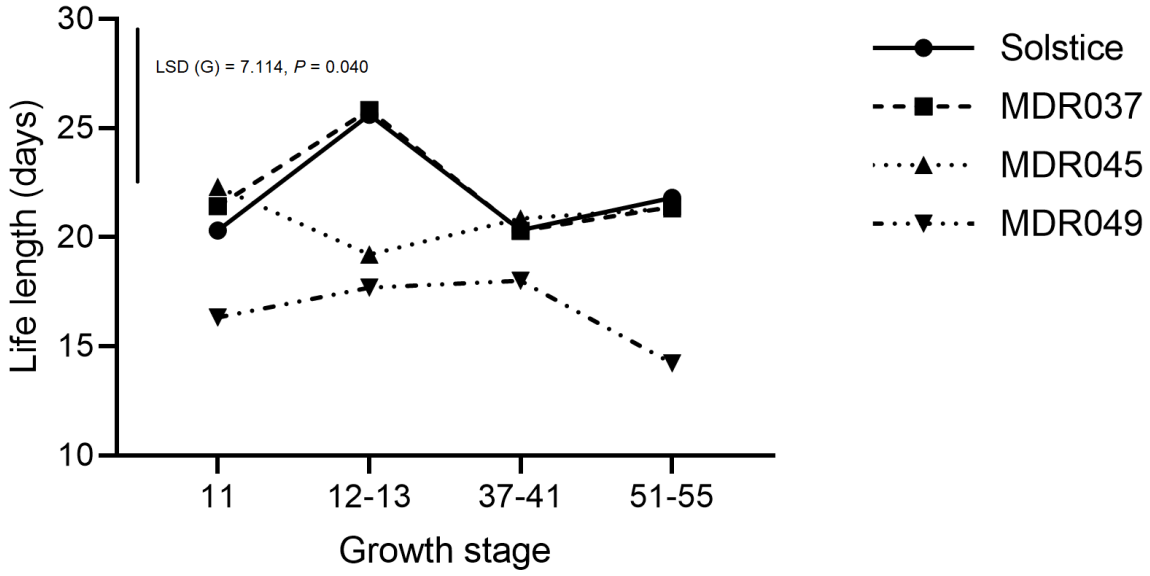


Figure 3.7. Average number of days till death of *Rhopalosiphum padi* on *Triticum monococcum* MDR037, MDR045 and MDR049 and *Triticum aestivum* Solstice at four growth stages; initial growth stages were 11, 12-13, 37-41 and 51-55. The least significant difference G represents the genotype.

3.4 DISCUSSION

The Optimal Defence Theory (ODT) assumes that defence is costly (McCall and Fordyce, 2010) and so allocation to defence will only occur when there is a low cost: benefit ratio (Stamp, 2003; McCall and Fordyce, 2010). The more important a tissue is for the fitness of the plant, the higher the concentrations of defence compounds are likely to be (Boege and Marquis, 2005). It has been suggested that younger leaves have higher defences than older leaves (Nicole M van Dam et al., 1995) because of their importance to the plant's establishment (Iwasa et al., 1996) and its higher photosynthetic ability (Wiedemuth et al., 2005). This is contradicted by my findings, where across all genotypes; *R. padi* took less time to produce their first nymphs and produced more nymphs on seedling wheat plants. It has been shown that *R. padi* is associated with feeding on cereal crop seedlings during warm

autumns leading to BYDV transmission (Riedell et al., 1997; Fabre et al., 2003), which could mean that they are more adapted to feeding on seedlings than on older plants.

It has previously been shown that certain varieties of *T. monococcum* seedlings have resistance to *R. padi* (Greenslade et al., 2016) and *S. avenae* (di Pietro et al., 1998; Migui and Lamb, 2004; Simon et al., 2017), however, post-alighting resistance in adult *T. monococcum* has only been studied towards *S. avenae* (Migui and Lamb, 2003, 2004) . Here I have shown that aphids on *T. monococcum* MDR049 have reduced mRGR and produce fewer nymphs than those on the susceptible Solstice and/or *T. monococcum* MDR037, and reduced life span was observed at all plant growth stage (bar GS 11 plants), showing that resistance is stable at later growth stages. This contradicts the study by Migui and Lamb, (2003) study, where no resistance to *R. padi* was observed in adult *T. monococcum*, however they only analysed the increase in aphid biomass. This emphasises the need for in depth investigations of both aphid development and reproductive success for each genotype, when exploring the interaction between plant resistance and aphids.

Antibiosis in MDR049 seedlings has been linked with the inability of aphids to carry out sustained feeding (Greenslade et al., 2016; Simon et al., 2017), and smaller vascular bundles (Simon et al., 2017), however, it remains to be tested whether this effect on feeding behaviour is consistent at different plant growth stages.

It has been shown for a number of plant species that they become more susceptible to herbivores with age as levels of defence compounds go down (Iwasa et al., 1996; McCall and Fordyce, 2010). Higher levels of defence compounds have been found in seedlings than adult plants in ribwort plantain (*Plantago lanceolata* (L.)) (Deane Bowers and Stamp, 1993), *C. officinale* (L.) (Nicole M. van Dam et al., 1995) and diesel tree (*Copaifera langsdorfii*

(L.)) (Macedo and Langenheim, 1989). The work in this chapter shows that *R. padi* on MDR045 antibiosis to *R. padi* is affected by plant growth stage, with no resistance present in GS 51-55 plants across all parameters on this genotype. This is in line with other examples of decreased herbivory on seedlings, such as moth (*Stenoma assignata* (M.)) larvae on *C. langsdorfii* (Macedo and Langenheim, 1989), and generalist slug (*Deroceras reticulatum* (M.)) on prostrate knotweed (*Polygonum aviculare* (L.)), acyanogeic white clover (*Trifolium repens* (L.)) and lettuce (*Lactuca sativa* (L.)) (Fenner et al., 1999).

On the other hand, in some species, adult plants are known to have higher defence compounds (Boege, 2005) with herbivores preferring to feed on and performing better on seedlings , including the grey garden slug (*Deroceras reticulatum* (M.)) on corn daisy (*Glebionis segeutum* (L.)), stinking willie (*Jacobaea vulgaris* (L.)), red clover (*Trifolium pratense* (L.)), chickweed (*Stellaria media* (L.)), catgrass (*Dactylis glomerata* (L.)) and poppy (*Papaver rhoeas* (L.) (Fenner et al., 1999). Additionally, Geometridae chewing insects were recorded at lower densities on adults of the tree species *Casearia nitida* (L.) (Boege, 2005). This shows that defence allocation can be dependent on growth stage , herbivory and plant genotype, with variations between plant species (Koricheva, 2002; Tiffin, 2002; Farnsworth, 2004; Boege and Marquis, 2005). In this work, results have shown that MDR049 has consistent pattern of resistance across the different plant growth stages, with some parameters showing an *R. padi* performing better on seedlings than older plants.

The affect of aphid infestation on wheat has been shown to be growth stage dependant, Wratten and Watt, (1984) have shown that *M. dirhodum* on GS40-69 wheat (Cultivar Bounty) reduced yield more than those on GS 65-73, however it is unknown whether this reduced affect is genotype dependant. The differences, between how MDR045 and MDR049 antibiosis was affected by plant growth stage is most apparent for nymph production. Antibiosis resistance affecting *R. padi* nymph production in MDR045 decreased with plant

maturity, whereas on MDR049, it increased with plant maturity, with increased significance between susceptible lines and MDR049 in GS 37-41 and GS 51-55 plants. Similarly, (Migui and Lamb (2004)) showed that *T. monococcum* TM44 and TM46 seedlings had antibiosis to *S. aveane* but only aphids on TM46 adult plants had reduced aphid development.

The increased resistance with plant maturity observed with MDR049 may be due to a form of Adult Plant Resistance (APR) (Dyck, 1979; Dyck and Samborski, 1979), as reported in response to pathogen infections. For example, the APR gene *Lr34/Yr18* expressed in adult *T. aestivum* confers resistance to stem rust in PI250413 (Dyck and Samborski, 1979) and to stripe rust in RL6058, RL6077 and Line902 (Singh, 1992). The effect of the APR gene alone can vary, depending on the level of infection (Ellis et al., 2014; Singh et al., 2014) but combining various APR genes can cause high levels of resistance to rust (Singh et al., 2014). Single APR genes can also interact with major resistance (*R*) gene mediated defences, increasing the phenotype associated with *R* genes and resulting in lower infection of the pathogen (Ellis et al., 2014; Singh et al., 2014). Little is known about APR towards insects, but my work suggests that resistance in MDR049, which is independent of the plant growth stage, may be due to the presence of *R* gene(s) providing resistance from seedling to adult plant, but in the adult plant the resistance is increased by the expression of APR gene(s).

3.4.1 Conclusion

The presence and level of resistance in *T. monococcum* MDR045 and MDR049 is dependent on the plant growth stage and genotype and resistance that reduces nymph production decreases with plant matuirty in MDR045 but increases in older plants in MDR049. This highlights the need to consider the variability of genotype responses over different growth

stages as well as examining both aphid development and reproductive success when investigating resistance to insect pest species.

Chapter 4

Identifying genes that underpin resistance responses to *Rhopalosiphum padi* infestation

4.1 INTRODUCTION

Plants have various defence mechanisms against insect feeding and damage. Biochemical traits such as the production of semiochemicals and toxins (Kogan and Ortman, 1978; Bi and Felton, 1995), and biophysical traits such as colour, increased trichome density and waxy surfaces can prevent damage from insect herbivory (Bahlmann et al., 2003; Wojcicka, 2013; Schröder et al., 2014). These traits are governed by changes in gene expression. Through gene expression, different cells use their DNA to create different RNAs and proteins, which underpin cellular function (Yamaguchi-Shinozaki and Shinozaki, 2006). Some of the proteins will be involved in the production of toxins and semiochemicals and hence affect the phenotype of the plant towards herbivores (Kosma et al., 2010).

Gene expression is not fixed and can change due to internal factors such as plant age, or external factors, including biotic and abiotic stress (Nejat and Mantri, 2017). Interactions with stressors trigger defence responses on a molecular and cellular level which can alter biochemical and biophysical traits.

During stylet penetration on host plants, aphid stylets secrete gelling saliva to aid movement intercellularly through the plant tissue to reach the phloem, whilst doing so, it occasionally disrupts a cell wall and secretes watery saliva into cells (Miles, 1999). The watery saliva may contain herbivore associated molecular patterns (HAMP). Pattern Recognition Receptors (PRR) use HAMPs to catalyse defence mechanisms in herbivore-plant interactions, this is called HAMP triggered immunity (HTI) (Hogenhout and Bos, 2011). This

form of immunity is analogous to pathogen associated molecular pattern (PAMP) triggered immunity (PTI) (Prince et al., 2014). Pattern recognition receptors can also recognise damage associated molecular patterns (DAMP) such as cell wall and intracellular fragments, and microbe associated molecular patterns (MAMP) such as the chaperonin GroEL from *M. persicae* symbiont *Buchnera aphidicola* (Chaudhary et al., 2014) to initiate HTI.

Herbivore associated molecular pattern recognition by PRRs causes the phosphorylation of respiratory burst oxidase-D (RBOHD), and activation of NADPH oxidase which produces reactive oxygen species (ROS) (G. Miller et al., 2009). These ROS trigger the phosphorylation of mitogen-activated protein kinase (MAPK) and activate phytohormone signalling pathways; jasmonic acid (JA), salicyclic acid (SA), and ethylene (ET) (Dodds and Rathjen, 2010; Hogenhout and Bos, 2011; Nejat and Mantri, 2017). Mitogen-activated protein kinase transfers these biotic stress signals to the nucleus leading to the activation of transcription factors that determine gene expression (Dodds and Rathjen, 2010; Nejat and Mantri, 2017). The production of ROS, phytohormone signalling and changes in gene expression lead to biochemical and biophysical defence responses. For example, extracts of *M. persicae* injected into *A. thaliana* increased callose deposition (Prince et al., 2014). Callose is a polysaccharide that forms occlusions in phloem sieve tube pores to prevent sap leaving the phloem, preventing aphid feeding (Will and Van Bel, 2006; Will et al., 2013; Sun et al., 2018). Increased callose deposits were also observed in SA-treated *A. thaliana* (Yi et al., 2014), which shows the involvement of phytohormones in HTI response to aphids. Another study showed that the salivary enzyme, pectinase from *S. avenae* on *T. aestivum* triggered HTI, eliciting sulcatol and sulcatone semiochemical production (Liu et al., 2009). *H. vulgare* treated with sulcatol, sulcatone and 2-tridecanone had reduced *R. padi* populations (Ninkovic et al., 2003).

Aphid saliva can contain HAMPs that trigger HTI, however, this saliva can also contain effector proteins. Effector proteins are small cystine rich proteins that prevent host detection of the invading organism, suppress the hosts immune system or aid the invading organisms' survival in the host (Rodriguez and Bos, 2012). The *M. persicae* effector protein *Mp55* expressed in *A. thaliana* reduced ROS hydrogen peroxide (H_2O_2) accumulation and aphids with *Mp55* produced more offspring (Elzinga et al., 2014). In susceptible plants, effector protein secretion allows aphids to carry out sustained feeding, silencing the salivary protein C002 in *Acyrthosiphon pisum* (H.) (pea aphid) caused a dramatic reduction in aphid feeding on broad bean (*Vicia faba* (L.)) plants (Mutti et al., 2008). The effector C002 has also been shown improve *A. pisum* survival (Mutti et al., 2006) and *M. persicae* reproduction (Bos et al., 2010).

In resistant plants, the secreted effectors are recognised by the presence of a nucleotide binding site (NBS) with leucine rich repeat (LRR) domains (NBS– LRR) in Resistance (R) receptor proteins, this recognition initiates defence responses. This is called effector triggered immunity (ETI). The *R* genes that encode NBS-LRR R proteins have been characterised for some aphid-plant interactions. The *R* gene *Mi-1.2* in *Solanum lycopersicum* (L.) (tomato), confers resistance to some *Macrosiphum euphorbiae* (T.) (potato aphid) clones, aphids on *Mi-1.2* transgenic lines had reduced fecundity compared to those feeding on a resistant variety (Rossi et al., 1998) which contained a gene tightly linked to *Mi-1.2.* Another *R* gene, *Vat* confers resistance to a biotype of *Aphis gossypii* (G.) (melon-cotton aphid) by causing a mechanical "block" in the aphid stylet, preventing feeding and the transmission of the cucumber mosaic virus (CMV) (Martin et al., 2003).

Triticum monococcum genotype MDR049 confers post-alighting resistance (antibiosis) to *R. padi* and *S. avenae,* whilst the MDR037 genotype is susceptible to these aphid species (Greenslade et al., 2016; Simon et al., 2017; Chapter 3). This resistance in MDR049 has been associated with reduced aphid feeding (Greenslade et al., 2016; Simon et al., 2017) which indicates that triggered immunity plays a role in this antibiosis. No *R* gene has been discovered in *T. monococcum* so it is unknown whether this is an HTI or ETI response that prevents aphid feeding. It is also unknown whether susceptibility in MDR037 is due to a lack of HTI or if this defence response is overcome by the secretion of *R. padi* effector proteins. This chapter reports studies to identify genes which are differentially expressed in *T. monococcum* MDR037 and MDR049. This used plants with either no *R. padi* infestation or plants with *R. padi* infestation sampled at three timepoints post-infestation (3h, 6h, 24h). The aim was to identify genes involved in defence mechanisms in susceptible and resistant *T. monococcum*.

4.1.1 Chapter hypotheses

This chapter addresses the following hypotheses:

1) Gene expression will differ between *Triticum monococcum* genotypes MDR037 and MDR049.
2) Gene expression will change in response to *Rhopalosiphum padi* feeding in both *T. monococcum* genotypes and these changes will be dependent on the time post *R. padi* infestation
3) Genes involved in defence responses will be expressed more in infested plants than in un-infested plants of both genotypes

4.2 METHODS

4.2.1 Experimental design

Triticum monococcum MDR037 and MDR049 were grown (section 2.2.2, 2.2.3) in a randomised block design to growth stage (GS) 12. Aphid infestation treatments (control, 3hours (h), 6h, 24h post infestation) were assigned randomly using GenStat® (2016, 18th Edition, © VSN International Ltd, Hemel Hempstead, UK) statistical software. Aphids were introduced onto plants by placing five aphids onto the first leaf within a clip cage (Section 3.2.2). Control plants were set-up in the same way, without aphids inside the clip cages. There were four replicates for each genotype and treatment.

4.2.2 Sample collection

After the allocated time post infestation, aphids were removed from the plant and the plant tissue that was within the clip cage was excised using sterile scissors. Excised plant tissue was placed into a frozen 2ml microcentrifuge tube with sterile 2mm ball bearings and snap frozen in liquid nitrogen. Samples were stored at -80^{o}C until RNA extraction.

4.2.3 RNA extraction and sequencing

RNA was prepared from frozen plant tissue using the Monarch® Total RNA Miniprep Kit protocol. Samples were ground into a fine powder using a genogrinder, all metal sample blocks were frozen prior to grinding to ensure samples remained frozen. Approximately 100mg of sample was use in each extraction. RNA protection reagent was added directly to the sample before further lysis by vortexing vigorously. Lysed tissue was centrifuged through a gDNA removal column which also removed cell debris. Ethanol (98%) was added to the supernatant before being centrifuged through an RNA purification column. RNA wash buffer

was centrifuged to remove salts before the optional DNase step was carried out on the column. The column was centrifuged with RNA priming and RNA wash buffers to remove contaminants. Total RNA was eluted with 50µl RNase free water and stored at -80^{o}C.

RNA quality was determined by gel electrophoresis on 1% agarose gel using TAE buffer (40 mM Tris-HCl, 1 mM EDTA) with 0.01% bleach to prevent RNA degradation (Aranda et al., 2012) for 50mins at 100V. For good quality RNA two separated bands, representing the 18S and 28S ribosomal RNA were observed under UV. RNA quantity was determined by nanodrop with the 230/280 and 260/280 ratio also indicating the quality of the prep.

Total RNA (500ng) was sent to Bristol Genomics Facility, Bristol, UK for library preparation and paired-end Illumina sequencing. High output paired-end transcriptome sequence of 75bp was carried out on the Illlumina NextSeq 500. Four technical replicates were done for each biological replicate.

4.2.4 RNA sequencing analysis

RNA sequences were analysed using the Galaxy web platform (version 19.05, https://usegalaxy.org/), RStudio: Integrated Development Environment for R (2016, Edition 1.1.453, ©RStudio Inc, Boston, Massachusetts,USA) and Blast2GO (version 5 PRO, BioBam Bioinformatics Solutions, Valencia, Spain) (Conesa et al., 2005).

For analysis, technical replicates for each biological replicate were combined and uploaded to Galaxy (Fig 4.1). In Galaxy, quality control was carried out using the FASTQC program, adaptors were trimmed and sequences under 20bp removed using the function Trimmomatic. The sequences were then subjected to quality control again and then aligned with the *Triticum urartu* genome, using the function HISAT2 which created a binary alignment map

(BAM) file for each biological replicate. The aligned sequences were assigned to genomic functions using the function FeatureCounts, which also estimated the read count of each of the genomic functions.

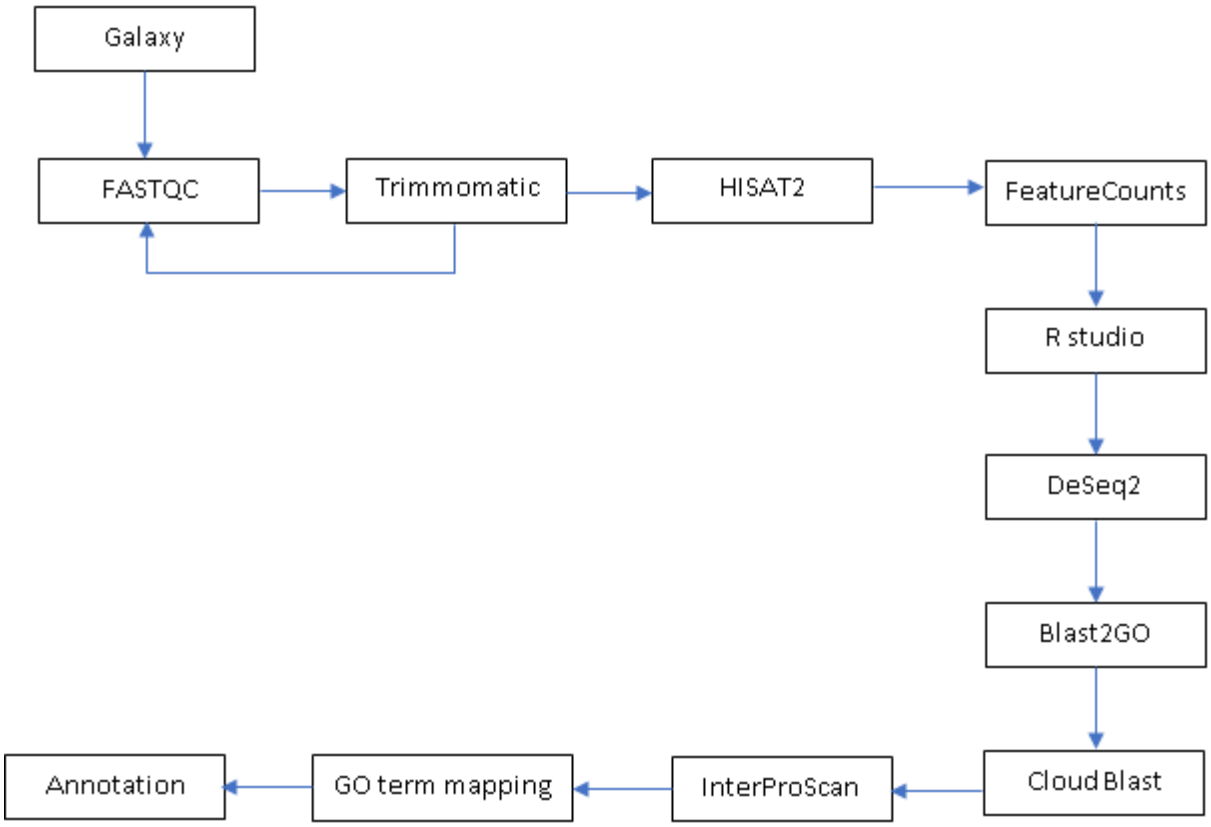


Figure 4.1 Schematic diagram of the analyses used to identify and characterise differentially expressed genes in *Triticum monococcum* MDR037 and MDR049 with and without *Rhopalosiphum padi* infestation.

FeatureCounts data was uploaded into RStudio, where differentially expressed genes were identified using the package Deseq2 (Fig 4.1). Annotations of sequenced samples and of the top 30 most significantly differentially expressed genes in response to *R. padi* infestation on MDR037 and on MDR049 was performed using Blast2GO (Fig 4.1) which assigns gene ontology (GO) annotation by BLAST against nucleotide and protein databases. Those that were assigned GO annotation terms were categorised by their proposed molecular function, cellular component and the biological process.

4.3 RESULTS

4.3.1 Mapping *Triticum monococccum* genes to *Triticum urartu*

Triticum monococcum genes were mapped to *Triticum urartu* genome because a well characterised genome for *T. monococcum* or *Triticum boeoticum* was not available. On average 64% of *T. monococcum* genes mapped to the *T. urartu* genome. The percentage of genes that mapped to the *Triticum urartu* genome ranged from 66% for MDR049 un-infested plants to 62% for MDR037 at 24 hours post aphid infestation none were significantly different to each other (Table 4.1).

Table 4.1. The average percentage of genes mapped to the *Triticum urartu* genome across four biological replicates of un-infested *T. monococcum* genotypes MDR049 and MDR037 and *R. padi* infested MDR037 and MDR049 at 3, 6 and 24 hours post infestation.

Genotype	**Hours post *Rhopalosiphum padi* infestation**	**Average % genes mapped to *Triticum urartu* genome**
MDR037	Un-infested (0hours)	64
MDR037	3	64
MDR037	6	64
MDR037	24	62
MDR049	Un-infested (0hours)	66
MDR049	3	64
MDR049	6	65
MDR049	24	63

4.3.2 Differential gene expression

A principle component analysis (PCA) was carried out to determine if gene expression was different between genotypes and treatments. This gave two distinct groups corresponding to the resistant (MDR049) and susceptible (MDR037) genotypes. Comparisons within the genotypes for different *R. padi* infestation timepoints show the same pattern for both genotypes, where expression 6 hours post infestation (hpi) was most similar to expression in control plants and expression in 3hpi plants was most similar to 24hpi plants. For both wheat genotypes, 24hpi gene expression was most dissimilar to the gene expression on control plants.

For MDR037 (on the right of the PCA fig 4.2) there was a clear separation between gene expression in control plants and plants at 3hpi, and a separation between gene expression in plants at 24hpi and plants at other timepoints (Figure 4.2). For MDR049 (on the left of the PCA fig 4.2) there was a clear separation between the gene expression in un-infested plants and plants at 24hpi.

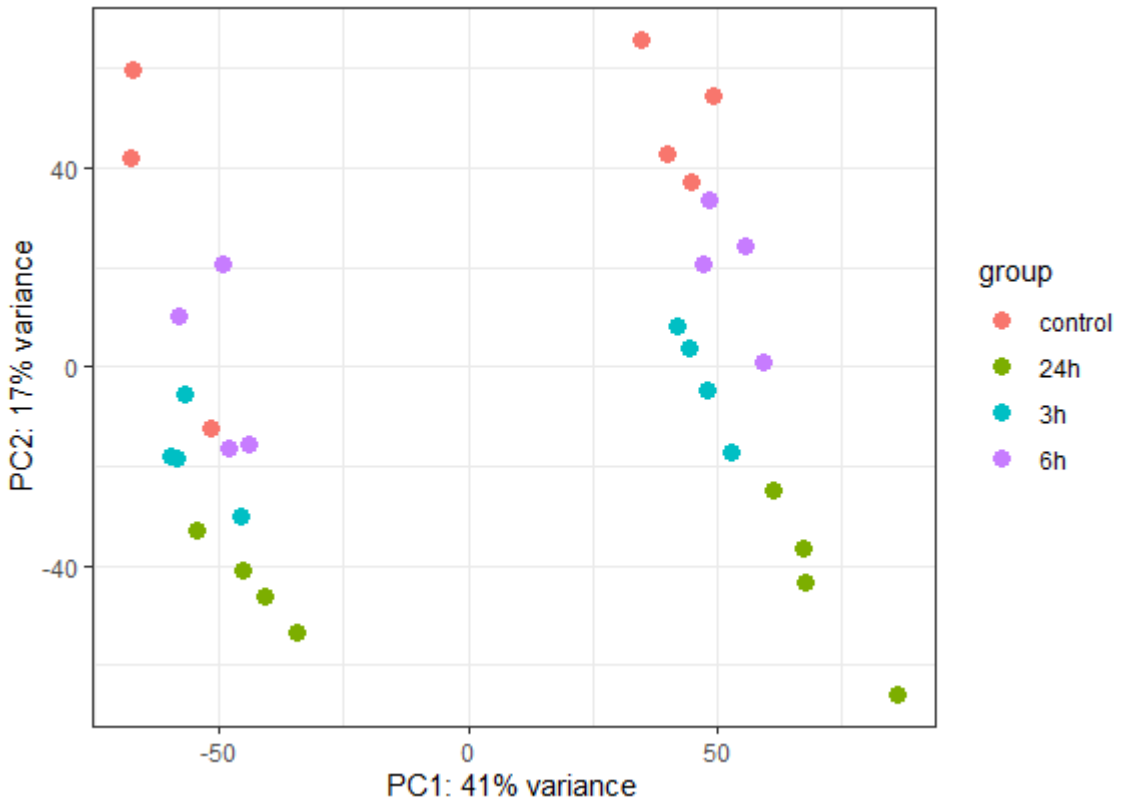


Figure 4.2. Principle Component Analysis of gene expression in *Triticum monococcum* MDR037 (right) and MDR049 (left) un-infested (control) and *Rhopalosiphum padi* infested plants at three timepoints (3h, 6h and 24h) post infestation

In un-infested MDR037 and MDR049, more genes were upregulated (positive log_2 fold-change) (1108) than downregulated (negative log_2 fold-change) (899) (Fig 4.3a). The genes with the highest log_2 fold-change were TRIUR3_02126, TRIUR3_17042, TRIUR3_09055, TRIUR3_30431 (downregulated), TRIUR3_32611, TRIUR3_07998 and TRIUR3_03466 (upregulated).

For MDR037 and MDR049 at 3hours post infestations (hpi), there were more genes downregulated (703) than upregulated (118) in infested plants (Fig 4.3b). The genes with the highest log_2 fold-changes were TRIUR3_24003, TRIUR3_35222, TRIUR3_26764 (downregulated), TRIUR3_07998 and TRIUR3_24247 (upregulated).

The number of differentially expressed genes decreased in MDR037 and MDR049 at 6hpi as only 62 genes were upregulated, and 72 genes were downregulated (Fig 4.3c). The

genes with the highest log_2 fold-changes were TRIUR3_00555 (downregulated), TRIUR3_07998 and TRIUR3_16082 (upregulated).

At 24hpi more genes were downregulated (1069) than upregulated (627) for MDR037 and MDR049 (Fig 4.3d). The genes with the highest log_2 fold-changes were TRIUR3_29741, TRIUR3_24052, TRIUR3_28947 (downregulated), TRIUR3_07998, TRIUR3_16140 and TRIUR3_17318 (upregulated).

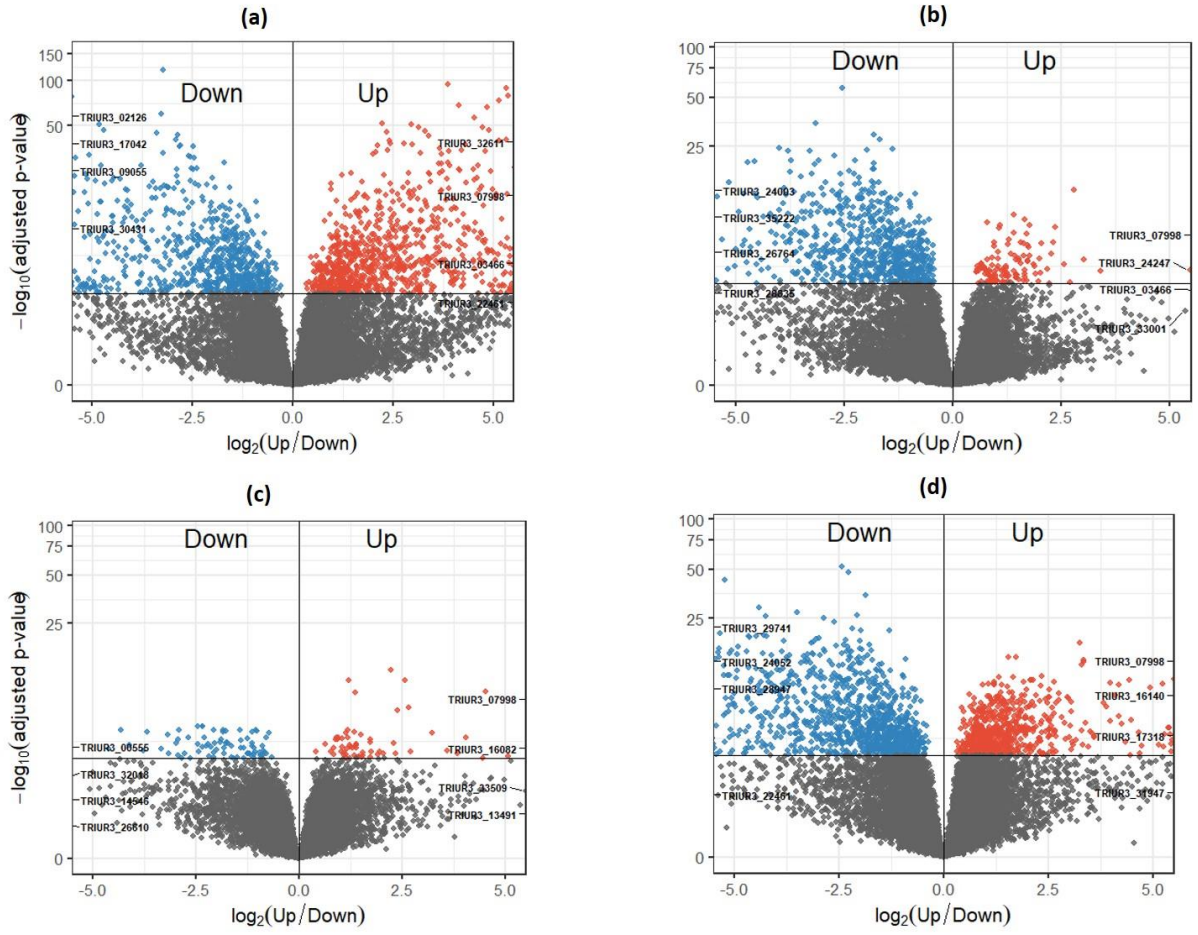


Figure 4.3. Volcano plots of down regulated and upregulated genes in *Triticum monococcum* MDR037 and MDR049 plants **a)** un-infested **b)** 3hours post *Rhopalosiphum padi* infestation (hpi) **c)** 6hpi **d)** 24hpi. Those in colour are genes that are significantly downregulated (blue) or upregulated (red) at significance level P<0.001.

To determine how gene expression varied between *T. monococcum* genotypes, *R. padi* infestation and the duration of infestation, a heat map of gene expression across all treatments

and genotypes was constructed (Figure 4.4) This separated the levels of expression in to five main clades; 1) genes that had a positive log_2 fold-change in MDR049 un-infested, 3hpi and 6hpi plants and MDR037 un-infested, and 6hpi plants, but a negative log_2 fold-change in MDR049 24hpi and MDR037 3hpi and 24 hpi plants. 2) genes that had a positive log_2 fold-change in MDR049 plants and a negative log_2 fold-change in MDR037 plants. 3) genes that had a positive log_2 fold-change in 3hpi and 24hpi plants and a negative log_2 fold-change in un-infested and 6hpi plants 4) genes that had a negative log_2 fold-change in MDR049 and a positive log_2 fold-change in MDR037. 5) genes that were that had a positive log_2 fold-change in un-infested, and 6hpi plants but a negative log_2 fold-change in 3hpi and 24 hpi plants. (Fig 4.4).

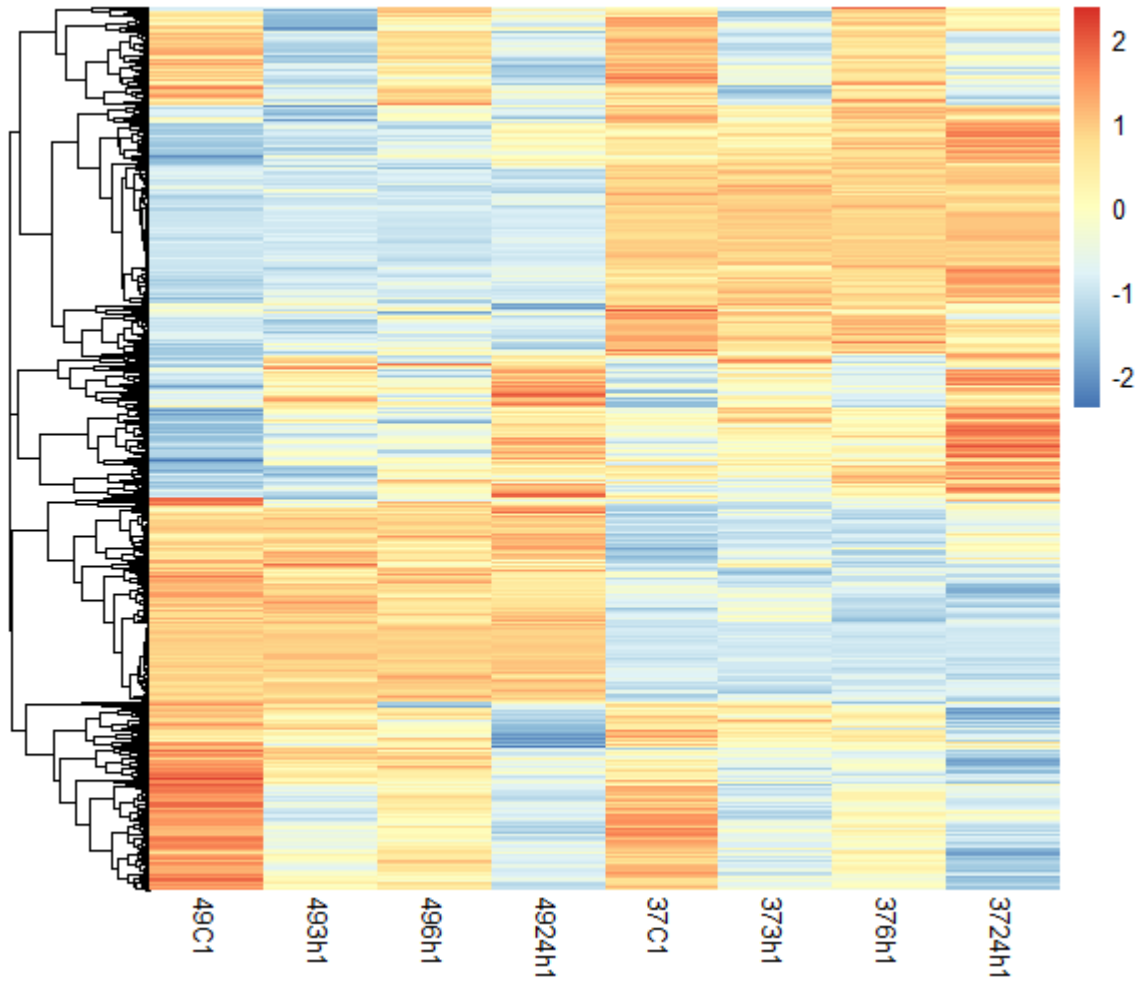


Figure 4.4. Heatmap of the $\log_2$ fold-change in expression of genes in *Triticum monococcum* genotypes MDR037 and MDR049, un-infested, 3hours, 6hours and 24hours post *Rhopalosiphum padi* infestation at a significance level of $P < 0.001$.

4.3.3 Gene expression in response to *Rhopalosiphum padi* infestation

To determine genotype specific responses to *R. padi* infestation, separate analyses on the individual genotypes was carried out (Appendix table B1). In both genotypes, gene expression was affected by duration (hours post infestation (hpi)) of *R. padi* infestation. The longest duration (24hpi) had the highest number of differentially expressed genes, and 6h had the fewest. In both genotypes, there were more similarities between differentially expressed genes in 3hpi and 24hpi than between 3hpi and 6hpi which had the fewest differentially expressed genes in common.The plant genotype also affected gene expression in response to *R. padi* infestation. A higher number of differentially expressed genes were observed in MDR049 at 3hpi and 6hpi than in MDR037. There were more differentially expressed genes at 24hpi in MDR037 than in MDR049 at 24hpi (Fig 4.5).

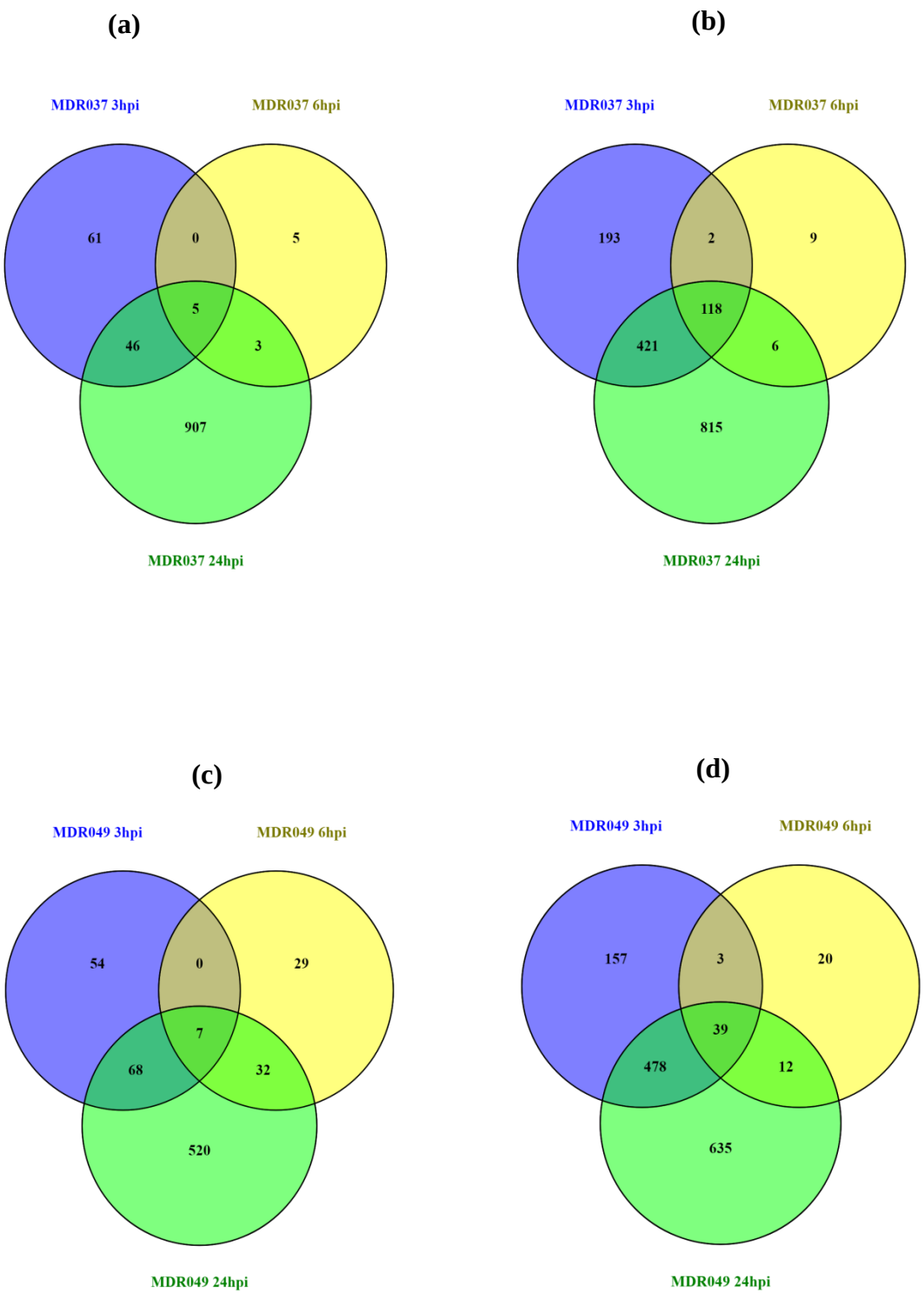


Figure 4.5. Venn diagram of *Triticum monococcum* genes that were **a)** upregulated in MDR037 **b)** downregulated in MDR037 **c)** upregulated in MDR049 **d)** downregulated in MDR049 at 3, 6 and 24hours post infestation in comparision to un-infestated *T. monococcum* MDR037 and MDR049 respectively. Only genes with a $\log_2$ fold change over 1 at significant level p <0.001 were included.

Genes that were differentially expressed in *R. padi* treatments in comparison to the control (Fig 4.5, Appendix table B1) were subject to gene ontology analysis identifying the molecular function, biological process and/or cellular component. Across all of the treatments most genes had no gene ontology terms associated with them. The top five gene ontology terms and the gene count are summarised in Figure 4.6 and 4.7. Across both genotypes and all treatments ATP binding and mtal ion binding (bar MDR037 6hours post inoculation) was the most common molecular functions, oxidation process for biological function and the most common cell component GO term was "integral component of membrane". Whilst photosynthesis was a very common biological function in most treatments, this was not the case in MDR049 3hpi where it was the 5th most common biological function GO term (Figure 4.6, 4.7)

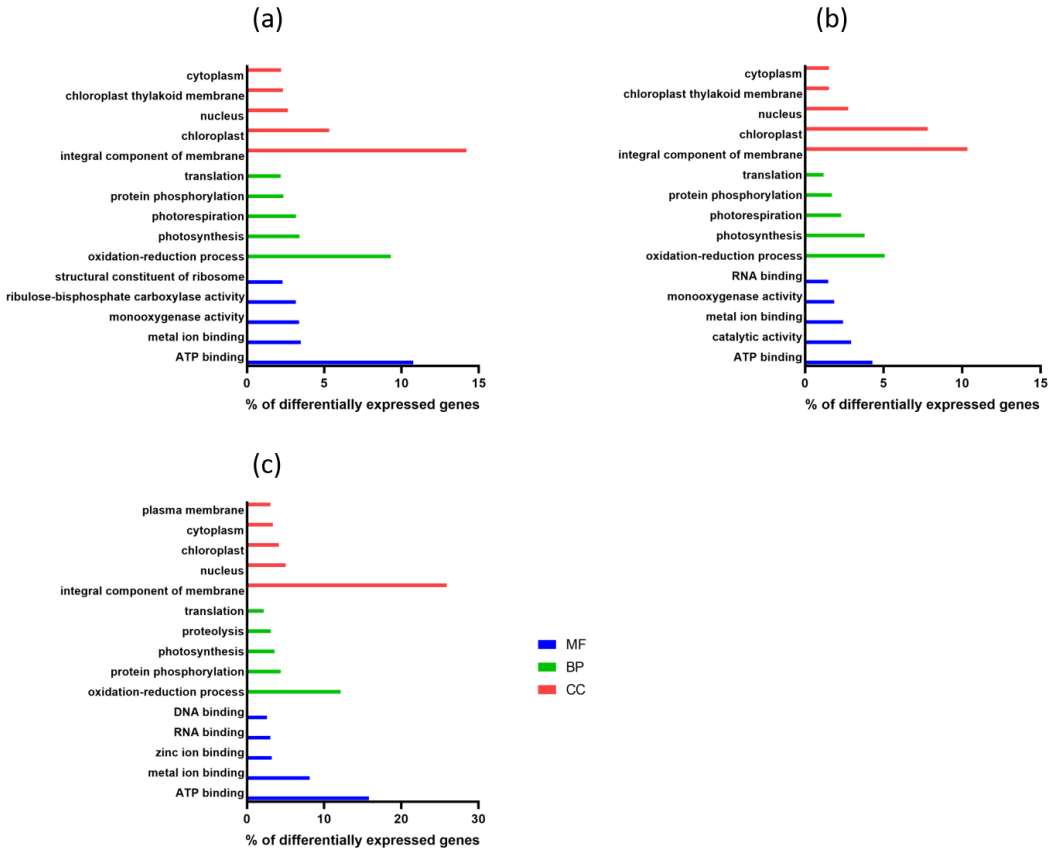


Figure 4.6. The five most abundant gene ontology terms (molecular function (MF), biological process (BP) and cell component (CC)) presence in differentially expressed genes in *Rhopalosiphum padi* infested *Triticum monococcum* MDR037 **a)** 3hours post inoculation (hpi) **b)** 6hpi **c)** 24hpi.

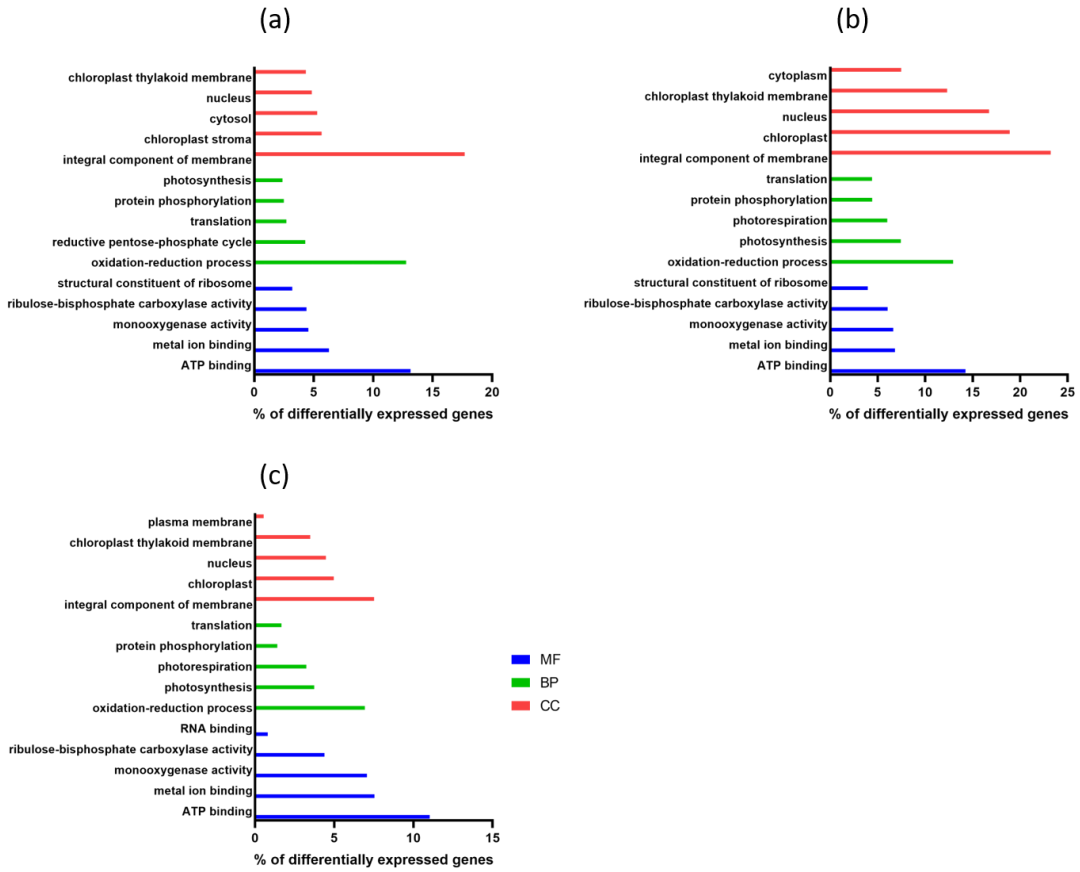


Figure 4.7. The five most abundant gene ontology terms (molecular function (MF), biological process (BP) and cell component (CC)) presence in differentially expressed genes in *Rhopalosiphum padi* infested *Triticum monococcum* MDR049 **a)** 3hours post inoculation (hpi) **b)** 6hpi **c)** 24hpi.

In each genotype, the 30 genes with the most significant differential expression in response to *R. padi*, were chosen for further analysis to determine their relative expression and their ontology. Of these genes, 27 were identified in both genotypes, showing some commonality in defence responses (Fig 4.8, 4.9). The three genes only showing differential expression in one genotype were TRIUR3_23540, TRIUR3_08259 and TRIUR3_10059 for MDR037 (Fig 4.8) and TRIUR3_08258, TRIUR3_03448 and TRIUR3_10822 for MDR049 (Fig 4.9). In un-infested plants, gene expression patterns were similar across the two genotypes, however at 3hpi and 6hpi, MDR049 had more genes that had a $\log_2$ fold-change of 1 or higher than

MDR037. At 24hpi MDR037 had more genes that had a $\log_2$ fold-change more than 1 or -1 (Fig 4.8, 4.9).

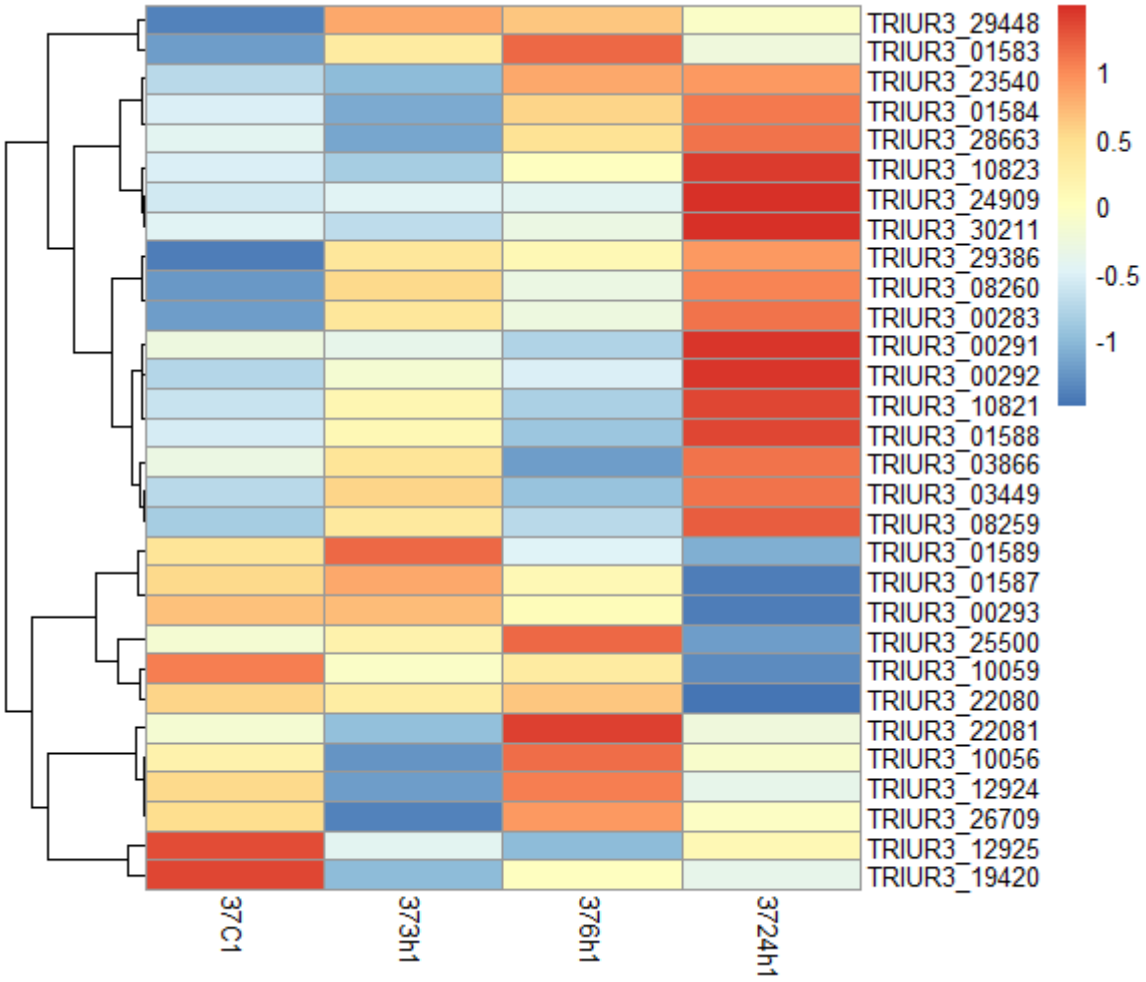


Figure 4.8. A heatmap of the $\log_2$ fold-changes of the 30 most significantly differentially expressed genes in *Triticum monococcum* MDR037 un-infested, and 3hours, 6hours and 24hours post *Rhopalosiphum padi* infestation.

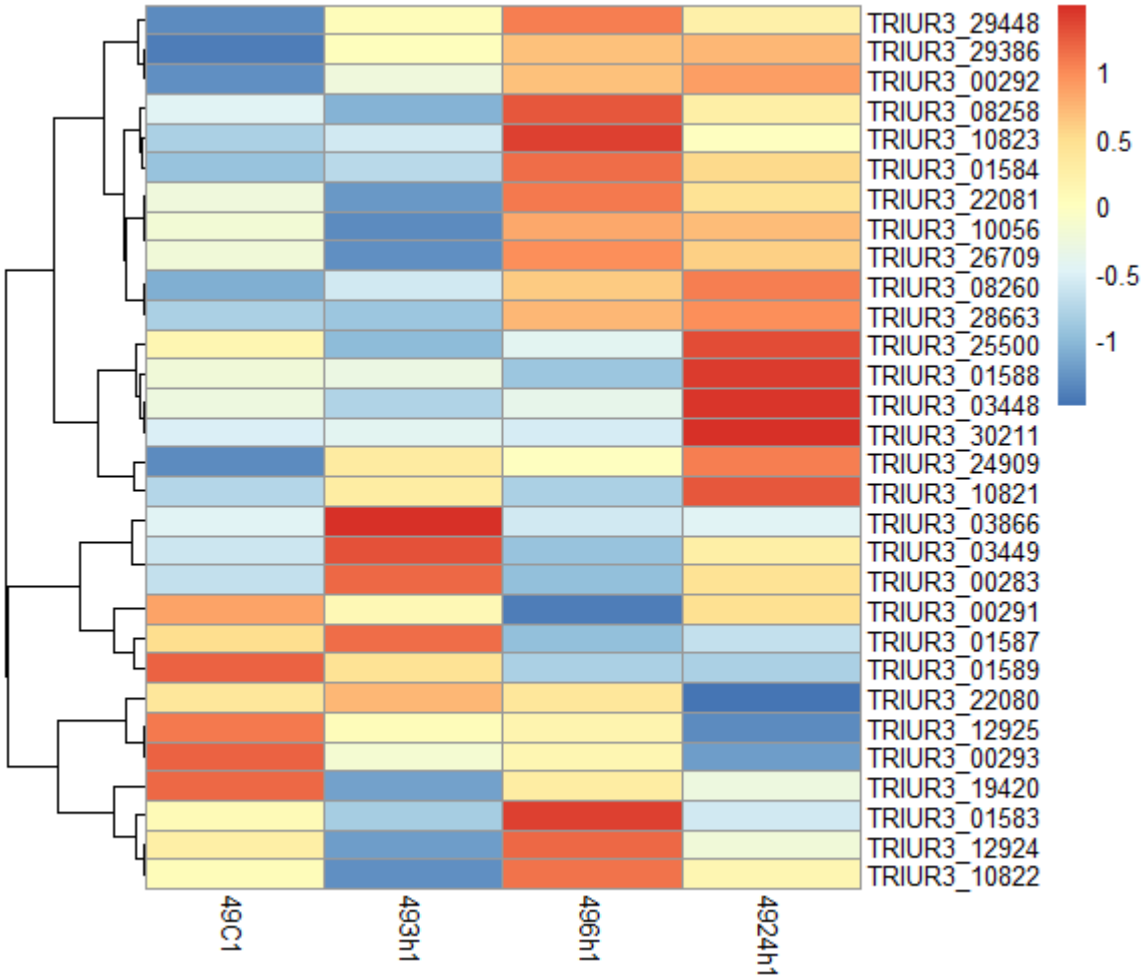


Figure 4.9. A heatmap of the $\log_2$ fold-changes of the 30 most significantly differentially expressed genes in *Triticum monococcum* MDR049 un-infested, and 3hours, 6hours and 24hours post *Rhopalosiphum padi* infestation

Most of the top 30 differentially expressed genes from each genotype (33 genes in total) did not have any gene ontology names. Of those that did, nine genes had a known molecular function, four had a known cellular component and 12 had the biological process associated with the gene (Table 4.2).

Table 4.2. Gene ontology of the top 33 differentially expressed genes in *Triticum monococcum* MDR037 and MDR049 un-infested and at three timepoints of response to *Rhopalosiphum padi* infestation (3hours post infestation (hpi), 6hpi and 24hpi). An X denotes that the gene was in the top 30 significantly differently expressed genes.

Sequence name	**Genotype differentially expressed in**		**Gene Ontology**		
	MDR037	**MDR049**	**Molecular Function**	**Cellular component**	**Biological Process**
TRIUR3_00283	X	X	ATP binding Mismatched DNA binding		Mismatch repair
TRIUR3_00291	X	X	Translation initiation factor activity	Ribosome	Regulation of translational initiation
TRIUR3_00292	X	X			
TRIUR3_00293	X	X			
TRIUR3_01583	X	X			
TRIUR3_01584	X	X		Integral component of membrane	Transmembrane transport
TRIUR3_01587	X	X	Protein binding		
TRIUR3_01588	X	X			
TRIUR3_01589	X	X	Nucleic acid binding ATP binding		
TRIUR3_03448		X			
TRIUR3_03449	X	X			
TRIUR3_03866	X	X		Endoplasmic reticulum	
TRIUR3_08258		X			
TRIUR3_08259	X				Mitotic spindle assembly checkpoint
TRIUR3_08260	X	X			
TRIUR3_10056	X	X			
TRIUR3_10059	X				
TRIUR3_10821	X	X	Protein kinase activity ATP binding		Protein phosphorylation
TRIUR3_10822		X			
TRIUR3_10823	X	X			
TRIUR3_12924	X	X	Metal ion binding		
TRIUR3_12925	X	X	Sucrose synthase activity		Starch metabolic process Sucrose metabolic process
TRIUR3_19420	X	X		Trans-Golgi network	Intracellular cholesterol transport
TRIUR3_22080	X	X	Glutathione		Glutathione

			synthase activity ATP binding		biosynthetic process
TRIUR3_22081	X	X			
TRIUR3_23540	X		Hydrolase activity Hydrolysing O-glycosyl compounds		Carbohydrate metabolic process
TRIUR3_24909	X	X			
TRIUR3_25500	X	X			
TRIUR3_26709	X	X			
TRIUR3_28663	X	X			Catalytic activity
TRIUR3_29386	X	X			
TRIUR3_29448	X	X			Hydrolase activity
TRIUR3_30211	X	X			RNA binding

The genes TRIUR3_00291, TRIUR3_12925 and TRIUR3_22080 were differentially expressed in both genotypes and from the GO terms identified may have a role in defense responses against *R. padi* infestation. The relative gene expression in MDR037 and MDR049 un-infested and infested plants are shown in Figure 4.9.

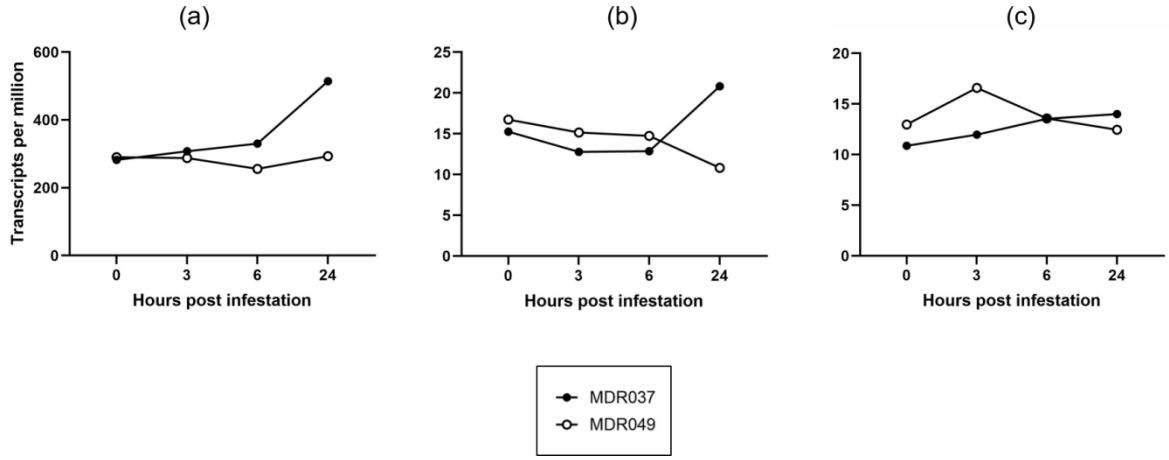


Figure 4.10. Transcripts per million of **a)** TRIUR3_00291, **b)** TRIUR3_12925 and **c)** TRIUR3_22080, in un-infested *Triticum monococcum* MDR037 and MDR049 plants un-infested, 3hours, 6hours and 24hours post *Rhoplaosiphum padi* infestation. Note different scales on y-axis.

4.4 DISCUSSION

This chapter has shown that gene expression in *Triticum monococcum* varies between genotypes and that *R. padi* infestation can alter gene expression. The 30 genes that had the largest differences in expression in response to *R. padi* infestation within each *T. monococcum* genotype were identified. Most of the top 30 differentially expressed genes were expressed in both genotypes, indicating that similar responses may be occurring to aphid infestation in the resistant and susceptible plant. The molecular function, biological process and the cellular component associated with the genes were also identified in some cases. This discussion focuses on three candidate genes; TRIUR3_00291, (Translational initiation factor) TRIUR3_12925 (sucrose synthase activity) and TRIUR3_22080 (Glutathione synthase activity), that were associated with defence related molecular functions and/or biological processes.

It should be noted that since only ca. 64% of genes aligned with the *Triticum urartu* genome, there may be other genes involved in MDR037 and MDR049 responses to *R. padi* infestation that did not map to the *T. urartu* genome and have not been captured in this study.

4.4.1 Candidate gene TRIUR3_00291

This gene was chosen because changes in a gene encoding a translation initiation factor might be expected to affect the expression of other genes involved in responses to aphids. If TRIUR3_00291 promotes translation initiation, then more translation would have occurred in MDR037 infested plants than in MDR049 infested plants. In MDR049, TRIUR3_00291 gene expression decreased as the duration of *R. padi* infestation increased. If TRIUR3_00291 inhibits translation initiation, there would be more translation in MDR049 infested plants, with translation initiation increasing as the duration of *R. padi* infestation increased.

4.4.2 Candidate gene TRIUR3_12925

This gene was chosen because it was expressed in both genotypes and had higher gene expression in un-infested plants than in infested plants of both genotypes and is associated with sucrose synthase activity. Sucrose synthase is part of an enzyme complex that catalyses callose and cellulose synthesis (Amor et al., 1995; Verma and Hong, 2001) (Figure 4.11). TRIUR3_12925 could be involved in the inhibition of sucrose synthase activity, or if the gene promotes synthase, the production of cellulose could explain this increased expression in un-infested plants. The activity of the annexin enzyme would have to be investigated to determine whether callose or cellulose synthesis occurred in these scenarios (Andrawis et al., 1992) (Fig 4.11).

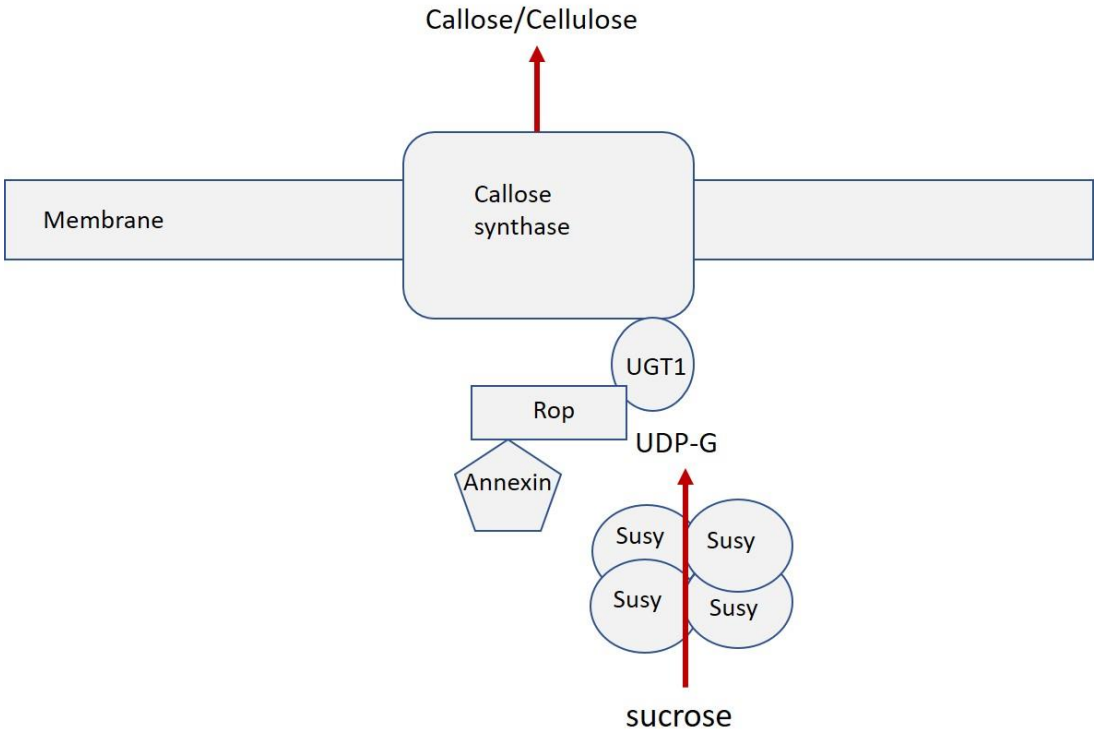


Figure 4.11. Schematic model of the callose synthase complex in plants, adapted from Piršelová and Matušíková (2013). The complex consists of callose synthase, UDP-glucose transferase (UGT1), annexin, Rho-like protein with GTP-ase activity (Rop) and sucrose synthase (SuSy). SuSy creates UDP-glucose (UDP-G) from sucrose. Rop may be involved with UGT1 regulation (Verma and Hong, 2001). Annexin controls whether callose or cellulose synthesis occurs (Andrawis et al., 1992).

Callose synthesis can lead to callose deposition around sieve pores, forming a structural defence against aphid feeding (Will and Van Bel, 2006; Will et al., 2013) so TRIUR3_12925 gene expression which was higher in MDR049 3hpi and 6hpi than in the respective MDR037 plants, might be playing a role in the defence of MDR049. This is supported by the finding that sucrose synthase genes *SUS1* and *SUS2* were upregulated in a wheat line (XN98-10-35) which is tolerant to *R. padi* and *S. avenae*. (Luo et al., 2014, 2019). Also increased sucrose in *Triticum aestivum* has been shown to decrease *R. padi* populations (Vassiliadis et al., 2018) and has been associated with decreased *S. avenae* weight and reproduction (Kang et al., 2018). A previous study on *T. monococcum – R. padi* interactions showed that *R. padi* on MDR049 spent less time feeding and was less fecund than *R. padi* on MDR037 (Greenslade et al., 2016). Higher TRIUR3_12925 gene expression in MDR049 at 3hpi and 6hpi could lead to increased callose synthesis and deposition in the phloem to prevent aphid feeding (Will and Van Bel, 2006; Piršelová and Matušíková, 2013; Will et al., 2013). Studies have shown that induced resistance in *A. thaliana* had higher callose synthase gene *AtGLS5* expression (Lü et al., 2011) and that a resistant pepper (*Capsicum* spp (L.) genotype had more callose deposition in both un-infested and *M. persicae* infested plants than susceptible *Capsicum* (Sun et al., 2018) Both of these studies demonstrated that *M. persicae* feeding on the resistant *A. thaliana* and *Capsicum* took longer to start feeding and spent less time feeding (Lü et al., 2011; Sun et al., 2018).

The lower TRIUR3_12925 expression in MDR037 at 3hpi and 6hpi could be the result of effector protein activity. The *M. persicae* effector protein *Mp55* has been shown to suppress callose deposition in *Ararbidopsis thaliana* and increase *M. persicae* reproduction (Elzinga et al., 2014). Feeding behaviour studies showed that *R. padi* on MDR037 start sustained phloem feeding earlier than those on MDR049 (Greenslade et al., 2016). Less

TRIUR3_12925 gene expression could lead to less callose deposition in the phloem, allowing un-inhibited aphid feeding.

At 24hpi, TRIUR3_12925 expression decreased in MDR049. Callose formation takes minutes to occur and reach its maximal level, however, it also take a long time to degrade (Furch et al., 2007), therefore this form of defence can be seen as a long term structural defence in response to aphid feeding, which may explain why there is downregulation in MDR049 24hpi, where sucrose synthase may no longer be needed, as callose synthesis was also not needed.

Overall the finding of the current study shows that TRIUR3_12925 gene expression changes with the time post *R. padi* infestation in both wheat genotypes is consistent with a proposed role in the defence response of callose sealing sieve tubes pores to prevent aphid feeding (Will and Van Bel, 2006; Will et al., 2013).

4.4.3 Candidate gene TRIUR3_22080

This gene was chosen because it was expressed in both genotypes and is associated with glutathione synthase and ATP binding molecular functions, as part of glutathione biosynthesis. Glutathione biosynthesis involves two ATP dependant steps, in one of these, glutathione synthase converts γ-glutamylcysteine to glutathione (Meister, 1988). Glutathione is an antioxidant that is involved in the glutathione-ascorbate pathway that detoxifies uncontrolled hydrogen peroxide oxidisation (Noctor and Foyer, 1998; Ferreira et al., 2014; Li et al., 2015) (Figure 4.12). Uncontrolled oxidation can cause oxygen species to build up, leading to cell death and early senescence in plants (Li et al., 2015).

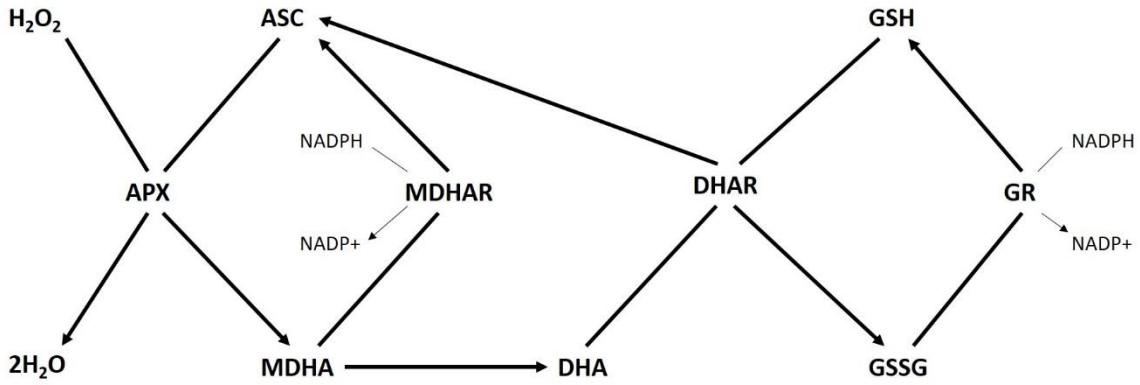


Figure 4.12. Simplified representation of the ascorbate – glutathione cycle in plant stroma, cytosol, mitochondria and apoplast adapted from Noctor and Foyer, (1998); Ferreira et al., (2014). Abbreviations are APX: ascorbate peroxidase, MDHA: monodehydroascorbate, ASC: ascorbate, MDHAR: monodehydroascorbate reductase, DHA: dehydroascorbate, DHAR: dehydroascorbate reductase, GSSG: oxidized glutathione, GR: glutathione reductase and GSH: reduced glutathione.

Increased ATP binding and glutathione synthase activity would increase glutathione biosynthesis, however, ascorbate biosynthesis would also have to increase to reduce oxygen species build up (Noctor and Foyer, 1998). Aphid infestation has been shown to increase reactive oxygen species (ROS) (Ni et al., 2001; G. Miller et al., 2009) such as hydrogen peroxide (H_2O_2). In the current study, TRIUR3_22080 gene expression increased in MDR049 at 3hpi and MDR037 at 6hpi. It can be hypothesised that TRIUR3_22080 promotes ATP binding and glutathione synthase activity to increase H_2O_2 detoxification.

The increase in TRIUR3_22080 expression in MDR049 at 3hpi plants is likely to be in response to an oxidative burst in response to *R. padi* infestation (Ni et al., 2001; G. Miller et al., 2009). ROS H_2O_2 can provide defence against herbivory by strengthening cell walls (Hückelhoven et al., 1999; Argandoña et al., 2001), transferring its toxicity to herbivores (Bi and Felton, 1995) and interacting with phytohormone signalling (Morkunas et al., 2011). Increased TRIUR3_22080 could aid the regulation of ROS to prevent the negative side effects of this defence response occurring.

TRIUR3_22080 gene expression in MDR037 increased dramatically at 6hpi. Recognition of HAMPs by PRR leads to an H_2O_2 oxidative burst (G. Miller et al., 2009). This oxidative burst has been shown to interact with JA signalling to produce proteinase inhibitors (Orozco-Cárdenas et al., 2001) that reduce *Diuraphis noxia* (K.), *Schizaphis graminum* (R.) and *R. padi* survival on *T. aestivum* (Tran et al., 1997). Herbivore associated molecular patterns may have been recognised at 3hpi in both genotypes, but H_2O_2 oxidative burst may have been dampened in MDR037 by the activity of effector proteins. *Macrosiphum euphorbiae* effector protein Me47 has been shown to have glutathione-S-transferase properties (Kettles and Kaloshian, 2016), glutathione-S-transferase is a dehydroascorbate reductase (DHAR) part of the ascorbate-glutathione pathway that detoxifies H_2O_2 (Noctor and Foyer, 1998; Ferreira et al., 2014) (Fig 4.11). *Myzus persicae* salivary effector proteins *Mp10* and *Mp55* have also been shown to reduce ROS production (Bos et al., 2010; Elzinga et al., 2014). If such effector proteins were present in *R. padi* saliva, this may have detoxified any H_2O_2 leading to less of an oxidative burst in MDR037 at 3hpi so less detoxification would have been needed. In this scenario, glutathione biosynthesis would not have been needed, leading to a lower TRIUR3_22080 expression. By 6hpi, other cues such as *R. padi* honeydew secretions and sustained *R. padi* feeding could have initiated triggered immunity responses, inducing an oxidative burst. It has been shown that hydrogen peroxide induction occurred earlier on *D. noxia* infestation resistant transgenic *T. aestivum* Tugela *DN* on susceptible plants (Moloi and van der Westhuizen, 2006). In MDR049 plants, effector proteins may have been recognised by R proteins which initiated effector triggered immunity (ETI), leading to an earlier defence response to *R. padi* infestation.

4.4.4 Conclusion

This chapter shows that the *T. monococcum* genotype and the duration of *R. padi* infestation affects gene expression, with a higher number of differentially expressed genes in MDR049 at the earlier timepoints, 3 and 6hpi, but more differentially expressed genes in MDR037 at 24 hpi.

Overall, of the candidate genes that were identified in this study, the three foremost were TRIUR3_00291, TRIUR3_12925 and TRIUR3_22080. TRIUR3_00291 was expressed more in MDR037 infested plants than MDR049 infested plants, this gene is associated with translation initiation, however it is unknown whether this gene promotes or is antagonistic to this process.

Both TRIUR3_12925 and TRIUR3_22080 are associated with proteins involved in initiation and regulation if defence responses. TRIUR3_12925 is associated with sucrose synthase activity as part of starch and sucrose metabolic processes. It has been hypothesised that this gene promotes sucrose synthase activity as part of the enzymatic complex that synthesises callose and cellulose. TRIUR3_12925 was expressed more in MDR049 un-infested, 3hpi and 6hpi than in the respective MDR037 plants, it is suggested that this influences callose synthesis and deposition in MDR049 in response to *R. padi* infestation.

TRIUR3_22080 is associated with ATP binding and glutathione synthase as part of glutathione biosynthesis. It has been hypothesised that this gene promotes glutathione biosynthesis as part of the ascorbate-glutathione pathway that regulates ROS which increases in response to herbivory. TRIUR3_22080 upregulation in response to *R. padi* infestation occurred earlier in MDR049 plants than MDR037 plants indicating a faster or un-inhibited defence response in MDR049.

CHAPTER 5

Field population dynamics of cereal aphids and their natural enemies on modern and ancestral wheat varieties.

5.1 INTRODUCTION

Aphid populations are governed by intrinsic factors, such as developmental rates, and extrinsic factors, such as biotic or abiotic interactions, which are elements external to the aphids. The host plant is an extrinsic factor that can affect the intrinsic factors of aphids, where plants with resistance traits can cause dramatic reductions in aphid development, reproductive success and survival, these plants are considered to have post-alighting resistance (antibiosis) (Painter, 1951). Examples of this are present in different genotypes within plant species such as the reduced infestation rates and nymph production of the pea aphid (*Acyrthosiphon pisum* (H.)) on pea (*Pisum sativum* (L.)) genotype P40 (Aznar-Fernández and Rubiales, 2018). Other examples include increased mortality and decreased reproduction in the soybean aphid (*Aphis glycines* (M.)) feeding on soybean (*Glycine max* (L.)) genotypes P574 and P746 (Wu et al., 2009) and reduced development and reproductive success of the foxglove aphid (*Aulacorthum solani* (K.)) feeding on genotypes Adams and William82 (Kobayashi and Murai, 2012). Antibiosis has also been found in cereal crops, for example, the bird cherry-oat aphid (*Rhopalosiphum padi* (L.)) had reduced reproductive success on Triticale (*Triticosecale* (W.)) genotypes N1185 and Okta Deizhana (Hesler and Tharp, 2005), and the russian wheat aphid (*Diuraphis noxia* (K.)) produced fewer nymphs on wheat (*Triticum aestivum* (L.)) genotypes PI 262660 and PI 137739 (Toit, 1989). Antibiosis affecting *D. noxia* and greenbug (*Schizaphis gaminum* (R.)) reproductive success was present in *T. aestivum* genotypes Mara, Fiorello, Carmen, Rannyaya, P. Oasis, P. Pasuco, B. Ombu, B. Cartriel, Favouits and C. Calquin (Castro et al., 1999).

Triticum monococcum genotype dependent antibiosis has been shown towards the English grain aphid (*Sitobion avenae* (F.)) (di Pietro et al., 1998; Simon et al., 2017), and *R. padi* (Migui and Lamb, 2003; Greenslade et al., 2016; chapter 3) in controlled environment studies as well as against the rose grain aphid (*Metopolophium dirhodum* (W.)) and *S. avenae* in the field (Sotherton and Lee, 1988; Hu et al., 2016), although for those studies, genotypes tested in the two different environment do not overlap.

The genotypes used in this study have shown varying levels of resistance and susceptibility to *R. padi* and *S. avenae* in controlled conditions (Greenslade *et al.* 2016; Simon *et al.* 2017; Chapter 2; Chapter 3) but have not been tested in the field until now.

Alate aphids are colonisers and use olfactory and visual cues to find their host plant (Bruce and Pickett, 2011). Host plant volatile cues influence whether an alate aphid alights onto the plant to colonise it. Alate aphid attraction to plants has been shown to vary between species and cultivar/genotypes (Nottingham et al., 1991; da Costa et al., 2011; Schröder et al., 2015). Antixenosis is a pre-alighting resistance in plants, sometimes also called non-preference, where in the case of aphids the plant is not attractive or can even be repellent to the host colonising form (Kogan and Ortman, 1978).

It has previously been shown that certain genotypes of *T. monococcum* confer antixenosis to *S. avenae* under field conditions (Sotherton and Lee, 1988; Hu et al., 2016), and antixenosis to *R. padi* has been shown to be genotype dependant under laboratory condition (Chapter 2) suggesting that host plant genotype can affect alate *R. padi* settlement.

Other external factors that affect aphid populations are predation and parasitism from higher trophic levels. Natural enemies, such as parasitoids, lacewing, and ladybirds have been proven to be an effective biocontrol method of aphids in glasshouses (Zehnder et al., 2006;

Boivin et al., 2012) and in field settings on upland cotton (*Gossypium hirsutum* (L.)) (Rosenheim, 2001) and barley (*Hordeum vulgare* (L.)) (Pålsson et al., 2019a). In a field study, *D. noxia* populations on *T. aestivum* were 2.9 to 11.2 times lower in the presence of predators and parasitoids than populations on *T. aestivum* where natural enemy exclusion cages were used. Ladybirds (Coccinellidae (L.)) were one of the most abundant predators observed in this study (Mohamed et al., 2000). Predation has also been estimated to reduce the effects of *R. padi* infestation on *H. vulgare* by at least 44% (Östman et al., 2003).

For effective population control, natural enemies must find their prey/host, and to do so they use a variety of olfactory and visual cues. Aphid infestation changes the semiochemicals that a plant produces. Semiochemicals that are only produced in the context of herbivory are called herbivore induced plant volatiles (HIPVs); these changes can affect the behaviour of both the herbivore and the natural enemies. The seven spotted ladybird (*Coccinella septempunctata* (L.)) has been shown to be attracted to *R. padi* infested barley (*Hordeum vulgare*) (Ninkovic et al., 2001). Parasitoids have been shown to use a combination of HIPV and pheromones from their host species to locate their host. The parasitoid *Aphidius ervi* (H.) is attracted to pea aphid (*Acyrthosiphon pisum* (H.)) infested broad bean plants (*Vicia faba* (L.)) (Du et al., 1998; Powell et al., 1998), as well as aphid sex pheromones, Nepetalactol and Nepetalactone, showing that they can use multiple strategies to locate their host (Powell et al., 1998). These interactions can be manipulated to increase aphid biocontrol. Synthetic slow-release HIPV caused an increase in the common green lacewing (*Chrysoperla carnea* (S.)) eggs and larvae presence on barley, leading to a reduction in the populations of *R. padi* and *S. avenae* (Pålsson et al., 2019b).

Wild and ancestral plants have been shown to produce different HIPVs and semiochemical profiles to their cultivated counterparts (Gouinguené et al., 2001; Gols et al., 2011). In some cases, such as for wild and cultivated cabbage, the parasitoids *Cotesia*

rubecula (M.) preferred the HIPVs emitted from wild plants (Gols et al., 2011). The same has been shown for the parasitoid *Cotesia marginiventris* (C.), which preferred HIPVs from ancestral maize species *Zea. mays* spp. *parviglumis* and *Zea mays* ssp. *Mexicana* than HIPVs from cultivated maize (*Zea mays* ssp. *Mays*) (de Lange et al., 2016). The HIPVs emitted from *Triticum monococcum* MDR037, MDR045 and MDR049 are distinct from modern *T. aestivum* var Solstice (Chapter 2), but it is unknown whether this affects natural enemy behaviour and presence in this system.

Aphid feeding on resistant plants can affect their development and fecundity, which in turn can affect natural enemy populations. In some cases, the effects can be additive, for example, the green lacewing species *Crysoperla plorabunda* (F.) was more effective in reducing *D. noxia* populations on resistant transgenic lines of *T. aestivum* than an isogenic susceptible line (Messina and Sorenson, 2001). This could be because initial aphid populations were lower due to reduced reproductive success. *Diuraphis noxia* feeding causes leaf rolling, with fewer aphids, leaf rolling may have been reduced, making aphids easier to locate. This additive affect was also shown in ladybird-aphid interactions where *C. septempunctata* predation of black bean aphid (*Aphis fabae* (S.)) had a larger effect on resistant broad bean variety (*Vicia faba* (L.)). *Coccinella septempunctata* feeding on *A. fabae* from the resistant line had lower fecundity (Shannag and Obeidat, 2008). This indicates that there may be longer term effects of predating on aphids on resistant plants.

Post-alighting resistance (antibiosis) to *S. avenae* has been identified in *T. monococcum* MDR045 and MDR049 (Simon et al., 2017) and to *R. padi* in MDR657, and MD049 (Greenslade et al., 2016). Antixenosis towards *R. padi* has been identified in MDR049 (Chapter 2). Susceptibility to *S. avenae* and *R. padi* has been identified in *T. monococcum* MDR037 and *T. aestivum* var. Solstice (Greenslade *et al.* 2016; Simon *et al.*

2017; Chapter 2; Chapter 3). All of these studies were all carried out under laboratory conditions, so it is unknown if these genotypes are resistant in the field and whether resistant plants would affect aphid-natural enemy interactions. This chapter presents three years of field trials investigating the field populations of three aphid species and the presence of three natural enemies on *T. aestivum* var. Solsice, *T. monococcum* MDR037, MDR657, MDR045 and MDR049.

5.1.1 Chapter hypotheses

This chapter addresses the following hypotheses:

1) Aphid (*Sitobion avenae*, *Rhopalosiphum padi* and *Metopolophium dirhodum*) populations in field conditions will vary between wheat genotypes
2) Natural enemy presence (parasitised aphids, lacewing eggs and larvae and ladybird beetles and larvae) will be dependant on wheat genotype.
3) Aphid populations and natural enemy presence is affected by meteorological factors.

5.2 METHODS

5.2.1 Experimental design

Observations of aphid and natural enemy presence on *Triticum monococcum* genotypes MDR037, MDR657, MDR045 and MDR049 and *Triticum aestivum* were carried out at Rothamsted Research, Hertfordshire, UK. Seeds were sown in Autumn at 250 seeds per plot for the 2017 field trial, and 300 seeds per plot for the 2018 and 2019 field trials. In 2017, the field trial was sown in a field named Long Hoos 4, in $0.6m^2$ plots sown at a drill speed of 0.7miles per hour (mph) (Fig 5.1). In 2018 the field trial was at Long Hoos 5, with $1m^2$ plots sown at a drill speed of 1.2mph (Fig 5.2). In 2019 the field trial was in Claycroft field with

$1m^2$ plots sown at a drill speed of 1.2mph (Fig 5.3). The reason for the smaller plot size in the first year was due to a limited availability of seed. Subsequent years used saved seeds, hand harvested from previous field season. The plots were in a randomised block design with four replicate plots for each genotype. At either side of the field trial was a strip of *T. aestivum* var. Hereward.

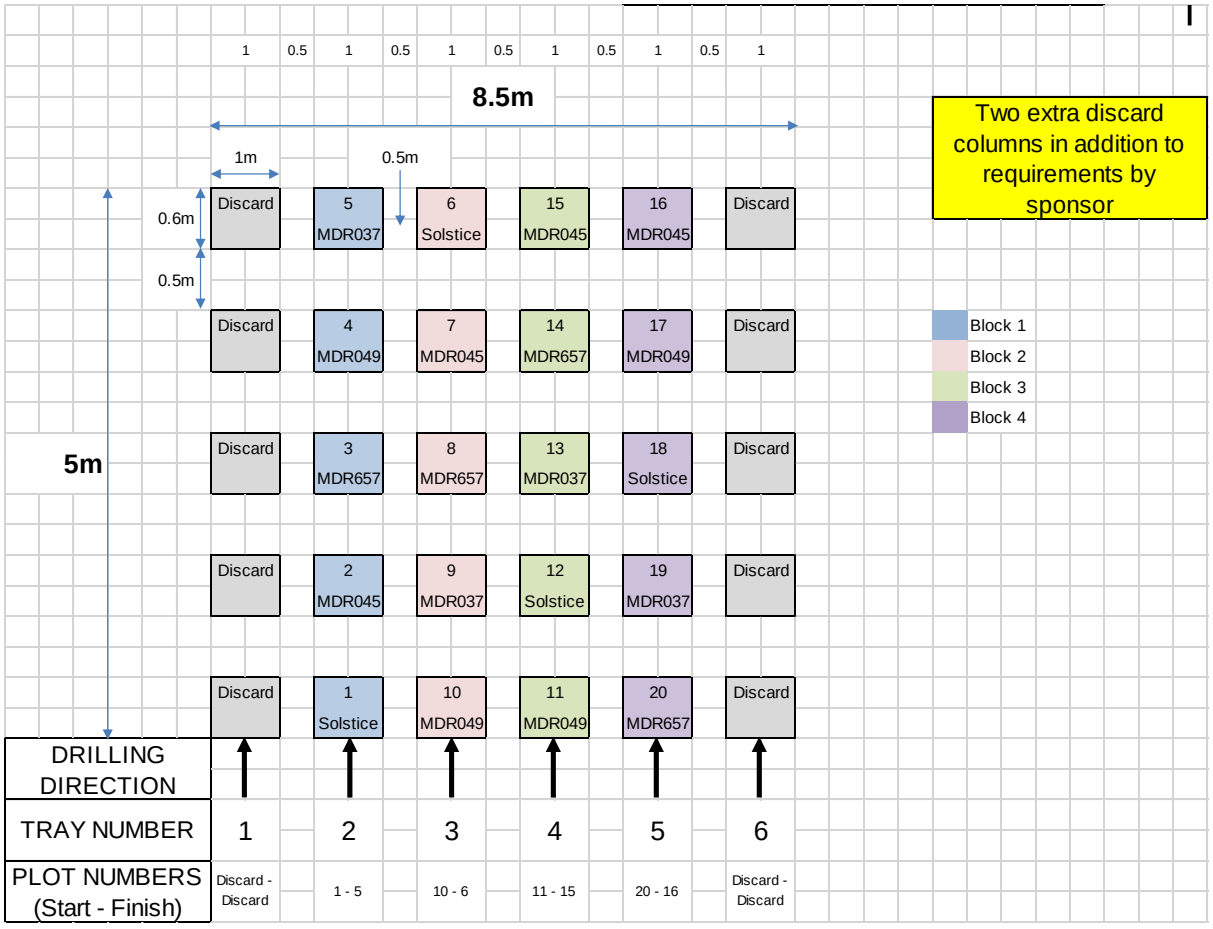


Figure 5.1. Experimental layout of the 2017 field trial of *Triticum aestivum* var. Solstice, *Triticum monococcum* MDR037, MDR657, MDR045 and MDR049 at Long Hoos 4 field, Rothamsted Research, Harpenden, Hertfordshire, UK.

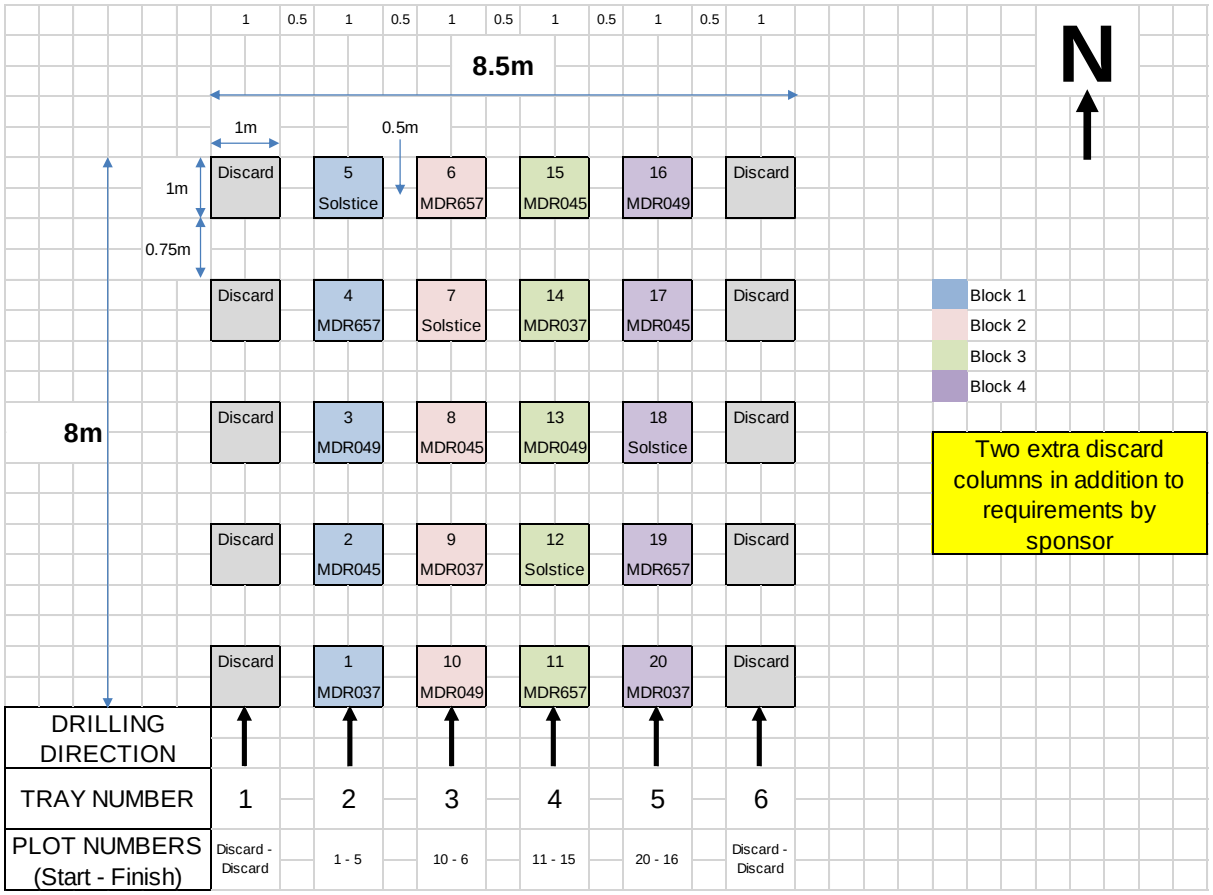


Figure 5.2. Experimental layout of the 2018 field trial of *Triticum aestivum* var. Solstice, *Triticum monococcum* MDR037, MDR657, MDR045 and MDR049 at Long Hoos 5 field, Rothamsted Research, Harpenden, Hertfordshire, UK.

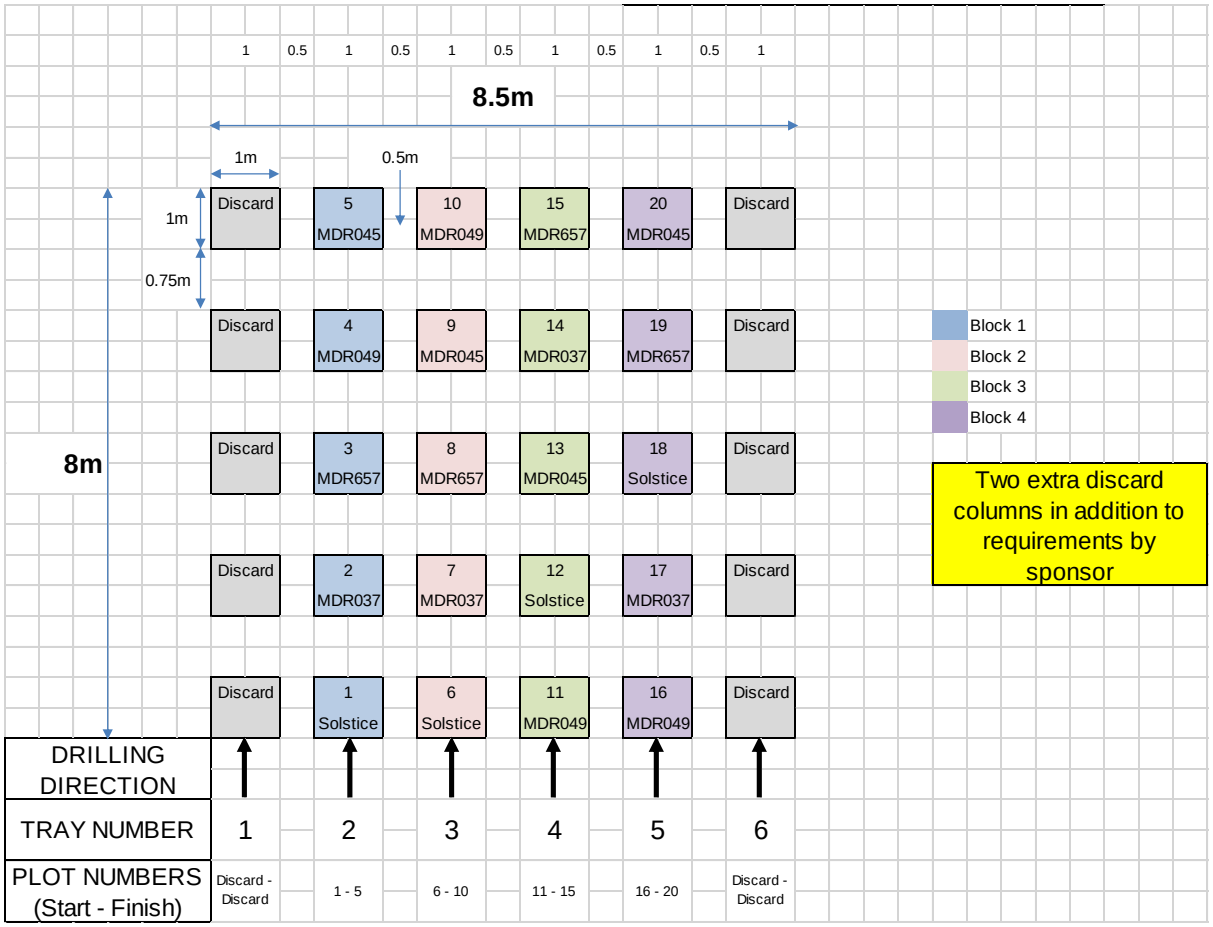


Figure 5.3. Experimental layout of the 2019 field trial of *Triticum aestivum* var. Solstice, *Triticum monococcum* MDR037, MDR657, MDR045 and MDR049 at Claycroft field, Rothamsted Research, Harpenden, Hertfordshire, UK

5.2.2 Measuring aphid populations and natural enemy presence

The number of cereal aphids (*Sitobion avenae*, *Rhopalosiphum padi* and *Metopolophium dirhodum*), ladybird beetles and larvae, lacewing eggs and larvae and parasitised mummified aphids per tiller was counted on 25 tillers in each plot on a weekly or biweekly basis, depending on weather. Each tiller was chosen at random using GenStat® (2016, 18th Edition, © VSN International Ltd, Hemel Hempstead, UK) statistical software. The sampling started either the first or second week of May when the aphid populations started to increase and finished in the last week of July when aphid populations had diminished.

Meteorological data on daily maximum temperature, minimum temperature, rainfall and temperature rainfall were provided by Rothamsted Research Long-term Experiments

National Capabilities (LTE-NCG) Electronic Rothamsted Archive (e-RA). Suction trap data for *S. avenae, R. padi* and *M. dirhodum* were provided by the Rothamsted Insect Survey (RIS).

5.2.3 Statistical analysis

Data were subjected to transformation to comply with assumptions of normality and equal variance. The number of aphids and natural enemy presence per 25 tillers and the number of alate aphids per year was subject to square root transformation. The proportion of aphid species and natural enemy families was subject to logit transformation. RStudio: Integrated Development Environment for R (2016, Edition 1.1.453, ©RStudio Inc, Boston, Massachusetts, USA) software was used for the following analysis. The number of aphids and natural enemy presence per 25 tillers and the proportion of aphid species and natural enemy families was analysed using linear mixed model (LMM) form of the restricted maximum likelihood model (REML) within the lme4 R package, as there were some missing values. The wheat genotype, field trial year and the week of recording were factors in this analysis.

To determine correlations between insect survey data and field trial data, data was $\log_2$ transformed, and Pearson's correlation analysis was carried out.

5.3 RESULTS

5.3.1 Aphid populations

Overall aphid population density was dependant on wheat genotype, field trial year and week. The lowest number of aphids per 25 tillers was observed on MDR045 and MDR049 (F = 1.68, P <0.001) and in the 2018 field trial (F = 1.99, P=0.04) (Fig 5.4). The highest number

of aphids per 25 tillers were observed in weeks 23 and 24 (F = 2.01, P <0.001) (Fig 5.4). There was no interaction between the field trial year and week (F = 0.67, P =0.249), and the genotype and week (F = 0.72, P =0.208), however, there was an interaction between the year and genotype (F = 1.28, P < 0.001) as fewer aphids observed in 2018 was most dramatic on MDR037 (Fig 5.4).

5.3.1.1 Field trial 2017

The lowest number of aphids per 25 tillers was observed on MDR045 and MDR049 (F = 1.34, P =0.039). The highest aphid populations were observed in weeks 22 to 28 (F = 0.98, P <0.001) (Fig 5.4a). There was no interaction between the genotype and week (F = 0.62, P =0.123)

5.3.1.2 Field trial 2018

The lowest number of aphids per 25 tillers was observed on MDR037 and MDR045 (F = 1.53, P =0.004). The highest aphid populations were observed in weeks 24 to 26 (F = 1.41, P <0.001) (Fig 5.4b). There was an interaction between the genotype and week (F = 1.20, P <0.001) as whilst populations on Solstice increase from week 22, populations on other genotypes were more sporadic.

5.3.1.3 Field trial 2019

The lowest number of aphids per 25 tillers was observed on MDR045 and MDR049 (F = 1.39, P =0.003). The highest aphid populations were observed in weeks 23 to 26 (F = 2.09, P <0.001) (Fig 5.4c). There was an interaction between the genotype and week (F = 1.13, P =0.002), populations on MDR045 and MDR049 were more static than those on Sosltice.

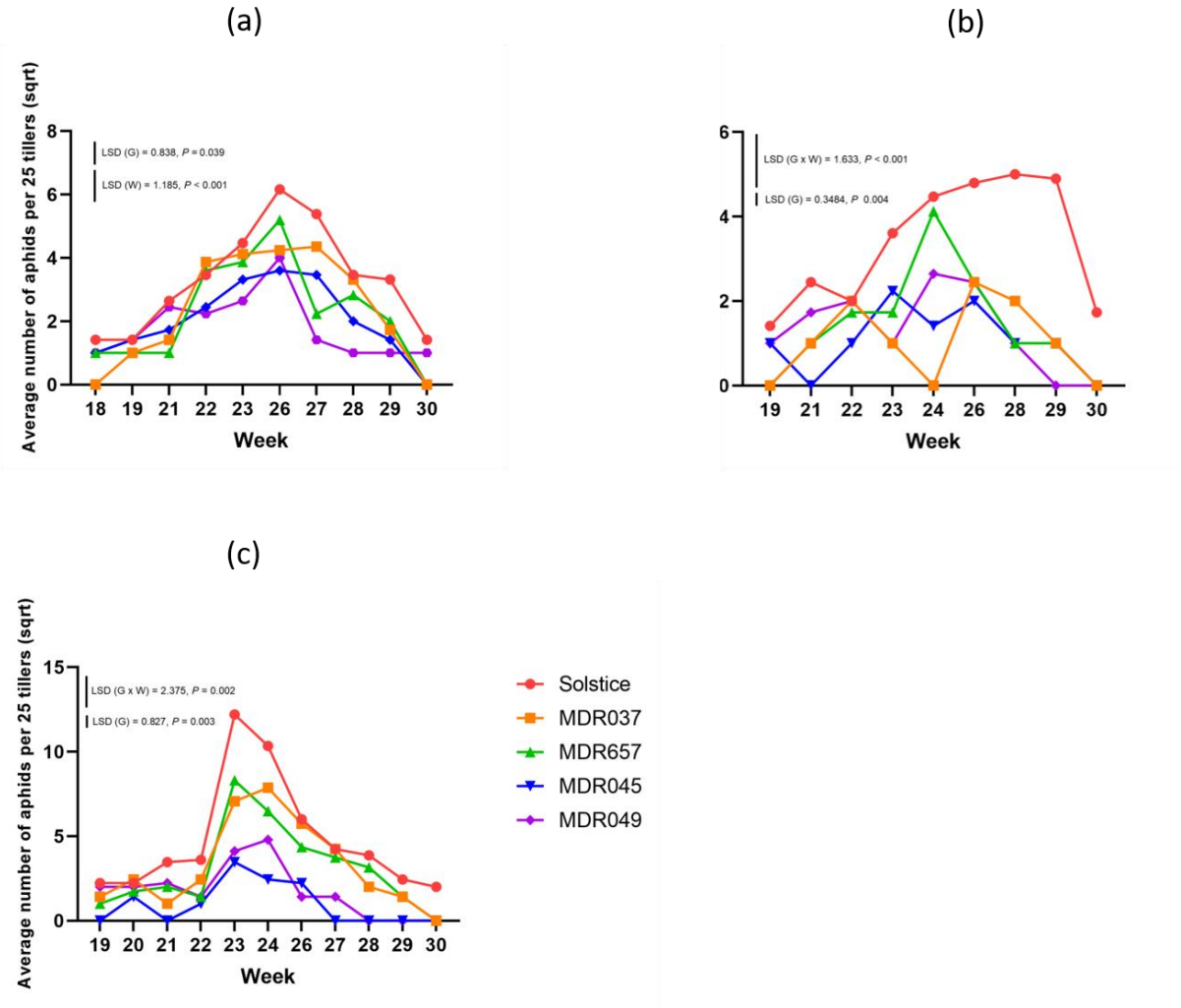


Figure 5.4. Average number of aphids (*Rhopalosiphum padi*, *Sitobion avenae* and *Metopolophium dirhodum*) per 25 tillers observed on *Triticum aestivum* Solstice, *Triticum moncoccum* MDR037, MDR657, MDR045 and MDR049 in field trial years **a)** 2017, **b)** 2018, **c)** 2019 from week 18 to week 30. All data was subject to square root transformation. The least significant G represents the genotypes, W represents the week.

5.3.1.4 Alate and apterous aphid populations

Winged (alate) aphids (which made up 6.5% of the field trial aphid populations) are often the initial colonisers of cereal crops, so their presence was recorded. The fewest number of alate aphids was observed on MDR045 and MDR049 ($F = 1.71$, $P < 0.001$) (Fig 5.5). There was no difference between the number of alate aphids present between the three field trials ($F = 2.90$, $P = 0.167$) (Fig 5.5). The highest number of alate aphids was observed in week 23 ($F =$

1.20, *P*=0.024) (Fig 5.5). There was an interaction between the field trial year and week (*F* = 1.11, *P*=0.019), however there was no interaction between the week and genotype (*F* = 1.00, *P*=0.607) nor between the year and genotype (*F* = 0.37, *P*=0.292) (Fig 5.5) Due to low numbers, the number of alate aphids per field trial year is presented.

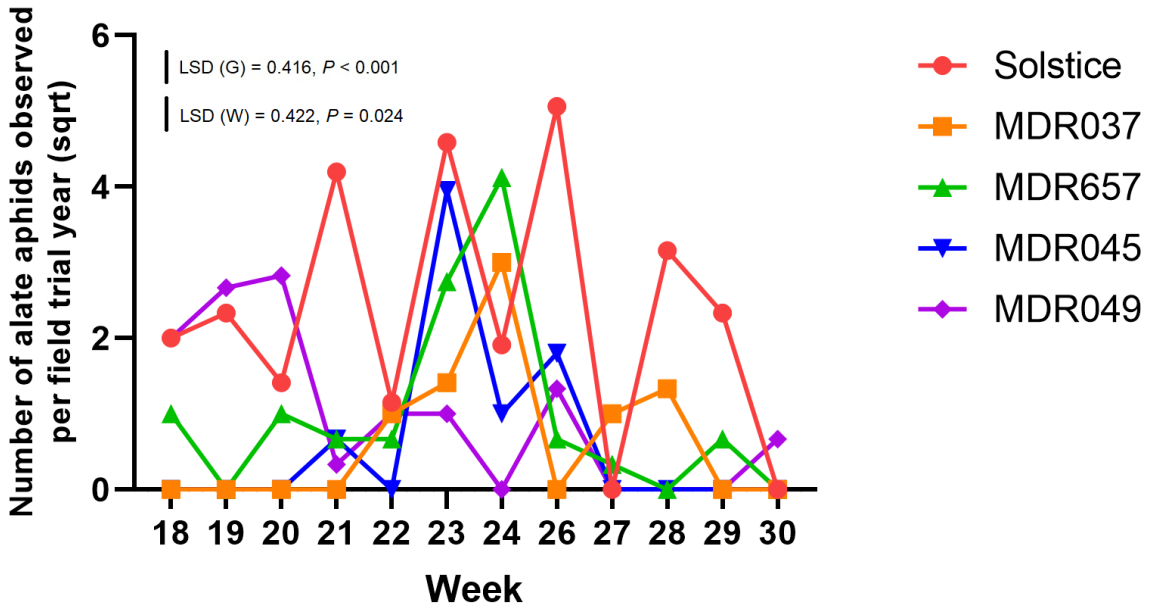


Figure 5.5. The number of alate aphids per field trial observed on **a)** *Triticum aestivum* Solstice, *Triticum moncoccum* MDR037, MDR657, MDR045 and MDR049. Data was subject to square root transformation. The least significant G represents the genotypes, W represents the week.

The highest proportion of alate aphids was present on MDR045 (*F* = 5.24, *P*<0.001) (Fig 5.6a) and in week 18 and 20 (*F* = 0.81, *P* <0.001) (Fig 5.6b). As with the number of alate aphids (Fig 5.6), there was no difference in the proportion of alate and apterous aphids between the field trial years (*F* = 1.18, *P* =0.21).

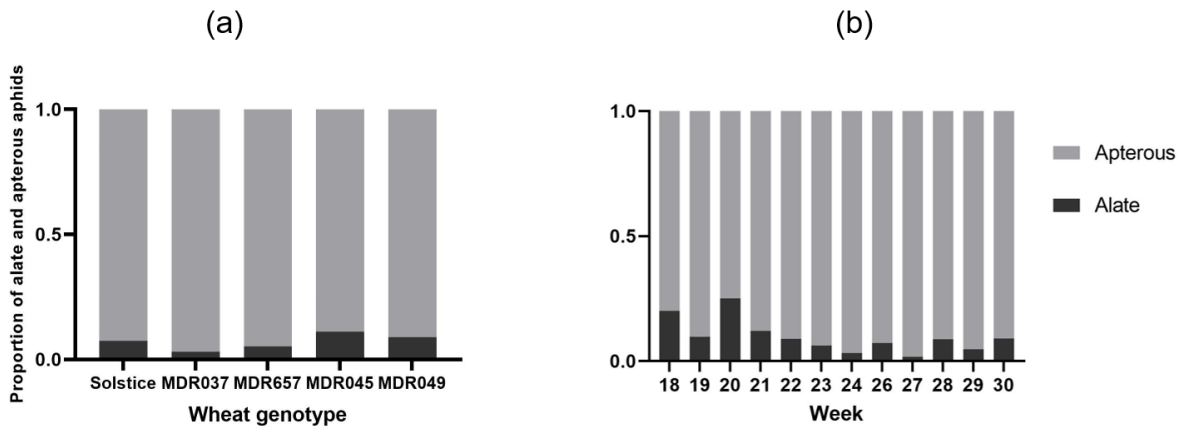


Figure 5.6. The proportion of alate and apterous aphids observed on **a)** *Triticum aestivum* Solstice, *Triticum moncoccum* MDR037, MDR657, MDR045 and MDR049 **b)** plants from week 18 to week 30.

5.3.1.5 Proportion of aphid species

The aphid population were compromised of *R. padi* which made up 22% of the aphid populations, *S. avenae* 53% and *M. dirhodum* 25% of the population across the three field trials. There was no difference in the proportion of *R. padi*, *S. avenae* and *M.dirhodum* across the wheat genotypes ($F = 2.47$, $P=0.63$) (Fig 5.7a). The highest proportion of *R. padi* and *M. dirhodum* were present in the 2017 field trial ($F = 14.74$, $P=0.006$) (Fig 5.7b). The highest proportion of *S. avenae* was present in week 24 ($F = 0.99$, $P=0.027$) (Fig 5.7c).

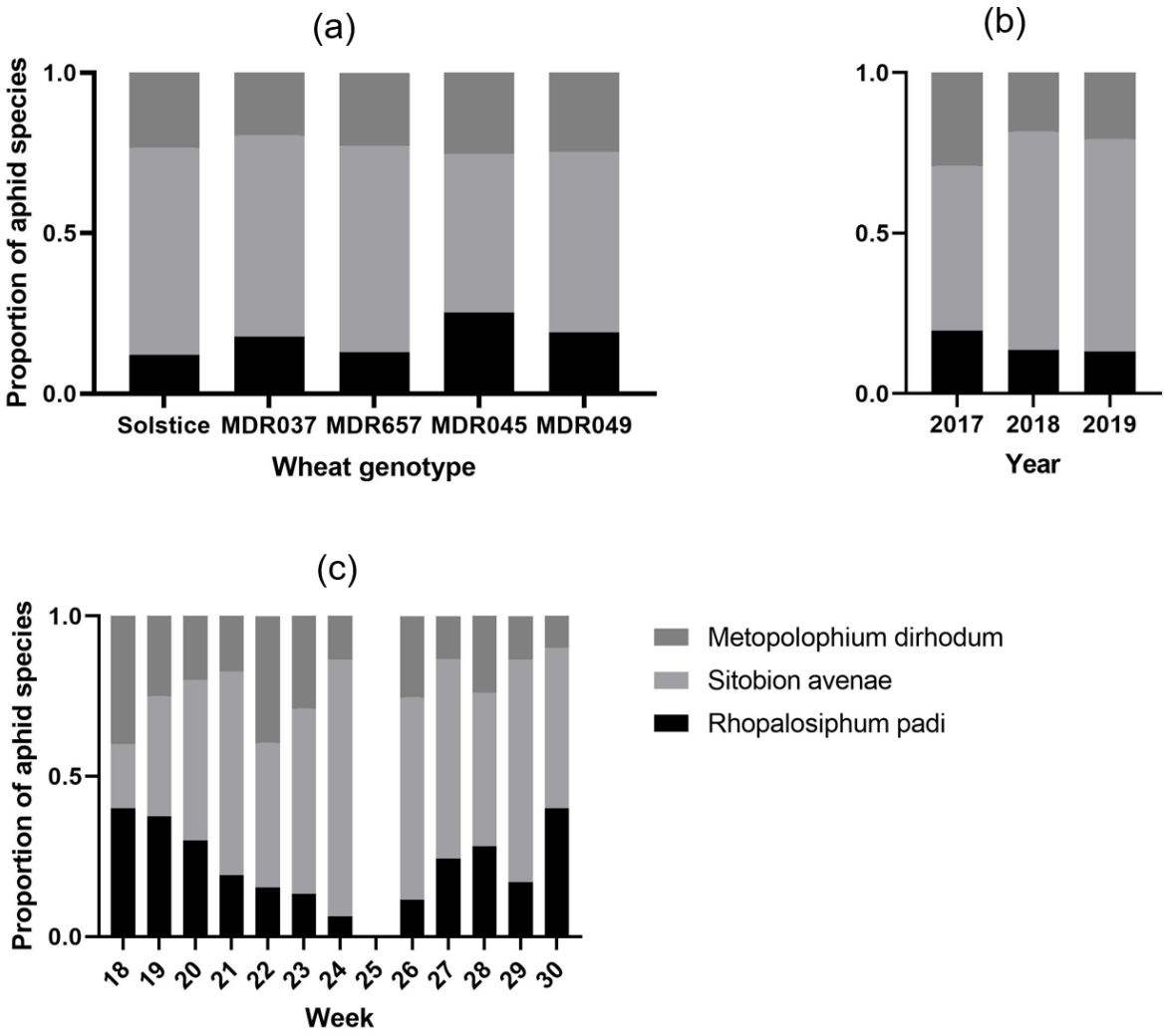


Figure 5.7. The proportion of *Rhopalosiphum padi, Sitobion avenae* and *Metopolophium dirhodum* observed on **a)** *Triticum aestivum* Solstice, *Triticum moncoccum* MDR037, MDR657, MDR045 and MDR049 **b)** plants in field trial year 2017, 2018 and 2019. **c)** plants from week 18 to week 30.

5.3.2 Natural enemy presence

Overall natural enemy presence was affected by wheat genotype and the week. The highest total natural enemy presence was observed on Solstice and the lowest on MDR045 ($F = 0.59$, $P < 0.001$) (Fig 5.8). There was no difference in the total natural enemy presence across the three field trial years ($F = 0.40$, $P = 0.842$) (Fig 5.8). The highest total number of natural enemies were observed on in week 26 ($F = 4.47$, $P = 0.034$) (Fig 5.8). There was no

interaction between the field trial year and week (F = 0.218, P =0.995), genotype and week (F = 0.70, P =0.465), nor year and genotype (F = 0.71, P =0.088)

5.3.2.1 Field trial 2017

The highest natural enemy presence per 25 tillers was on Solstice (F = 0.94, P <0.001) and during weeks 23 to 28 (F = 0.99, P <0.001) (Fig 5.8a). There was no interaction between the week and genotype (F = 0.43, P =0.134)

5.3.2.2 Field trial 2018

The highest natural enemy presence per 25 tillers was on Solstice (F = 0.72, P <0.001) and during weeks 24 to 26 (F = 0.84, P =0.029) (Fig 5.8b). There was an interaction between the week and genotype (F = 0.71, P < 0.001)

5.3.2.3 Field trial 2019

The highest natural enemy presence per 25 tillers was on Solstice (F = 0.56, P =0.001), however there was no difference in natural enemy presence across the field trial weeks (F = 0.48, P =0.193) (Fig 5.8c). There was an interaction between the week and genotype (F = 0.55, P =0.006)

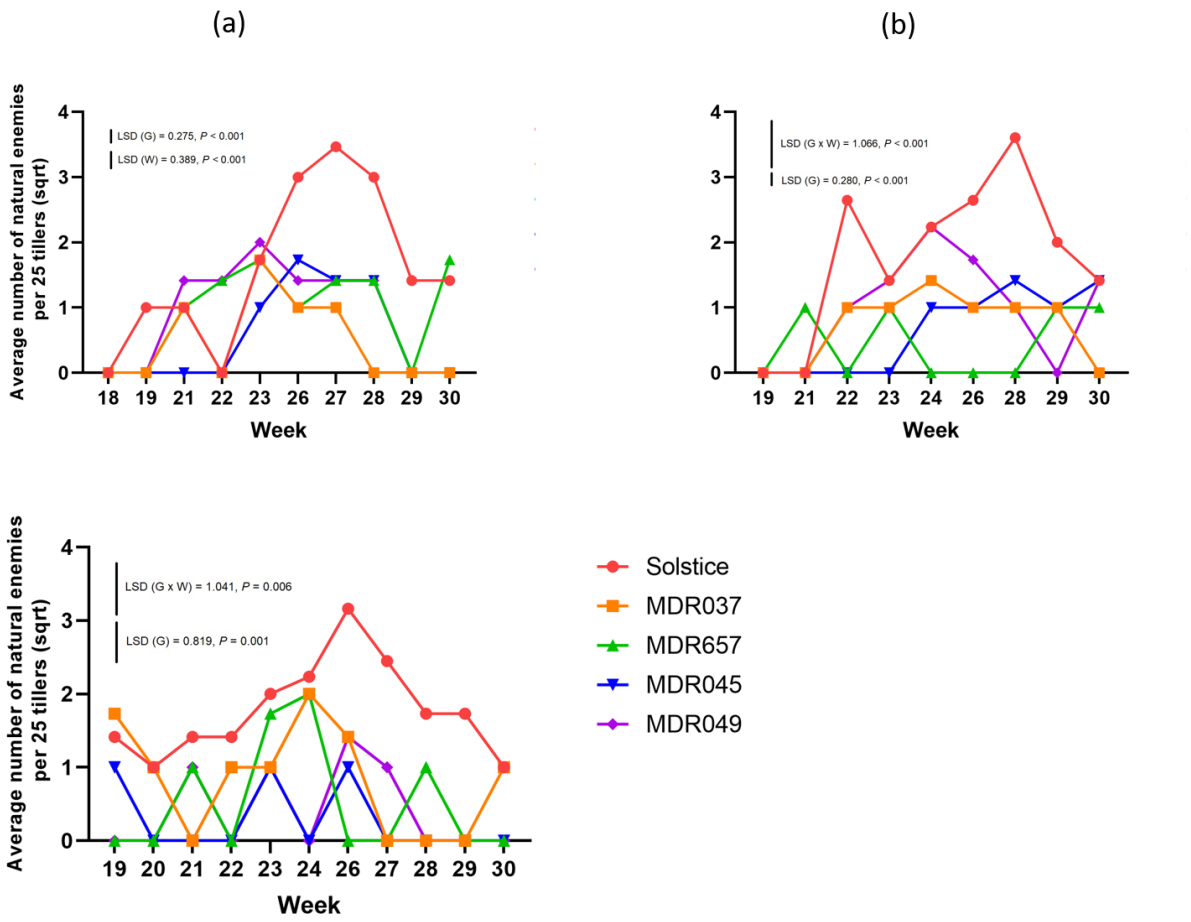


Figure 5.8. Average number of natural enemies presence (parasitised mummified aphids, Ladybird and Chrysopidae eggs and larvae) per 25 tillers observed on *Triticum aestivum* Solstice, *Triticum moncoccum* MDR037, MDR657, MDR045 and MDR049 in field trial years **a)** 2017, **b)** 2018, **c)** 2019 from week 18 to week 30. All data was subject to square root transformation. The least significant G represents the genotypes, W represents the week

5.3.2.4 Proportion natural enemies

Natural enemy populations were comprised parasitised mummified aphids which made up 61% of natural enemy populations, Chrysopidae eggs and larvae 25%, and Ladybird adults and larvae 14%.

The highest proportion of parasitised aphids and the lowest proportion of ladybirds were observed on Solstice and MDR049 (F = 0.59, P <0.001) (Fig 5.9a). The highest proportion of parasitised aphids was overserved in the field trial year 2018 and the highest proportion of Chrysopidae eggs and larvae were observed in 2017 (F = 0.99, P = 0.006) (Fig 5.9b). The

largest proportion of ladybirds was observed in week 19 and the largest proportion of parasitised aphids were observed in week 24 ($F = 2.68$, $P = 0.047$) (Fig 5.9c).

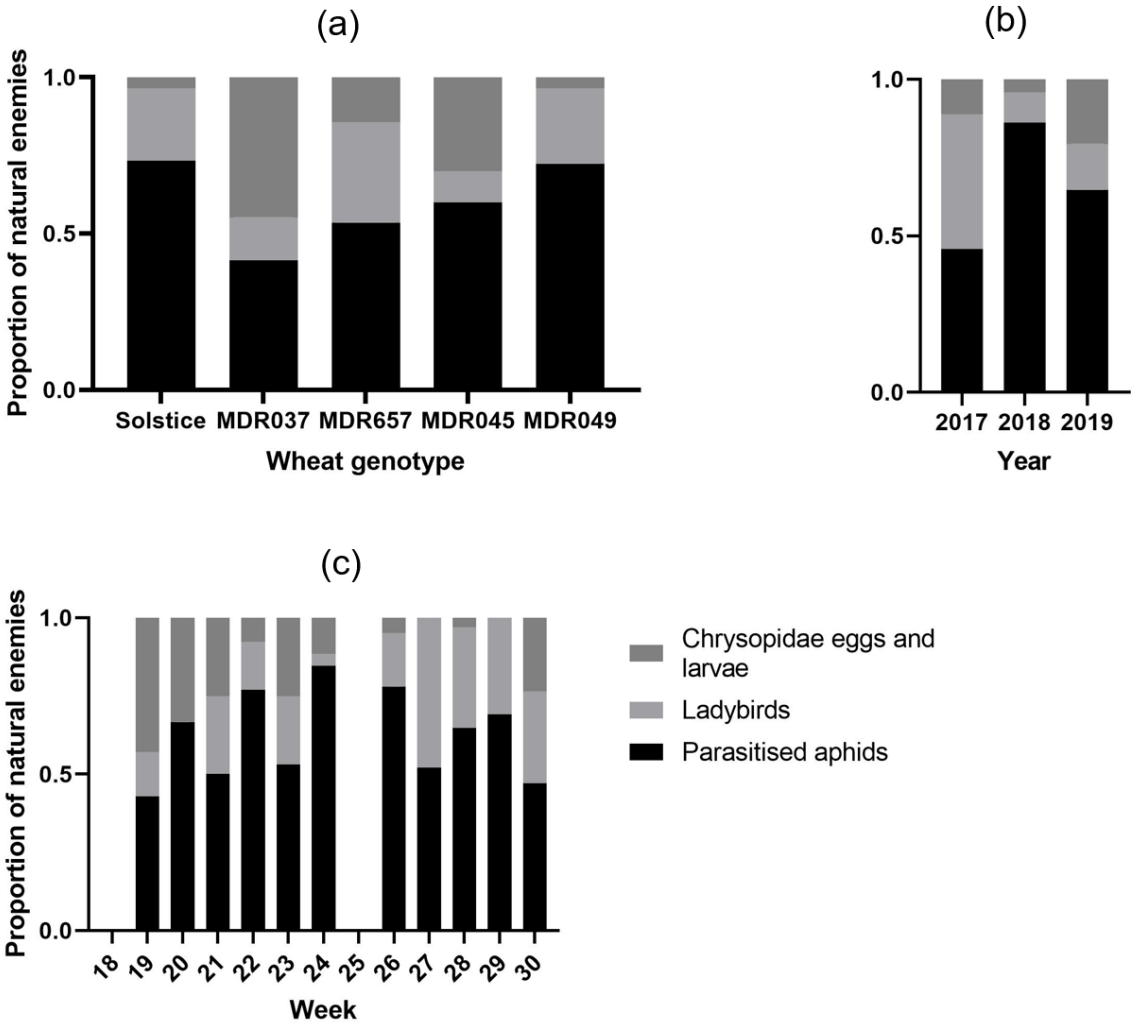


Figure 5.9. The proportion of natural enemies (parasitised mummified aphids, Ladybird adults and larvae and Chrysopidae eggs and larvae) presence observed on **a)** *Triticum aestivum* Solstice, *Triticum moncoccum* MDR037, MDR657, MDR045 and MDR049 **b)** plants in field trial year 2017, 2018 and 2019. **c)** plants from week 18 to week 30.

5.3.3 Aphid and natural enemy populations

Aphids made up 73.1% of all insects recorded across all of the field trials. The lowest proportion of aphids were observed on Solstice and MDR049 ($F = 20.70$, $P = 0.032$), in the 2019 field trial ($F = 20.83$, $P < 0.001$) and week 30 ($F = 16.47$, $P = 0.004$) (Fig 5.10).

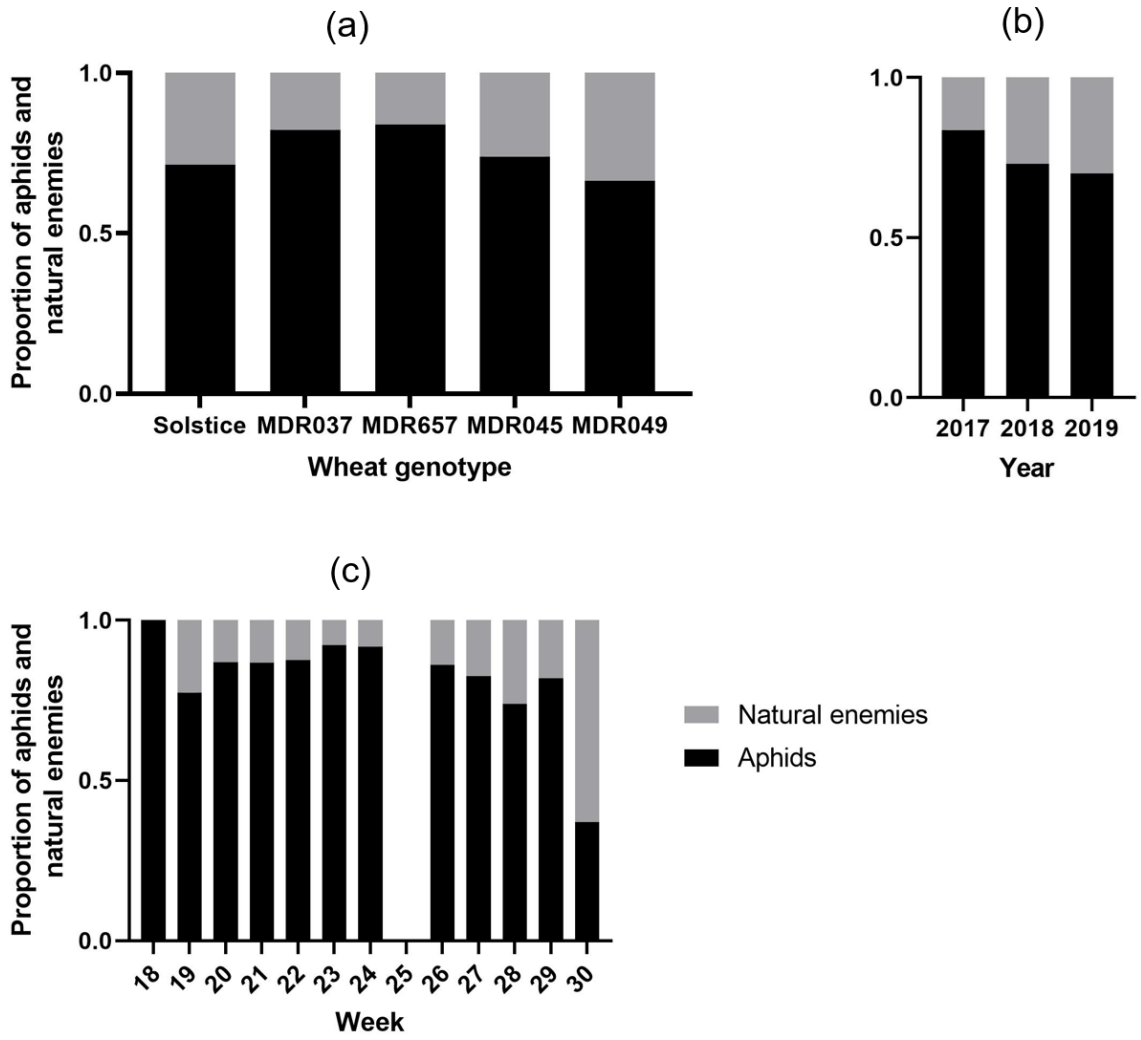


Figure 5.10. The proportion of aphids (*Rhopalosiphum padi, Sitobion avenae* and *Metopolophium dirhodum*) and natural enemies (parasitised aphids, Ladybird adults and larvae and Green lacewing eggs and larvae) presence observed on **a)** *Triticum aestivum* Solstice, *Triticum moncoccum* MDR037, MDR657, MDR045 and MDR049 **b)** plants in field trial year 2017, 2018 and 2019. **c)** plants from week 18 to week 30.

To determine whether there was a correlation between the average number of aphids and natural enemies observed throughout all three field trials, a Pearsons correlation was carried out.

There was a positive correlation between the number of aphids and natural enemies in the crop ($R = 0.54$, $P < 0.001$) (Fig 5.11).

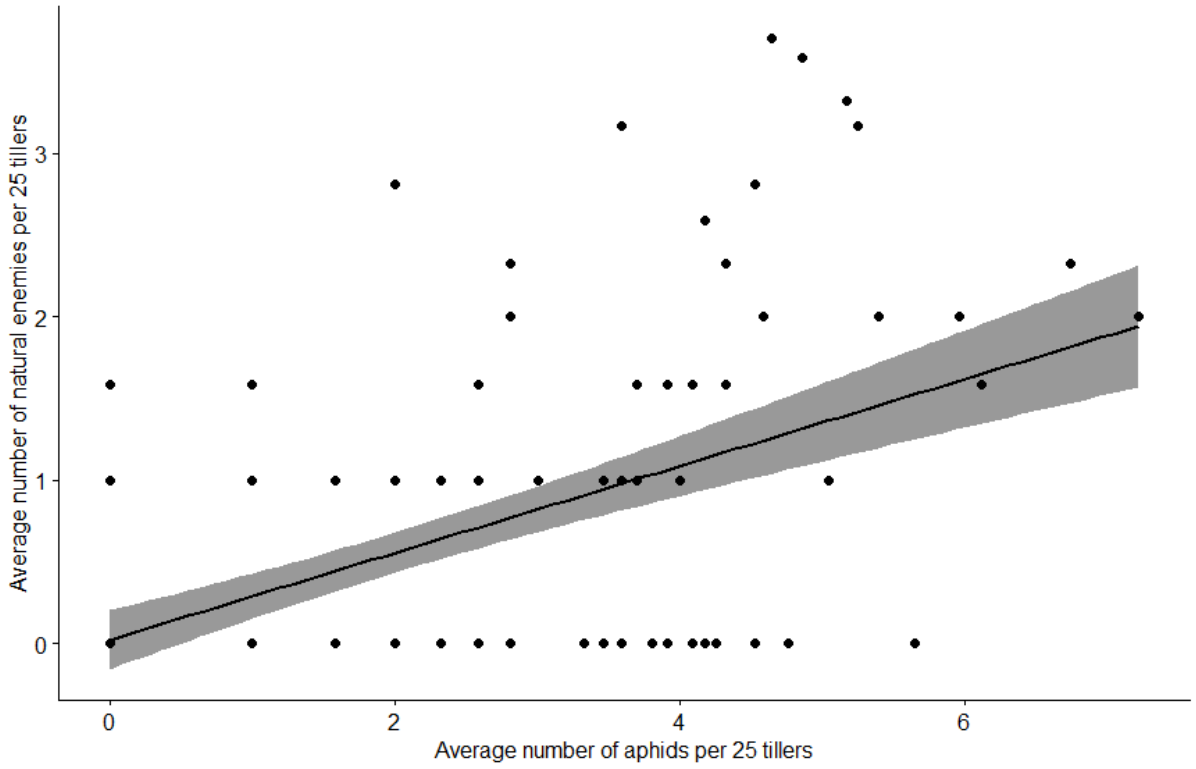


Figure 5.11. Correlation between the average number of aphids per 25 tillers and the average number of natural enemies per 25 tillers. Shading represents confidence intervals.

5.3.4 Temperature and rainfall during field trials

Data on temperature and rainfall was obtained for the three summers insects were monitored in the field trials from the Rothamsted Long-term Experiments National Capability (LTE-NCG) e-Rothamsted Archive. The summer of 2018 had the highest maximum temperatures and the least amount of rain in June and July (Table 5.1).

Table 5.1. Average maximum temperature, minimum temperature, rainfall and rainfall duration ± SD for May, June and July 2017, 2018 and 2019 at Rothamsted Research, Harpenden, Hertfordshire, UK.

	2017			**2018**			**2019**		
	May	**June**	**July**	**May**	**June**	**July**	**May**	**June**	**July**
Maximum temperature (°C)	17.74 ± 4.21	21.52 ± 4.38	21.77 ± 3.03	18.67 ± 3.70	21.54 ± 3.27	26.06 ± 3.10	16.32 ± 3.16	19.30 ± 4.19	23.16 ± 4.08
Minimum temperature (°C)	8.65 ± 3.11	12.01 ± 2.43	13.21 ± 1.60	7.86 ± 3.39	10.87 ± 2.43	13.81 ± 1.55 c	6.34 ± 3.02 a	10.16 ± 2.81 b	12.97 ± 2.95 c
Rainfall (mm)	2.26 ± 5.06	1.29 ± 3.72	2.38 ± 4.78	2.00 ± 4.91	0.12 ± 0.28	0.49 ± 1.17	1.16 ± 2.44 a	2.86 ± 4.38 ab	1.08 ± 2.33 b
Rainfall duration (hr)	1.23 ± 2.64	0.75 ± 2.00	1.12 ± 2.20	0.79 ± 1.56 a	0.11 ± 0.24 ab	0.28 ± 0.72 b	0.72 ± 1.36 a	1.32 ± 2.41 ab	0.19 ± 0.43 b

A Pearsons correlation was carried out to determine if size aphid and natural enemy populations observed in the field were correlated with meterological factors, but no correlations were oberserved for temperature, rainfall and rainfall duration (Appendix Fig C2, C3).

5.3.5 Aphid populations in Rothamsted Research suction trap

The highest total number of alate aphids (*Rhopalosiphum padi*, *Sitobion avenae* and *Metopolophium dirhodum*) recorded in the suction trap was in 2019 and the lowest in 2017. Within each year the highest number of aphids was in week 27 in 2017 and 2018, and week 25 in 2019. *Sitobion avenae* was the most abundant aphid across all three years (Table 5.2).

Table 5.2. Total number of alate *Rhopalosiphum padi*, *Sitobion avenae*, *Metopolophium dirhodum* observed in Rothamsted Insect Survey suction trap (RIS) at Rothamsted Research, Harpenden, Hertfordshire, UK and the total number observed in the field trials (FT) from week 18 to 30 in 2017, and week 19 to 30 in 2018 and 2019. A (-) symbol represents a week where FT aphid numbers were not recorded.

Year	Week	*Rhopalosiphum padi*		*Sitobion avenae*		*Metopolophium dirhodum*		Total aphids	
		RIS	FT	RIS	FT	RIS	FT	RIS	FT
2017	18	0	4	1	0	0	8	1	12
	19	3	8	0	0	0	0	3	8
	20	1	-	1	-	0	-	2	-
	21	10	4	4	4	4	4	18	12
	22	10	4	0	0	8	0	18	4
	23	1	12	1	8	3	8	5	28
	24	2	-		-	29	-	31	-
	25	3	-	3	-	6	-	12	-
	26	4	12	7	0	16	4	27	16
	27	8	0	48	4	18	0	74	4
	28	3	4	16	4	0	12	19	20
	29	4	4	18	0	4	0	26	4
	30	2	0	3	0	0	0	5	0
2018	19	3	0	2	0	0	8	5	8
	20	1	-	0	-	1	-	2	-
	21	6	4	2	4	2	0	10	8
	22	9	4	0	0	4	4	13	8
	23	0	0	0	12	7	12	7	24
	24	1	0	1	0	2	0	4	0
	25	6	-	3	-	22	-	31	-
	26	7	4	22	8	45	0	74	12
	27	39	-	93	-	78	-	210	-
	28	17	8	80	8	23	8	120	24
	29	7	0	35	4	4	0	46	4
	30	0	0	16	0	0	0	16	0
2019	19	0	0	5	0	1	4	6	4
	20	8	12	17	0	0	8	25	20
	21	2	4	26	4	9	8	37	16
	22	15	4	53	4	2	0	70	8
	23	15	8	15	32	3	12	33	52
	24	7	8	14	4	14	12	35	24
	25	116	-	78	-	16	-	210	-
	26	23	16	46	8	32	4	101	28
	27	4	0	21	4	17	0	42	4
	28	8	0	20	0	23	4	51	4
	29	1	0	11	0	9	4	21	4
	30	0	0	12	0	9	0	21	0

To determine whether alate populations observed in the field were correlated with the populations captured by the suction traps, a linear regression was carried out for each aphid species.

There was a positive correlation between the number of alate *S. avenae* observed in field trials and in the suction traps ($R = 0.43$, $P = 0.05$) (Fig 5.12b). There was no correlation

between insect survey presence and field observations for alate *R. padi* ($R = 0.48$, $P = 0.06$) and *M. dirhodum* ($R = 0.09$, $P = 0.68$) and total aphids ($R = 0.14$, $P = 0.44$) (Fig 5.12a, c, d)

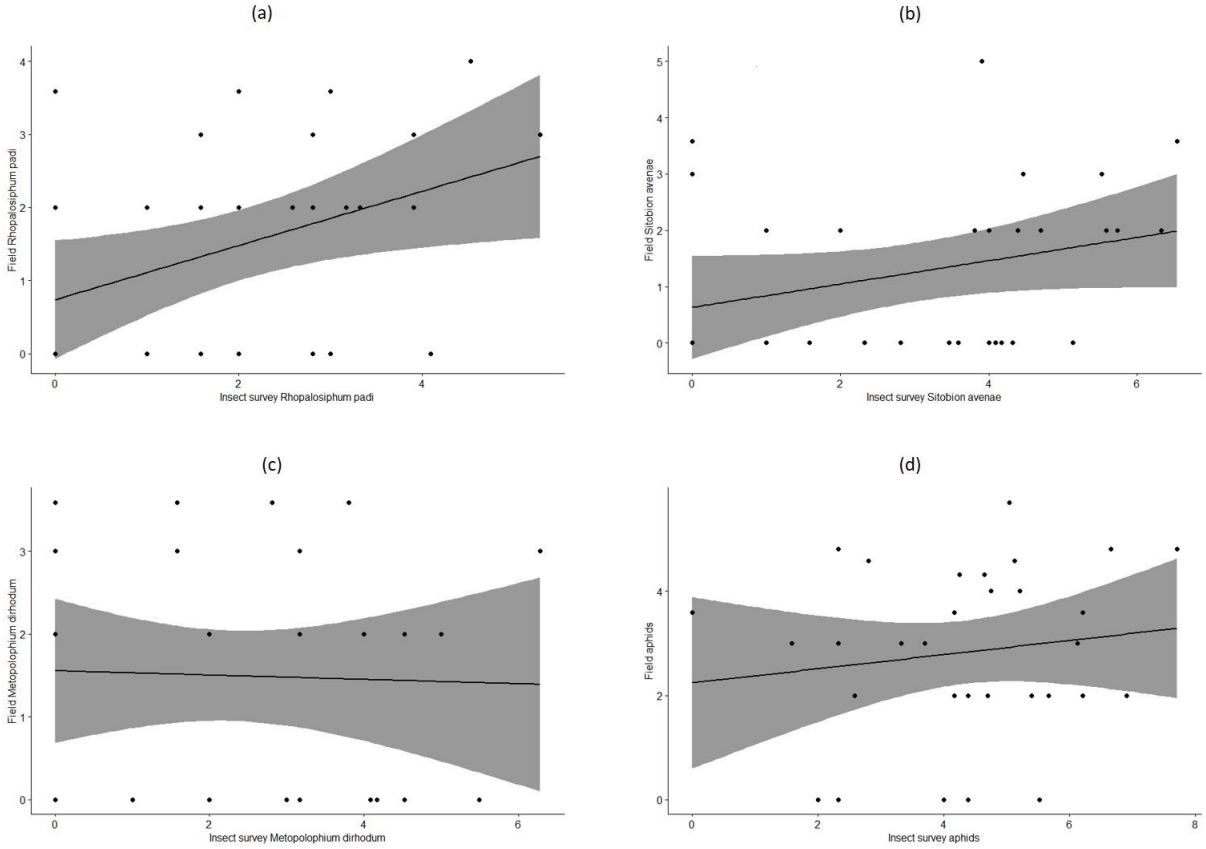


Figure 5.12. Correlation between the total number of alate **a)** *Rhopalosiphum padi* **b)** *Sitobion avenae* **c)** *Metopolophium dirhodum* **d)** aphids observed in the Rothamsted Insect Survey suction trap at Rothamsted Research, UK, and the total number of alate *S. avenae* observed in the field trials from week 18 to 30 in 2017, 2018 and 2019. Shading represents confidence intervals.

5.4 DISCUSSION

Aphid populations and natural enemy presence on five wheat genotypes were recorded across three years. A caveat of these field studies is the size of the field plots ($0.6m^2$ - $1m^2$). With small plot sizes it is hard to know whether aphid and natural enemy presence and interactions observed in these studies are applicable on a large field scale.

5.4.1 Aphid populations and natural enemy presence on wheat genotypes

Under field conditions *Triticum monococcum* has been previously shown to confer and antibiosis (post-alighting resistance) to *Sitobion avenae* and *Metopolophium dirhodum* and antixenosis (pre-alighting resistance) to *S. avenae*. In these studies only one *T. monococcum* genotype was investigated, the identity of which is not given (Sotherton and Lee, 1988; Hu et al., 2016). It has been shown that aphid infestation varies between cereal crop genotypes. For example, aphid (*Rhopalosiphum padi, Sitobion avenae* and *Metopolophium dirhodum*) infestation varied significantly between 122 Watkins land races in field trials (Aradottir et al., 2017). Under laboratory conditions, antibiosis and antixenosis in *T. monococcum* has been shown to be genotype dependant (di Pietro *et al.* 1998; Greenslade *et al.* 2016; Simon *et al.* 2017; Chapter 2; Chapter 3). *Triticum monococcum* genotypes MDR657 and MDR049 have been shown to confer antibiosis to *Rhopalosiphum padi* (Greenslade et al., 2016), MDR045 and MDR049 have been shown to confer antibiosis to *Sitobion avenae* (Simon et al., 2017), and MDR049 has been shown to confer antixenosis to *R. padi* (Chapter 2). In these studies, MDR037 was shown to be susceptible to aphid colonisation and infestation (Greenslade *et al.* 2016; Simon *et al.* 2017; Chapter 2). This chapter shows that smaller *R. padi, S. avenae* and *M. dirhodum* populations were observed on MDR045 and MDR049 in field conditions. As aphid populations on MDR657 were similar to those observed on MDR037, it is concluded that this genotype did not confer resistance to these aphid species in the field trials.

Lower aphid populations observed on MDR045 and MDR049 could be due to antixenosis or antibiosis, or both. Antixenosis relies on semiochemical and/or visual cues that prevent alate aphids alighting on the plant (Kogan and Ortman, 1978; Schröder et al., 2014). It has been shown that semiochemical production can vary between crop genotypes (Weaver et al., 2009; Dong et al., 2015; Mattiolo et al., 2017) and that this can affect cereal aphid behaviour towards different crop genotypes, for example, under controlled conditions, alate

R. padi only moved towards *Zea mays* (L.) (maize) genotype 6Q-121 semiochemicals and not semiochemcials from genotype 78-15B and CRN 3505 (Schröder et al., 2015). Fewer alate aphids were observed on MDR045 and MDR049 which indicates non-preference and that antixenosis was present in these genotypes, this, coupled with a reduced rate of reproduction (Chapter 3) would lead to lower populations as shown here, hence lower aphid number on MDR045 and MDR049 are likely due to a combination of antixenosis and antibiosis.

The highest number of alate aphids was observed on Solstice and MDR037, this suggests that the semiochemicals produced from these plants in the field were attractive to alate aphids. This work shows that the extrinsic factor of host genotype can affect aphid populations under field conditions.

The lowest number of aphids was observed on MDR045 and MDR049, however, the natural enemy presence of parasitised aphids and lacewing eggs and larvae presence on MDR049 was comparable to their presence on MDR037 and MDR657. This indicates that MDR049 recruited more of these natural enemies in relation to its aphid population. Natural enemies use HIPVs to locate their host/prey. Parasitoids *Aphidius gifuensis* (A.), *Encarsia formosa* (G.), ladybird *Coccinella septempunctata* (L.) have been shown to move towards HIPVs (Ninkovic et al., 2001; Tan and Liu, 2014) and the green lacewing *Chrysopa nigricornis* (B.) and lacewing *Hemerobius* (L.) were attracted to the HIPV methyl salicylate (James and Price, 2004). The lower aphid numbers observed on MDR049 may not only be due to antibiosis and/or antixenosis, but increased predation and/or parasitism. It has been shown that natural enemies perform better on resistant plants, in a glasshouse study, lacewing *Chrysoperla plorabunda* (F.) larvae reduced aphid populations more on resistant transgenic wheat line (Messina and Sorenson, 2001), lacewing eggs on resistant plants also had higher larval emergence and the proportion of female larvae increased on transgenic resistant *T. aestivum* than on the isogenic susceptible plants (Farid et al., 1998). Therefore, it is hypothesised that

the lower number of aphids present on MDR049 are due to a combination of fewer alate aphids colonising, combined with slower aphid population growth, and more natural enemy presence. To further investigate natural enemy population effects on MDR049, exclusionary and inclusionary cage studies could be carried out in controlled or field conditions.

Natural enemy presence was also dependant on wheat genotype as the highest presence was on Solstice and the lowest presence on MDR045. Natural enemies recorded was made up of parasitised aphids, lacewing eggs and larvae and ladybird adults and larvae. Parasitised aphids and lacewing eggs and larvae were most abundant on Solstice. Solstice had the highest aphid densities out of all the wheat genotypes studied. Higher levels of *Acyrthosiphon pisum* survival have been linked with increased *Aphis ervi* parasitism (Hufbauer, 2001, 2002) and parasitised aphids have been positively correlated with aphid numbers (Garratt et al., 2010). Some studies, such as de Lange *et al.* (2016) have shown natural enemy preference for ancestral genotypes, such as the parasitoid *C. marginiventris* which was more attracted to HIPV from ancestral maize than modern maize (de Lange et al., 2016). This work shows that parasitoids and lacewing eggs and larvae do not prefer ancestral genotypes.

Ladybird beetles and larvae were most abundant on the ancestral MDR037 genotype indicating that this natural enemy may have a preference for ancestral genotypes. It has been shown that ladybird *Stethorus gilvifrons* (M.) movement towards red spider mite (*Tetranychus urticae* (K.)) infested sweet potato (*Ipomoea batatas* (L.)) depended on the cultivar (Gencer et al., 2009) and that the ladybird *C. septempunctata* preferred volatiles from the *R. padi* infested *Hordeum vulgare* cultivar that their prey had fed on (Glinwood et al., 2011). This shows that wheat genotype and the production of HIPV can affect natural enemy behaviour.

5.4.2 Aphid populations and natural enemy presence across field trial years and weeks

Aphid populations varied between years, the lowest number of aphids was observed in the summer of 2018, which had the highest average maximum and minimum temperature, as well as having the least average rainfall in June and July. The UK Meteorological Office declared a heatwave on 23rd June 2018. A heatwave is "A marked unusual hot weather (Max, Min and daily average) over a region persisting at least two consecutive days during the hot period of the year based on local climatological conditions, with thermal conditions recorded above given thresholds." (*Guidelines on the Defintion and Monitoring of Extreme Weather and Climate Events*, 2016). The number of alate aphids observed did not change between field trial years, however, the lowest number of aphids was observed in the 2018 field trial. This indicates that the heatwave and meterological conditions affected aphid development and reproductive success on plants in the 2018 field trial.

Climate change scenarios predict more frequent and intense summer droughts (Dai, 2013) which can affect aphid populations, as temperature can affect aphid development and fecundity (Strazyński and Ruszkowska, 2015). Each aphid species has an optimal developmental and fecundity temperature (Table 5.3). Aphids experiencing above optimal temperatures can experience heat stress which can affect their development, reproduction and longevity.

Table 5.3. The optimal development and reproductive temperatures of *Rhopalosiphum padi*, *Sitobion avenae* and *Metopolophium dirhodum*

Aphid species	Optimal development temperature (°C)	Optimal reproduction temperature (°C)	References
Rhopalosiphum padi	25	25	(Dean, 1974; Park et al., 2017)
Sitobion avenae	25	18	(Asin and Pons, 2001)
Metopolophium dirhodum	20	20	(Dean, 1974)

Temperatures of 27.5°C have been shown to decrease *S. avenae* and *M. dirhodum* developmental rates, reduced *S. avenae* and *R. padi* fecundity and caused 100% nymph mortality in *M. dirhodum* (Asin and Pons, 2001). The average temperature in July 2018 was 26°C, which is sub-optimal especially for *S. avenae* reproduction and *M. dirhodum* development and reproduction. Therefore, the weather may have affected aphid development and reproduction. The maximum temperature, minimum temperature, rainfall and rainfall duration were not correlated with aphid numbers so these meteorological factors alone did not affect aphid populations. These results are presented in the Appendix (Appendix Fig C2, C3)

It has been shown that abiotic stress such as heat or drought can affect plant host quality which in turn can affect herbivore populations (Awmack and Leather, 2002). Ivestigations on the affect of drought on spruce aphid (*Elatobium abietinum* (Wlk.)) interactions with spruce trees (*Picea sitchensis* (Bong)) has shown that whilst intermittent drought improved spruce aphid (*Elatobium abietinum* (Wlk.)) development (Banfield-Zanin and Leather, 2015c), population growth (Banfield-Zanin and Leather, 2014) and reproductive success (Banfield-Zanin and Leather, 2015b), *E. abietinum* had reduced reproductive success and took longer to produce their first nymph on *P. sitchensis* that experienced severe

prolonged drought (Banfield-Zanin and Leather, 2015a, 2015b). It is unknown how intermittent the drought conditions were in the 2018 field trial, and how this affected plant nutitional quality for aphids. The lower aphid numbers on the 2018 plants is supported by the plant vigour hypothesis which states that herbivores would perform better on vigorous plants than on drought-stressed plants (Price, 1991) due to higher nutrient availability (Price, 1991) and lower abscission rates (Preszler and Price, 1995). Drought stress has been shown to affect aphid feeding behaviour, *R. padi* feeding on drought stressed *H. vulgare* had a lower proportion of probes where phloem feeding occurred and salivated for longer than those on well-watered plants (Ponder et al., 2001). On drought stressed *H. vulgare, S. graminum* had slower development rate than those on well-watered plants (H. Marino Cabrera et al., 1995). It has also been shown that *R. padi* and *S. avenae*, on drought-stressed *T. aestivum* produced fewer nymphs and had a lower intrinsic rate of increase (Pons and Tatchell, 1995). Therefore, it is hypothesised that drought-stressed plants, were lower quality host-plants, reduced aphid feeding and aphid development and reproductive success in the 2018 field trial.

There was an interaction between the field trial year and the wheat genotype. Within the *T. monococcum* genotypes, MDR037 had the highest number of aphids in the 2017 and 2019 field trials. In 2018, MDR037 had the second lowest aphid population with lower aphid presence than MDR049. Overall aphid numbers in 2018 were reduced by 52.8% from 2017 and 74.3% from 2019. The aphid population on MDR037 in the 2018 field trial reduced 80.2% compared to 2017 and 90.3 % compared to aphid numbers on MDR037 plants in the 2019 field trial. MDR037 has also been shown to be susceptible to *R. padi* and *S. avenae* infestation under controlled conditions (Greenslade et al., 2016; Simon et al., 2017). This presents a hypothesis that drought/heat stress reduced MDR037 suitability as a host to aphid infestation.

A simulated heatwave showed that a peak temperature of 30°C for one hour daily reduced *M. persicae* abundance on sweet pepper (*Capsicum annuum* (L.)) but had no effect on adult ladybird *Coleomegilla maculata* (T.) (Sentis et al., 2013). This chapter also shows that the number of total natural enemy presence did not change between field trial years; however, the abundance of the three types of natural enemies did change, affecting the proportion of individual natural enemies over the three field trials. The highest proportion of parasitised aphids was observed in 2018 and the lowest in 2017. The endosymbiont *Hamiltonella defensa* (M.) presence in aphids has been shown to provide protection against parasitism (Bensadia et al., 2006; Vorburger et al., 2013), however, defence against *A. ervi* parasitism that was held in *A. pisum* with *H. defensa* at 20°C, was reduced at 25°C and aphids were susceptible to parasitism at 30°C (Bensadia et al., 2006). It has also been shown that *A. ervi* develop faster at 25°C and 30°C (Malina et al., 2010). The 2018 field trial had the highest maximum temperature with the average July temperature being 26°C. Therefore, the increased presence of parasitised aphids in the 2018 field trial may be due to a combination of reduced aphid defences against parasitism and increased parasitoid development due to higher temperatures. As the aphid parasitism increased in 2018, the lower number of aphids observed in 2018 may be a combination of reduced aphid development and reproductive success on water/heat stressed plants and increased parasitism.

Lacewing eggs and larvae were most abundant in the 2017 field trial, lacewings perform well over a wide range of temperatures and produce the highest numbers of eggs between 20°C and 27°C (Pappas and Koveos, 2011) making them versatile. It has been shown that there is a positive correlation between the temperature and *Chrysopodes lineafrons* (A.P.) development rates (Silva et al., 2007).

Ladybird beetles and larvae were most abundant in the 2019 field trial. This field trial also had the highest number of aphids observed. It has been shown that the ladybirds *C. septempunctata* and *Harmonia axyridis* (P.) (harlequin ladybird) egg laying increased as *Aphis gossypii* (G.) populations increased (Hironori and Katsuhiro, 1997). Field ladybird populations have also been positively correlated with *A. pisum* densities (Evans and Youssef, 1992).

Correlations between ladybird and aphid densities (Evans and Youssef, 1992), and parasitised aphids and aphid densities (Garratt et al., 2010) can also explain why ladybird and parasitised aphid presence was highest in week 23 and 24 respectively, Aphid densities were highest in week 23 and 24, which is in the beginning of June. In England, aphid populations on *T. aestivum* were shown to have peaked in late June/early July in the 1990's (Holland and Thomas, 1997) and *M. dirhodum* populations peaking mid-July (Howard and Dixon, 1992) which contradict this study. Both studies showed that aphid populations increased for the first 5-7 weeks of the field trials, with aphid populations peaking during these periods (Howard and Dixon, 1992; Holland and Thomas, 1997). The present study showed that overall aphid populations increased in the first 7 weeks, with aphid populations peaking during the 5th and 6th week into the field trial.

Aphid populations decreased after week 24, this could be the effects of increased predation and parasitism in weeks 23 and 24, the highest number of lacewing eggs and larvae were observed in week 27 which may have also contributed to the decline in aphid populations.

Alate aphids initially colonise plants, therefore the alate: apterous ratio was highest in week 18 and 20, this ratio decreased until 24 weeks when apterous and overall aphid populations

peaked. This was also observed in *R. padi* populations on wheat in Tikkurila, Finland where alate: nymph populations were highest during the second week of the field trial (Leather and Lethi, 1982).

5.4.3 Conclusion

This chapter has demonstrated that aphid populations are dependent on the wheat genotype with *T. monococcum* MDR045 and MDR049 possessing antixenotic and antibiotic traits against aphid infestation. In addition to this MDR049 attracted more natural enemies in relation to its aphid populations which would have also played a role in reduced aphid populations on MDR049.

Solstice and MDR037 are attractive to alate aphids, with the largest number of this morph present on these genotypes. However, in the 2018 field trial, MDR037 had one of the lowest aphid populations. As there was a heatwave in 2018, it is hypothesised that abiotic stress reduced MDR037 susceptibility to aphid colonisation.

Natural enemy presence was correlated with aphid presence with parasitised aphid and lacewing eggs and larvae presence being highest during the weeks where aphid populations were most abundant.

Chapter 6

General Discussion

6.1 DISCUSSION

This work set out to build on the studies of Greenslade *et al.* (2016); Aradottir per comms which showed that one week old *Triticum monococcum* (L.) genotypes MDR045 and MDR049 conferred antibiosis (post-alighting resistance) to *Rhopalosiphum padi* (L.). The aims were to investigate MDR045 and MDR049 resistance to *R. padi* and to unravel the determinants of this resistance.

The objectives were:

1) To establish if *Triticum monococcum* genotypes that confer antibiosis to *Rhopalosiphum padi*, emit volatile organic compounds that prevent aphid alighting (pre-alighting resistance) and if so, to identify which compounds are involved (Chapter 2).

2) To study whether antibiosis shown by one-week-old *T. monococcum* genotypes is also present in older plants (Chapter 3)

3) To identify differences in gene expression in resistant and susceptible *T. monococcum* that occur in response to *R. padi* infestation and identify candidate genes that underpin resistance traits (Chapter 4).

4) To determine whether *T. monococcum* genotypes that confer resistance under controlled conditions are resistant in field conditions and whether they more aphid natural enemies than susceptible genotypes. (Chapter 5).

In this thesis, antibiosis was identified in *T. monococcum* MDR045 and MDR049 to *R. padi*, but in MDR045 this resistance was dependant on the plant growth stage. *Triticum*

monococcum MDR049 seedlings conferred antixenosis to alate *R. padi.* Both MDR045 and MDR049 showed resistance phenotypes in field-based studies. Candidate genes that were involved in responses to *R. padi* infestation in susceptible MDR037 and resistant MDR049 were identified.

6.1.1 Identifying antixenosis in *Triticum monococcum*

Antixenosis relies on olfactory and/or visual cues that elicits a behavioural response in insects that deters them from alighting onto a plant (Kogan and Ortman, 1978). *Triticum monococcum* has been previously shown to confer antixenosis to *Sitobion avenae* and *Metopolophium dirhodum* under field conditions, however, the *T. monococcum* genotype used in these experiments was not disclosed (Sotherton and Lee, 1988; Hu et al., 2016). This is important as antixenosis can be genotype dependant, for example, in a study done by Hesler and Tharp (2005), among the five *Ttiticosecale* (W.) genotypes, four (Okto Derzhavina, N1185, N1186 and Stniism 3) conferred some antixenosis to *R. padi*. The current work showed for the first time that *T. monococcum* genotype MDR049 confers antixenosis to alate *R. padi* and that this form of resistance in *T. monococcum* is genotype dependant as alate *R. padi* had neutral/no behavioural response to MDR045 volatiles and was attracted to the volatiles of MDR037. Behavioural differences were not observed between species as *R. padi* was also attracted to the volatiles of *T. aestivum* var Solstice. Solstice and MDR037 have been shown to be susceptible to aphid infestation, whereas MDR049 confers antibiosis to *R. padi* (Greenslade et al., 2016). As *R. padi* were only attracted to volatiles from susceptible plants, this suggests that *R. padi* can discriminate between susceptible and resistant wheat genotypes.

Antennae from alate *R. padi* responded to different compounds in un-infested MDR037 (heptanal and decanal) and un-infested MDR049 (2,4-diemthyl-1-pentene, 4,4-

dimethyl-2-pentene, octanal, 4-ethylbenzaldehyde and decanal) volatile profiles. Qualitative semiochemical differences between genotypes have been shown in barley (*Hordeum vulgare* (L.)) where cultivar Su3 produced the semiochemical benzaldehyde but cultivar Su4 did not (Dong et al., 2015). Although decanal was physiologically active to *R. padi* in volatile profiles from both the resistant and susceptible plants, more decanal was produced in MDR037 (425pg/µl) than in MDR049 (200pg/µl). Quantitative semiochemical difference have been shown in *Triticum aestivum* (L.), where the cultivar Reeder produced more (Z)-3-hexenyl acetate than cultivar Conan (Weaver et al., 2009). The amount of a compound produced can also affect aphid behaviour, for example, alate *R. padi* was only attracted to (Z)-3-hexenyl acetate at 1000ng/µl and not at lower concentrations (Schröder et al., 2015). Therefore, it is likely that the qualitative and quantitative differences between the physiologically active VOCs were responsible for the different behavioural responses from *R. padi.*

Herbivore infestation changes plant semiochemistry, producing herbivore induced plant volatiles (HIPV) and causing quantitative changes in semiochemical production (Dicke and Baldwin, 2010). It has been shown that cereal aphids *R. padi, M. persicae* and the corn aphid (*Rhopalosiphum maidis* (F.)), prefer odours from un-infested plants over plants with HIPV (Quiroz et al., 1997; Bernasconi et al., 1998; Verheggen et al., 2013). This work shows that *R. padi* attraction to Solstice and MDR037 is affected by *R. padi* presence, as alate *R. padi* were attracted by volatiles from un-infested plants, but had no behavioural response to volatiles from *R. padi* infested Solstice and MDR037. Studies on the electrophysiological responses of the antennae of alate *R. padi* using GC-EAG identified qualitative differences in active compounds present in un-infested MDR037 (heptanal and decanal) and infested MDR037 (ethylbenzene, octanal, linalool) volatile profiles.

There was no difference in the alate aphid behavioural responses between the volatiles of un-infested and infested MDR045 (neutral/no behavioural response) or un-infested and infested MDR049 (repellent). Herbivore induced plant volatile production does not always affect aphid behaviour, for example, the cotton aphid (*Aphis gossypii* (G.)) had no behavioural response to both un-infested and *A. gossypii* infested pepper (*Caspium* (L.) spp) cultivar Sweet Pepper Hybrid Green Belt (da Costa et al., 2011). There were qualitative differences between physiologically active compounds in un-infested MDR049 (2,4-diemthyl-1-pentene, 4,4-dimethyl-2-pentene, octanal, 4-ethylbenzaldehyde and decanal) and infested MDR049 (heptanal, naphthalene, decanal, 4-ethylacetophenone) volatile profiles. Slightly more decanal was produced from infested MDR049 (500pg/µl) than in un-infested MDR049 (200pg/µl). Higher quantities of a compound in infested plants were also shown in the peach (*Prunus persica* (L.)) antixenotic cultivars Rubira and WFP, which produced higher amounts of (E,E)-α-farnesene when infested with the peach potato aphid (*Myzus persicae* (S.)) (Staudt et al., 2010). Tenfold changes in compound concentrations have been shown to change *A. fabae* and *R. padi* behaviour in olfactometry studies (Webster et al., 2010; Schröder et al., 2015), however, it is unknown if such small quantitative changes in decanal (± 300pg/µl) produced from un-infested and infested MDR049 would affect *R. padi* alate behaviour.

Alate aphids are produced under certain environmental conditions such as aphid over-crowding (Dixon and Glen, 1971; Wratten, 1977), these alate aphids can leave the plant easily to start a new colony, as alate *R. padi* were only attracted to un-infested susceptible plant volatiles, it is suggested that alate *R. padi* are able to discriminate between more and less suitable hosts.

Some volatile organic compounds were electrophysiologically active to *R. padi* in volatile profiles of one plant but not others, despite them being tentatively identified in those plants. Compound concentration can affect the electrophysiological responses of aphid antennae (Birkett et al., 2006), so compounds may not have been identified, especially in an EAG signal with a 'noisy' background. The identification of the tentatively identified compounds would need to be confirmed and the quantity of these compounds in the volatile profiles to be evaluated in future work.

It has been shown that mixtures of compounds can affect aphid behaviour, for example, Webster *et al.* (2010) showed that individual VOCs from *Vicia faba* were repellent to *Aphis fabae*, but when these compounds were in a blend, they were attractive. Blends of the confirmed compounds identified through GC-EAG and tentative compounds within blends were not tested for *R. padi* behavioural responses. Further studies should investigate alate *R. padi* behavioural responses to blends to understand the role of composition and ratios of VOCs in *R. padi* attraction to its host plant.

6.1.2 Aphid populations and natural enemy presence in field studies

Triticum monococcum has been shown to confer antibiosis to *S. avenae* and the rose grain aphid (*Metopolophium dirhodum* (W.)), and antixenosis to *S. avenae* (Sotherton and Lee, 1988; Hu et al., 2016) under field conditions, however, it is not known what genotypes were used in these experiments. Under controlled conditions, antibiosis has been shown to be *T. monococcum* genotype dependant with MDR657 and MDR049 conferring resistance to *R. padi* (Greenslade et al., 2016) and MDR045 and MDR049 conferring resistance to *S. avenae* (Simon et al., 2017). In this thesis (chapter 2) MDR049 was also shown to confer antixenosis to *R. padi.* In all these studies, MDR037 has been shown to be susceptible to aphid colonisation and infestation (Greenslade et al., 2016; Simon et al., 2017). Field studies over

three seasons showed variation in populations of *R. padi, S. avenae* and *M. dirhodum* on *T. monococcum,* where the smallest aphid populations were observed on MDR045 and MDR049. Although MDR657 was shown to confer antibiosis in controlled conditions (Greenslade et al., 2016), this resistance was not observed under field conditions. The lowest number of alate aphids was observed on MDR045 and MDR049, therefore it is considered that these genotypes confer antixenosis to aphids (Chapter 5). This shows that antixenosis in MDR049 observed in laboratory experiments, is also present under field conditions.

Although alate *R. padi* had no behavioural response to the VOCs of infested Solstice, MDR037 and MDR045 under controlled conditions, in the field, the highest number of alate aphids were observed on Solstice and MDR037, and fewest on MDR045 and MDR049. Aphid population recordings in the field were comprised of three species; *R. padi, S. avenae* and *M. dirhodum*, but only *R. padi* behaviour towards *T. monococcum* MDR037, MDR045 and MDR049 volatiles was investigated. It has been shown that different aphid species perceive different compounds (Visser and Yan, 1995) so *S. avenae* and/or *M. dirhodum* may have had different behavioural responses to MDR045 volatiles than *R. padi* did. Additionally, the semiochemicals produced under controlled conditions may qualitatively and/or quantitatively differ to those produced in the field.

Fewer alate aphids colonising plants could, depending on host plant suitability, lead to less oviposition and lower overall aphid populations. The highest proportion of alate were also observed on MDR045 and MDR049. This indicates that the smaller aphid smaller populations may also be due to antibiosis, *R. padi* and *S. avenae* on these genotypes have been shown to spend less time feeding and take longer to experience sustained feeding (Greenslade et al., 2016; Simon et al., 2017). The inability to feed on MDR045 and MDR049 could cause alate aphids to leave the plants before producing nymphs. A small experiment was done to look at the aphid behaviour on the *T. monococcum* genotypes. Locomotion

studies looked at the proportion of time *R. padi* spent stationary, walking and off the plant, as well as the position of the antenna. When alate *R. padi* were placed on MDR045 and MDR049 they spent more time off the plant than those placed on MDR037 (Appendix Fig C1). When on the plant *R. padi* spent less time with their antenna backwards on MDR045 and MDR049 than those on MDR037 (Appendix Fig C1). When aphids have their antenna backwards, this is an indication that their stylets have pierced the plant epidermis. Fewer alate aphids on MDR045 and MDR049 could be due to fewer alate aphids alighting onto the plant due to repellent semiochemicals being produced, or alate aphids alighting onto plants did not stay to larviposit.

Allelochemicals produced from *R. padi* infested *Triticum aestivum* Solstice, *T. monococcum* MDR037, MDR045 and MDR049 have been shown to affect aphid behaviour (chapter 2). Herbivore induced plant volatiles also affect the behaviour of natural enemies that prey on aphids, for example, the seven spotted ladybird (*Coccinella septempunctata* (L.)) preferred *R. padi* infested *H. vulgare* to un-infested plants (Ninkovic et al., 2001). Another study showed that the parasitoids (*Aphidius gifuensis* (A.)) and (*Encarsia formosa* (G.)) preferred volatiles from peach-potato aphid (*Myzus persicae* (M.)) infested tomato (*Solanum lycopersicum* (L.)) leaves than un-infested leaves (Tan and Liu, 2014). In the field trials, three types of natural enemies were recorded, parasitised aphids, lacewing eggs and larvae, and ladybird adults and larvae. Natural enemy presence was highest on Solstice, which also had the highest number of aphids. This has also been seen in other studies, such as on *A. gifuensis* and *E. formosa* that were attracted to volatiles from leaves with higher aphid densities and leaves where aphids had been feeding for longer (Tan and Liu, 2014), and another study demonstrated that ladybird populations were positively correlated with aphid densities (Evans and Youssef, 1992).

Despite having the lowest aphid populations, MDR049 attracted a similar number of natural enemies as MDR037 and MDR0657. It is suggested, therefore, that MDR049 attracts more natural enemies in relation to its aphid densities. Individual HIPV compounds have been shown to attract natural enemies. For example, methyl salicylate is a HIPV (Arimura et al., 2002; Zhu and Park, 2005) which has been shown to be attractive to the seven spotted ladybird (*Coccinella septempunctata* (L.)) (Arimura et al., 2002), green lacewing (Chrysopidae) and hoverfly (Syrphidae (L.)) (Mallinger et al., 2011).

The compound, 4-ethylacetophenone, was identified in the volatile profiles of *R. padi* infested Solstice and MDR049 which indicates that it is an HIPV. This compound was also produced by mirid bug (*Apolygus lucorum* (M.)) infested castor bean (*Ricinus communis* (L.)) (Xiu et al., 2019). Methyl salicylate has been shown to be attractive to the parasitoid *Peristenus spretus* (C.), in olfactometer studies, and its presence in field trials increased parasitism by *A. lucorum* (Xiu et al., 2019). *Triticum aestivum* Solstice had the highest natural enemy presence and *T. monococcum* MDR049 had natural enemy presence similar to that of susceptible genotypes. This suggests that the semiochemicals produced from infested Solstice and MDR049 plants may attract more natural enemies in relation to their aphid densities and would be an interesting research focus in the future.

The field trials have shown that the weather can affect aphid populations on *T. aestivum* and *T. monococcum.* A heatwave was declared in the summer of 2018, and there was a dramatic reduction in the number of alate and apterous aphids observed in the field in 2018, especially on *T. monococcum* MDR037. The adverse weather conditions caused aphid populations on MDR037 to decrease to numbers similar to those on the resistant MDR045 and MDR049. Alate populations on plants did not change over the field trial years, this indicates that the smaller aphid populations on MDR037 in field trial 2018 were due to apterous aphid populations and population growth on these plants.

6.1.3 The effect of plant age on antibiosis

In seedlings, *T. monococcum* genotypes MDR045 and MDR049 have been shown to confer antibiosis to *R. padi* (Greenslade *et al.* 2016; Aradottir per comms). As these studies were carried out on one-week-old seedlings, it was unknown whether antibiosis would also be present in older plants. This thesis presents work that shows antibiosis to *R. padi* in MDR049 seedlings which confirms the findings of Greenslade *et al.* (2016), and additionally confirms it in MDR045. The antibiosis was not consistent in MDR045 over time, in GS 51-55 (Zadoks et al., 1974) MDR045 plants, *R. padi* had no reduced mean relative growth rate (mRGR), nymph production or aphid life length. Other studies on *T. monococcum* genotypes showed that TM44 and TM46 only conferred antibiosis to *Sitobion avenae* (F.) at seedling stage and not as adult plants (di Pietro et al., 1998; Migui and Lamb, 2004). No antibiosis in GS 51-55 MDR045 is in line with the optimal defence theory that younger plants will have better anti-herbivory defence than older plants (Boege and Marquis, 2005).

In contrast to this, *R. padi* on MDR049 had reduced mRGR, nymph production and longevity across all plant ages and had increased resistance affecting nymph production at GS 51-55. It is suggested that this may due to the Adult-Plant Resistance phenomenon, this phenomenon has been observed in plant-pathogen interactions where adult plants from flag leaf (growth stage (GS) 39) to late milk grain (GS77) stage (Zadoks et al., 1974) confer resistance to pathogens which is either not present or diminished seedlings (Dyck, 1979; Dyck and Samborski, 1979; Mercado Vergnes et al., 2006).

In seedlings, antibiosis in MDR045 and MDR049 has been linked to increased salivation and reduced sap ingestion behaviours (Greenslade et al., 2016; Simon et al., 2017). Barley Yellow Dwarf Virus (BYDV) causes root and shoot death in plants (Esau, 1957; Erion and Riedell, 2012) and is transmitted to plants via aphid salivation (Miller et al., 2002). *Rhopalosiphum padi* is one of the major UK vectors for BYDV (Leather et al., 1989; Halbert

et al., 1992; Chapin et al., 2001; Tanguy and Dedryver, 2009; Yu et al., 2013), so increased salivation on MDR045 and MDR049 plants may increase BYDV transmission. It is not known how susceptible MDR045 and MDR049 are to BYDV infection. Further investigation should investigate *R. padi* feeding behaviours on older MDR045 and MDR049 and whether this affects that rate of BYDV transmission.

In the field, the genotype and week of recording affected aphid populations. The field trials seeds were not sown on the same day each year, so the week of recording does not directly correspond to the plant growth stage, unfortunately, this means that we are not able to decipher whether aphid population changes over the course of the field trials were due to the environmental temporal changes, and/or due to innate plant temporal factors. As plants mature various biochemical changes occur, it has been shown *T. aestivum* had higher concertrations of phenolics when infested with *R. padi* at seedling stage than *T. aestivum* infested at 21 days post anthesis (Ramos et al., 2017). Some phenolics such as ferulic acid are involved in defence against insects (Abdel-Aal et al., 2001), for example, reducing greenbug (*Schizaphis graminum* (R.)) survival on *H. vulgare* (H. M. Cabrera et al., 1995). Ferulic acid presence has also been shown to reduce with plant maturity, in *T. aestivum* post anthesis (Abdel-Aal et al., 2001) indicating reduced defence against aphids. A field study demonstrated that *A. gossypii* populations were dependant on the interactions between cotton (*Gossypium hirsutum* (L.)) genotype and plant age (Fernandes et al., 2012). Although the effect of plant growth stage on aphid and natural enemy populations in the field cannot be deciphered, it is unlikely that plant growth stage had no effect on aphid field trials.

6.1.4 Candidate gene TRIUR3_12925 involvement in defence responses

Aphid feeding behaviour can affected by biophysical barriers in the phloem such as callose sealing and protein plugging which block the pores in sieve tube elements, preventing sap from escaping the phloem (Will and Van Bel, 2006). Callose is synthesised in response to damage by an enzymatic complex that is made up of callose synthase, UDP-glucose transferase, annexin, Rho-like protein with GTP-ase activity (Rop) and sucrose synthase (SuSy) (Verma and Hong, 2001; Piršelová and Matušíková, 2013). The gene TRIUR3_12925 is hypothesised to promote sucrose synthase and callose synthesis in *T. monococcum.* In the initial stages of *R. padi* infestation (3hours and 6 hours post infestation (hpi)), TRIUR3_12925 was expressed more in MDR049 than MDR037. Studies have shown that over expressing callose synthase genes (*GSL1* and *GSL5*) in *A. thaliana* leaves cause more callose deposition (Begum et al., 2016) and that resistant pepper (*Capsicum baccatum* (L.)) genotype has more callose deposition than susceptible genotype (Sun et al., 2018). Other studies showed that higher callose synthase gene expression in resistant *Arabidopsis thaliana* (L.) (*AtGLS5*) and pepper (*Capsicum baccatum* (L.)) (*CalS1* and *CalS7*) caused aphids on these plants to spend more time salivating and less time feeding (Lü et al., 2011; Sun et al., 2018). Aphids feeding on resistant *T. monococcum* MDR045 and MDR049 spent more time salivating and less time feeding than those on the susceptible MDR037 (Greenslade et al., 2016; Simon et al., 2017). This suggests that these feeding behaviours are in part due to increased callose synthesis and deposition. Inducing sieve element occlusions in *V. faba* has been shown to make Vetch aphids (*Megoura viciae* (B.)) salivate, *M. viciae* saliva contains calcium-binding proteins in which can reduce occlusions in *V. faba* (Will et al., 2007). It is unknown whether *R. padi* saliva also contain calcium-binding proteins, but it demonstrates that increased *R. padi* salivation on MDR049 (Greenslade et al., 2016) may be due to more occlusions. Increased salivation and reduced feeding has been associated with reduced

development and reproductive success (Greenslade et al., 2016; Simon et al., 2017). Therefore, higher expression of TRIUR3_12925 in GS 13 MDR049 may play a role in increasing callose synthesis and deposition to prevent *R. padi* feeding, leading to the reduced development and reproductive success on GS 12-13 MDR049 (chapter 2).

To confirm this, TRIUR3_12925 expression in MDR037 and MDR049 un-infested, 3hpi, 6hpi and 24hpi plants would need to be studied through RT-qPCR, and determine its effect on callose deposition by overexpressing this gene and determining callose deposition by aniline blue staining leaves and observing deposition by UV epifluoresence microscopy as seen in Kissoudis *et al.* (2016).

6.1.5 Candidate gene TRIUR3_22080 involvement in defence response

When pattern recognition receptors (PRR) detect herbivore presence (by recognising herbivore associated molecular patterns (HAMP) or microbe associates molecular patterns (MAMP)), and/or plant damage (by recognising damage associated molecular patterns (DAMP)), reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) are produced (G. Miller et al., 2009). Hydrogen peroxide can provide defence by strengthening cell walls (Hückelhoven et al., 1999; Argandoña et al., 2001), transferring toxicity to the herbivore (Bi and Felton, 1995) and interacting with phytohormone signalling pathways (Morkunas et al., 2011). However, H_2O_2 can be harmful to host plants in high amounts, leading to cell death and early senescence (Li et al., 2015). The ascorbate-glutathione pathway detoxifies H_2O_2 to prevent these adverse effects. It is hypothesised that the gene TRIUR3_22080 promotes ATP binding and glutathione synthase activity to increase glutathione biosynthesis in *T. monococcum*. In MDR049, TRIUR3_22080 was expressed more in plants at 3 hours post infestation, than in un-infested plants, possibly in response to increased H_2O_2 presence in plant cells. The increase in TRIUR3_22080 expression occurred later in MDR037, at 6 hours

post infestation. Hydrogen peroxide induction has been shown to occur later in susceptible *D. noxia* infested *T. aestivum* than transgenic resistant plants (Moloi and van der Westhuizen, 2006). This later induction may be due to effector protein activity as the potato aphid (*Macrosiphum euphorbiae* (T.)) effector protein Me47 has been shown to have glutathione-S-transferase properties to reduce the presence of H_2O_2 (Kettles and Kaloshian, 2016). Resistance (*R*) proteins can recognise aphid salivary effectors to initiate defence responses such as H_2O_2 induction as part of effector triggered immunity (ETI). Therefore, in resistant genotypes defence responses may occur earlier and be more effective than responses in susceptible plants.

Glutathione-S-transferase is a dehydroascorbate reductase (DHAR) protein that is involved in the ascorbate-glutathione pathway that detoxifies H_2O_2. Induced gene expression of glutathione-S-transferase (*GST1*) has been shown to increase HIPV green leaf volatileS (GLV) (*E*)-2-hexenal, (*Z*)-3-hexenal, allo-ocimene and (Z)-3-hexenol production in *A. thaliana* (Kishimoto et al., 2005). This evidences that proteins that are part of the ascorbate-glutathione pathway can affect HIPV production. Increased TRIUR3_22080 expression which is hypothesised to promote glutathione biosynthesis which is one of the first steps in the glutathione-ascorbate pathway may be linked to HIPV production. Cereal aphids, *R. padi*, *M. persicae* and the corn aphid (*Rhopalosiphum maidis* (F.)) have been shown to move away from HIPV (Quiroz et al., 1997; Bernasconi et al., 1998; Verheggen et al., 2013), and natural enemies C. *septempunctata* and *A. ervi* have been shown to move towards HIPV (Du et al., 1998; Powell et al., 1998; Ninkovic et al., 2001). Therefore, earlier responses to the detoxification of H_2O_2 may induce HIPV production earlier in MDR049 than MDR037. The expression of this gene and its effect on HIPV production in MDR037 and MDR049 would need to be confirmed through RT-qPCR and air entrainment studies.

6.1.6. General Conclusions

This work has identified that at seedling stage *T. monococcum* MDR045 and MDR049 confer antibiosis to *R. padi* confirming (Greenslade et al., 2016) previous work. It is speculated that higher TRIUR3_12925 expression in MDR049 seedlings increased callose synthesis and deposition in sieve element pores preventing *R. padi* sustained feeding leading to reduced development, reproduction and life length. Antibiosis was not present in GS 51-55 MDR045, but it is suggested that defence responses along with the presence of adult plant resistance (APR) genes in MDR049 caused consistent resistance to *R. padi* and in some aphid life history traits, increased antibiosis to *R. padi* in GS 51-55 plants.

This work also demonstrates that MDR049 confers antixenosis to *R. padi* irrespective of aphid infestation but that alate *R. padi* was only attracted to un-infested Solstice and MDR037 volatiles. It is hypothesised that the qualitative and quantitative differences in semiochemical production caused these genotype dependent and *R. padi* infestation dependent behaviours.

Field studies demonstrated that MDR045 and MDR049 are resistant to *R. padi* infestation. It is hypothesised that reduced alate aphid colonisation and decreased population growth on MDR045 underpins this resistance. In addition to this, it is suggested that MDR049 recruits more natural enemies relative to its aphid population than the other *T. monococcum* genotypes studied.

6.1.7. Future work

To determine whether TRIUR3_12925 is involved in the resistance observed in MDR045 and MDR049 seedlings, future work should confirm TRIUR3_12925 gene expression levels in infested and un-infested MDR049 and MDR037 through RT-qPCR. To establish whether this gene is plays a major role in callose synthesis and deposition, callose staining (Elzinga et al.,

2014; Schenk and Schikora, 2015; Kissoudis et al., 2016) and electrical penetration graph (Tjallingii, 1985; Tjallingii and Hogen Esch, 1993) studies on aphid feeding should be carried out on TRIUR3_12925 knock-out and control plants.

To determine whether alate behaviours observed was due to qualitative and quantitative differences in semiochemical production, aphid behaviour to individual physiologically active compounds as well as blends of confirmed physiologically active compounds in specific ratios would need to be investigated. In addition, the physiologically active compounds in un-infested and infested Solstice and MDR045 volatile profiles should be determined using GC-EAG.

It is speculated that increased TRIUR3_22080 expression could lead to HIPV production through activating the glutathione-ascorbate pathway. To address this, the volatiles from *R. padi* infested TRIUR3_22080 knock-out and control plants would need to be analysed to determine whether this gene is involved in HIPV production.

To investigate the extent of natural enemy populations affect on field aphid populations across the difference *T. monococcum* genotypes, artificial aphid infestation and natural enemy introduction within exclusionary cages should be set up under field conditions across multiple years.

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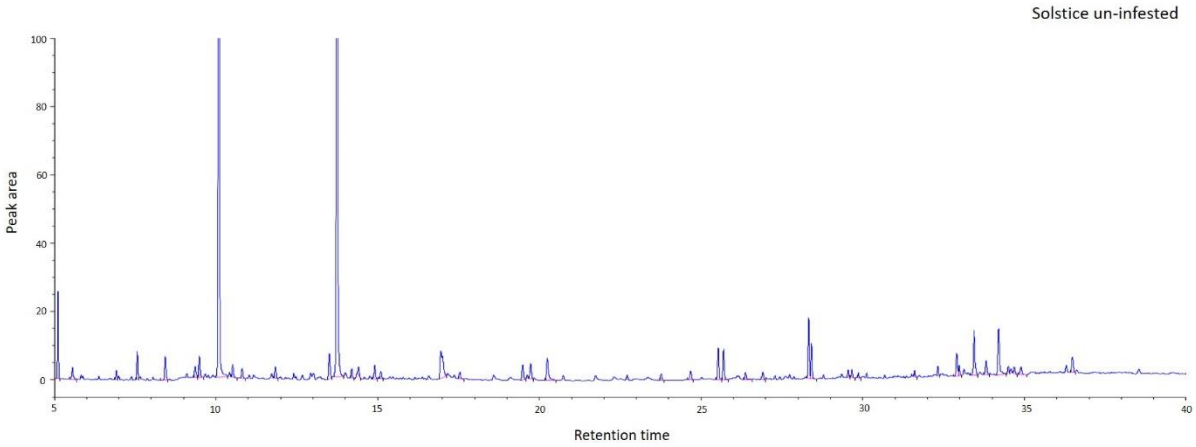
APPENDIX

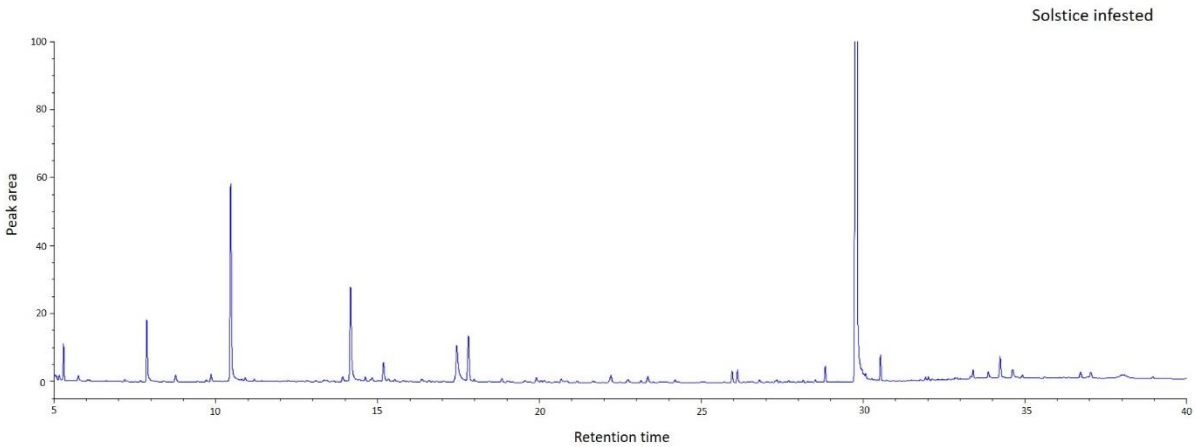
Appendix A

After volatile organic compounds were collected from un-infested and *Rhopalosiphum padi* (L.) infested *Triticum aestivum* (L.) var. Solstice, *Triticum monococcum* (L.) MDR037, MDR045 and MDR049 and eluted in re-distilled diethyl ether, the volatiles were run on gas chromatography (GC) to determine the number of compounds and the relative amounts (peak area) of compounds in the volatile profiles of these plants. The GC traces of un-infested and infested Solstice, MDR037, MDR045 and MDR049 are provided below (Fig A1).

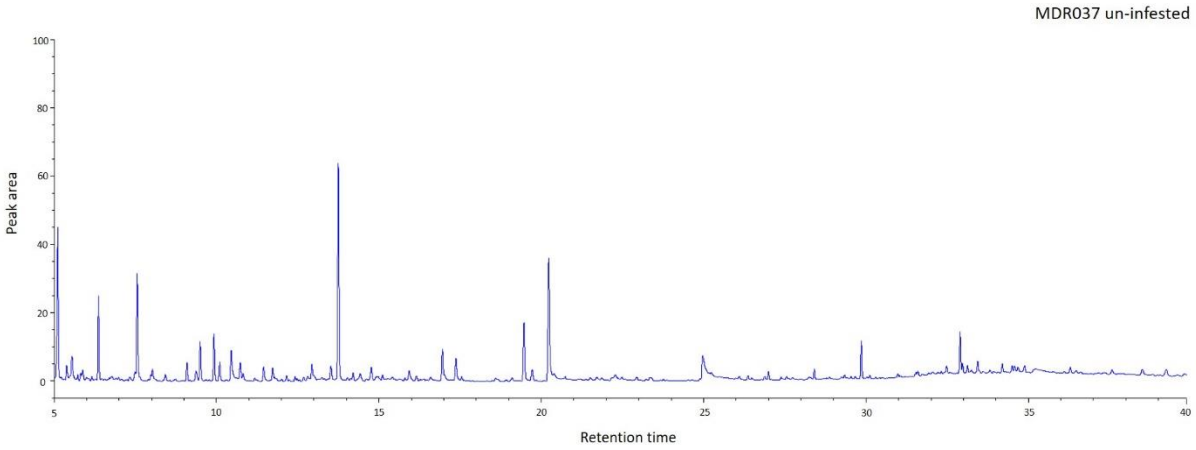
(a)



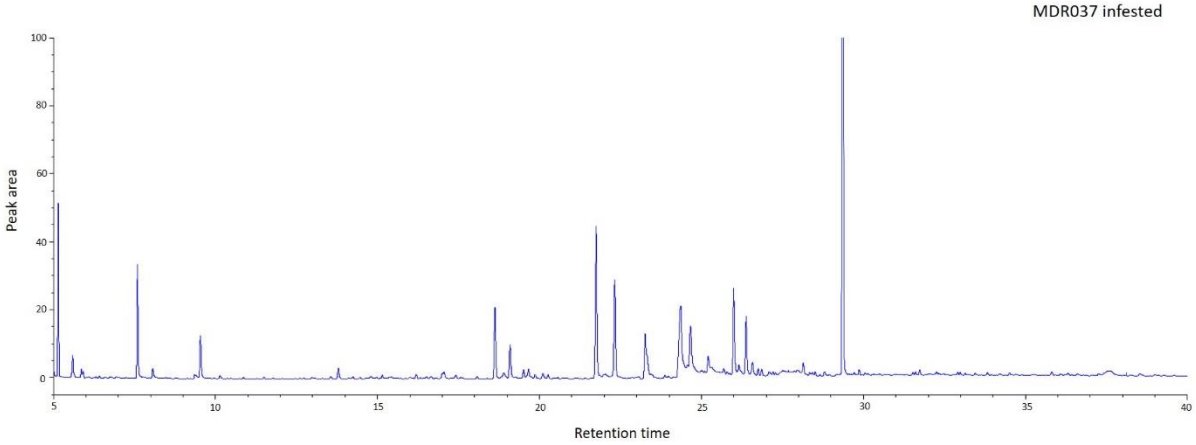
(b)



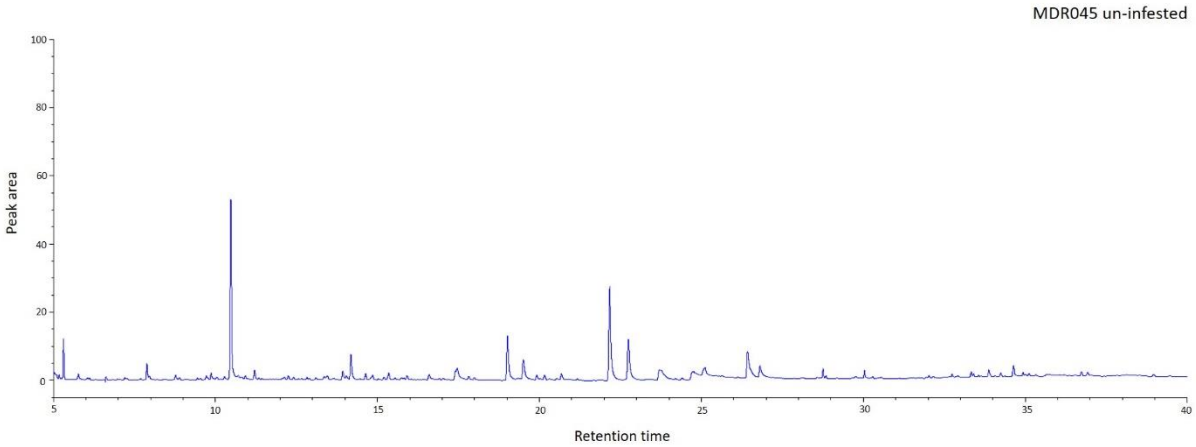
(c)


MDR037 un-infested
Peak area
Retention time

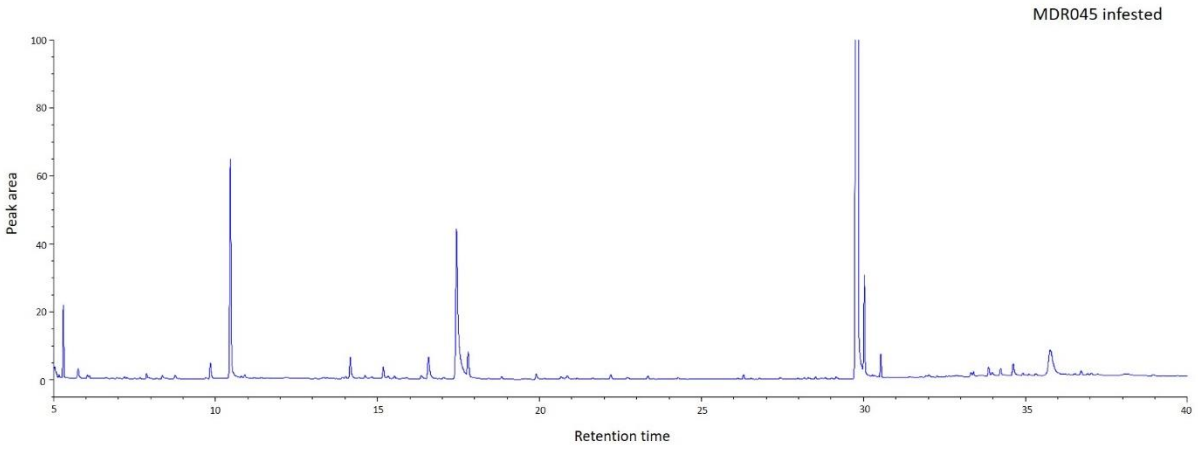
(d)


MDR037 infested
Peak area
Retention time

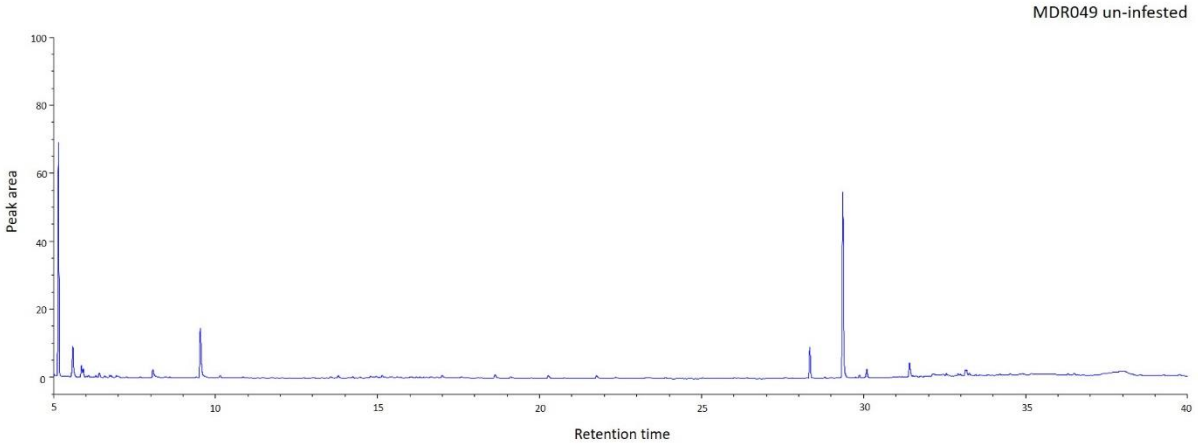
(e)


MDR045 un-infested
Peak area
Retention time

(f)



(g)



(h)

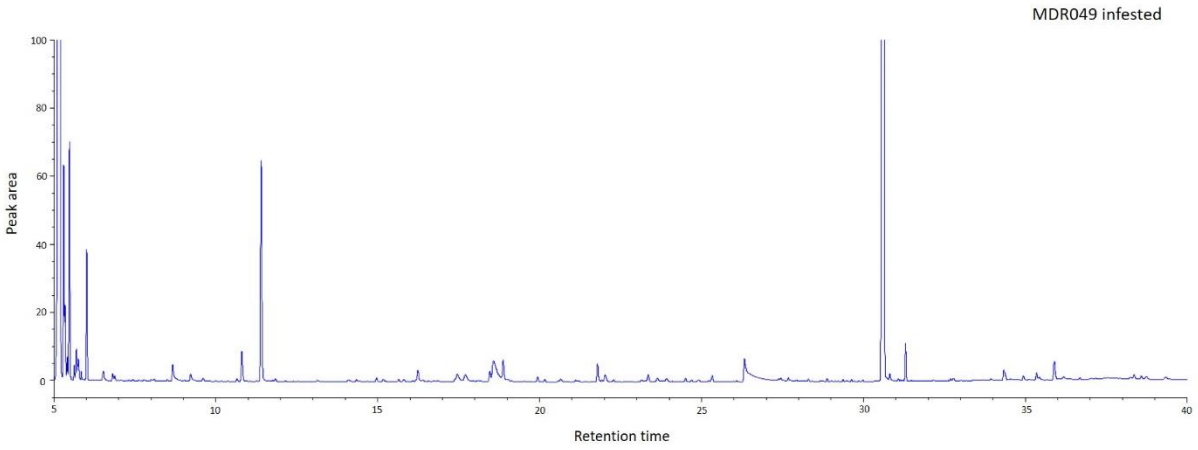


Figure A1. Gas Chromatography (GC) traces from a non-polar HP-1 GC of 4µl aliquot of entrainment samples **a)** *Triticum aestivum* Solstice **b)** *Rhopalosiphum padi* infested *T. aestivum* Solstice **c)** *Triticum monococcum* MDR037 **d)** *R. padi* infested *T. monococcum* MDR037 **e)** *T. monococcum* MDR045 **f)** *R. padi* infested *T. monococcum* MDR045 **g)** *T. monococcum* MDR049 **h)** *R. padi* infested *T. monococcum* MDR049

Compounds in un-infested and *R. padi* infested *T. monococcum* MDR037 and MDR049 that elcited a physiological response from alate *R. padi* antennae in gas chromatography electroantennography studies were quantified by co-injecting the synthetic compound with the entrainment sample, with the aim of doubling the peak area. This would prove the compounds identity in the plant volatile profile and estimate compound concentration. Co-injections started at a concentration of 5ng/µl and were decreased until the peak height was double that from the semiochemical profile (Fig A2-A13).

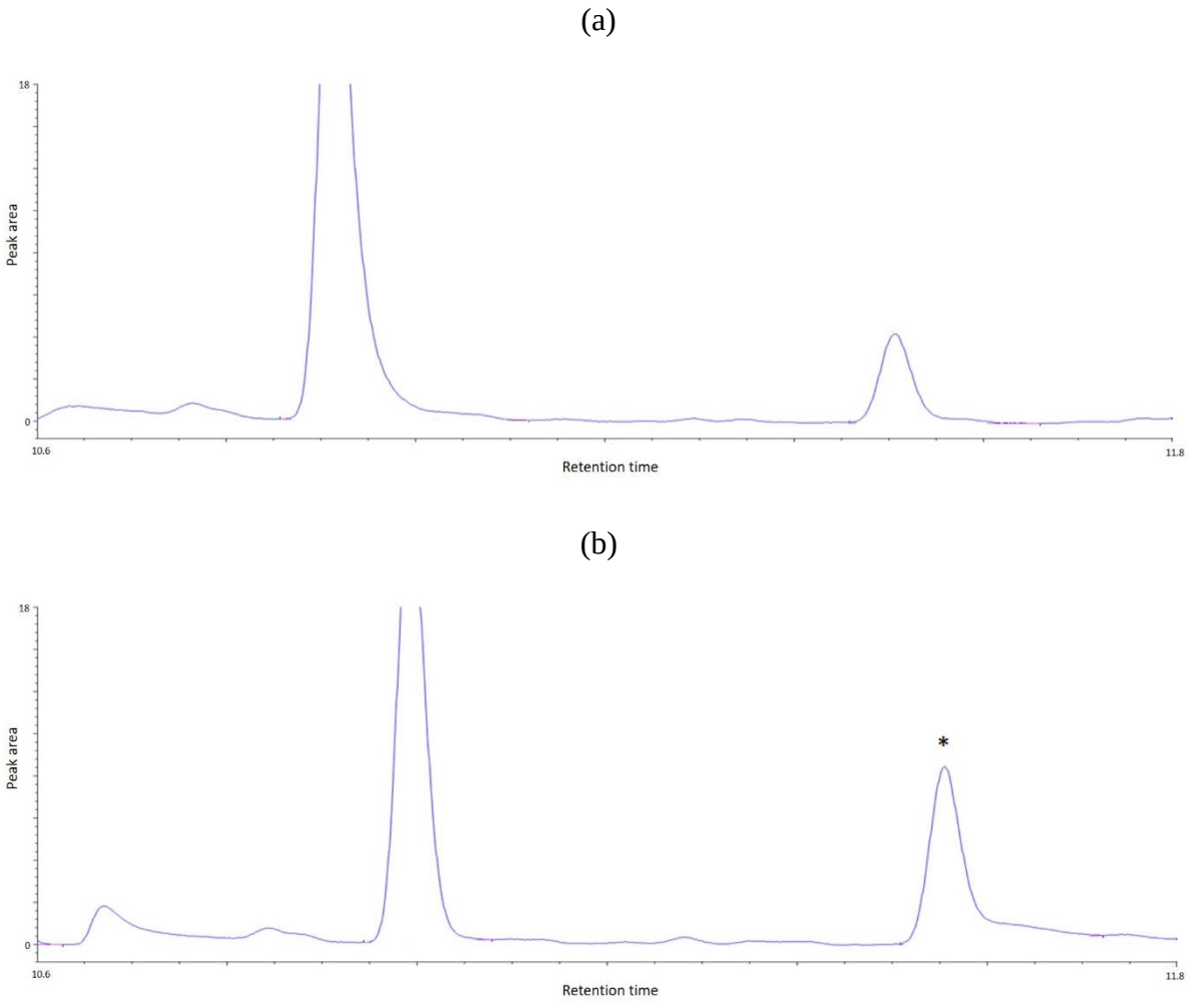
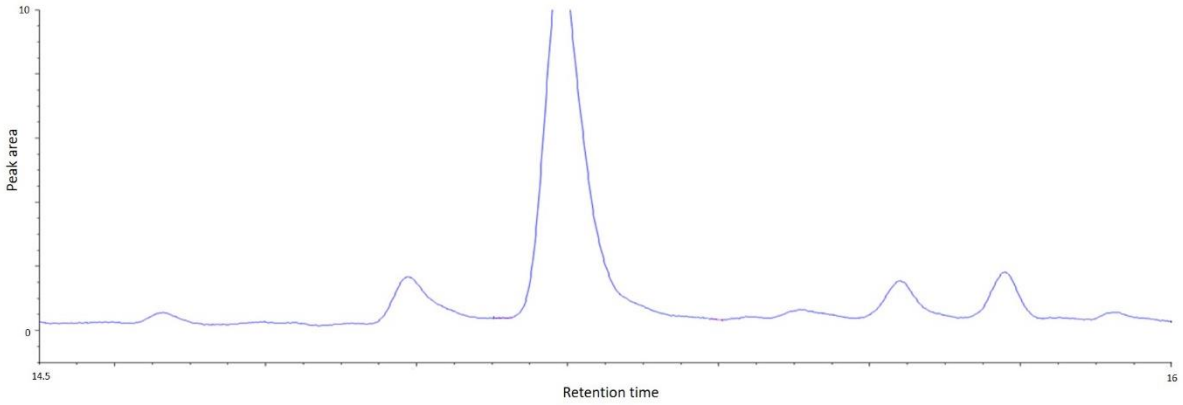


Figure A2. Gas Chromatography (GC) trace on a non-polar GC (HP-1) of a) air entrainment sample of *Rhopalosiphum padi* infested *Triticum monococcum* MDR037 b) air entrainment sample of *R. padi* infested *T. monococcum* MDR037 co-injected with ethylbenzene (100pg/µl). * denotes peak enhancement

(a)



(b)

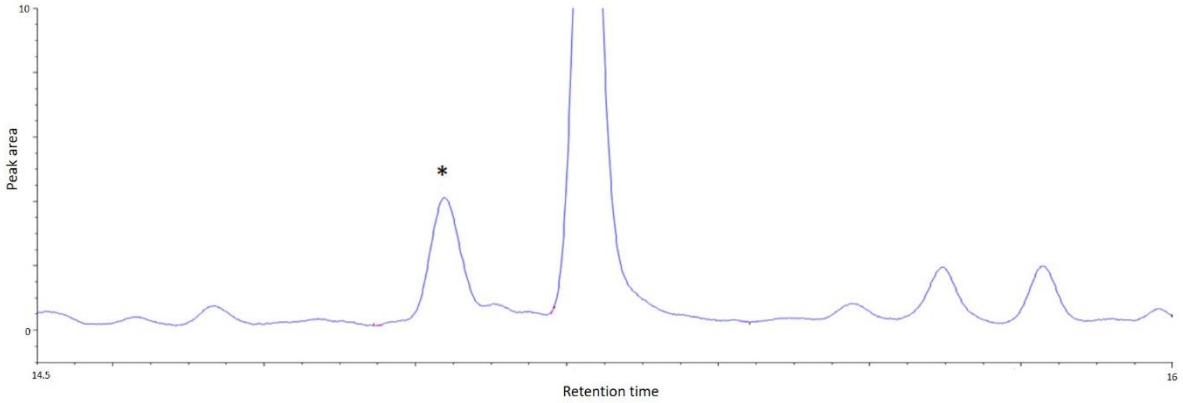
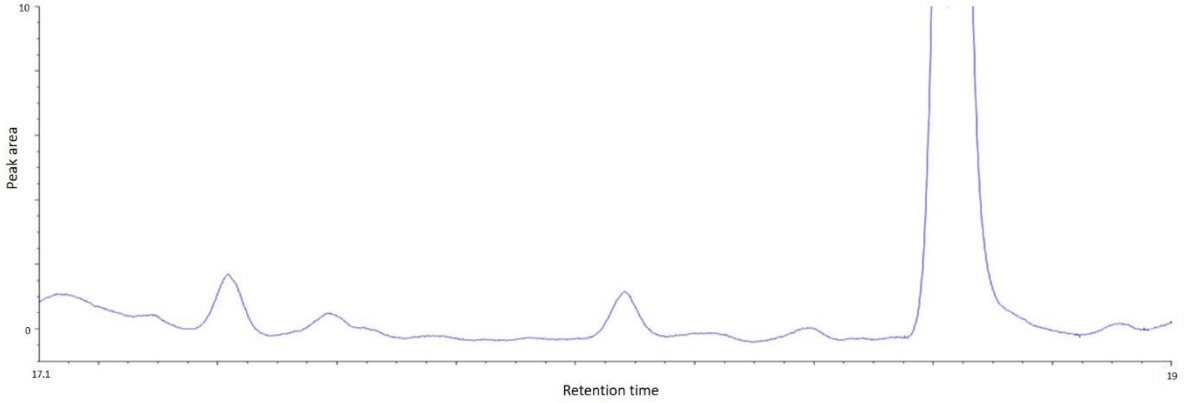


Figure A3. Gas Chromatography (GC) trace on a non-polar GC (HP-1) of a) air entrainment sample of *Rhopalosiphum padi* infested *Triticum monococcum* MDR037 b) air entrainment sample of *R. padi* infested *T. monococcum* MDR037 co-injected with octanal (125pg/µl). * denotes peak enhancement

(a)



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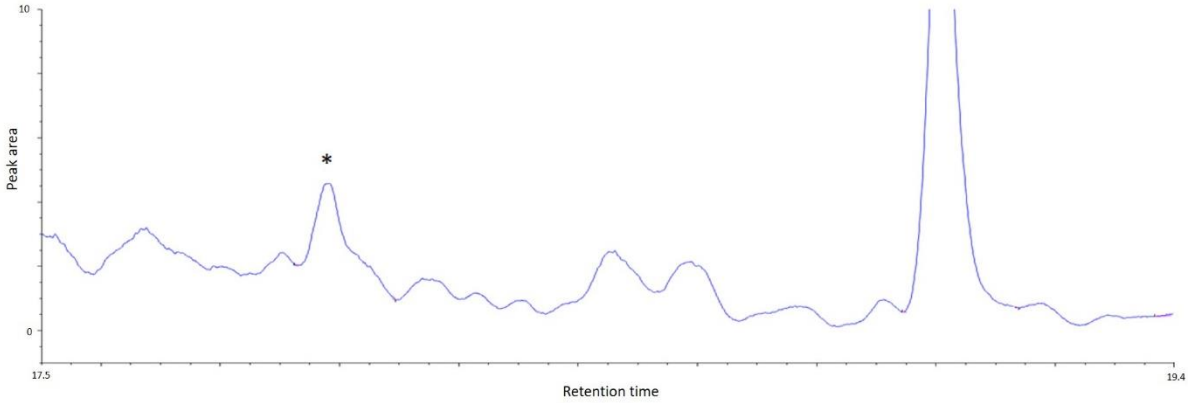
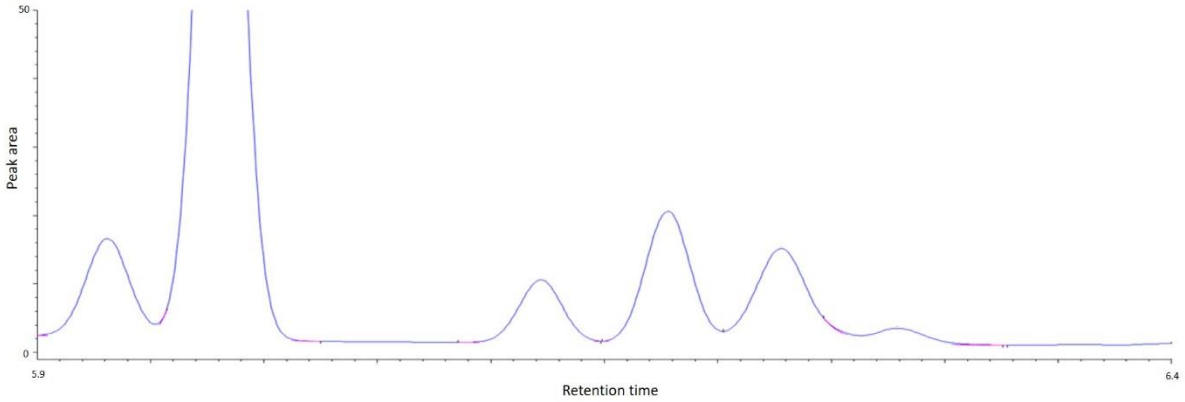


Figure A4. Gas Chromatography (GC) trace on a non-polar GC (HP-1) of a) air entrainment sample of *Rhopalosiphum padi* infested *Triticum monococcum* MDR037 b) air entrainment sample of *R. padi* infested *T. monococcum* MDR037 co-injected with Linalool (275pg/µl). * denotes peak enhancement.

(a)



(b)

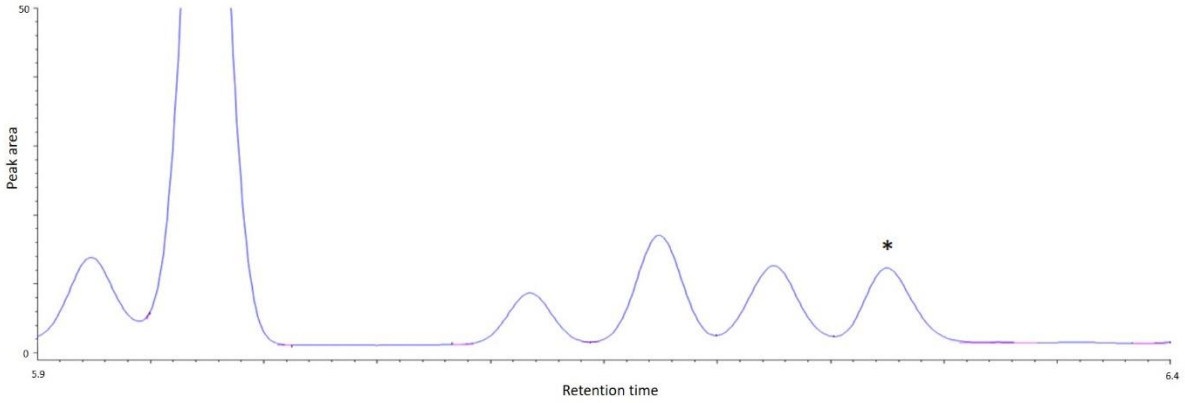
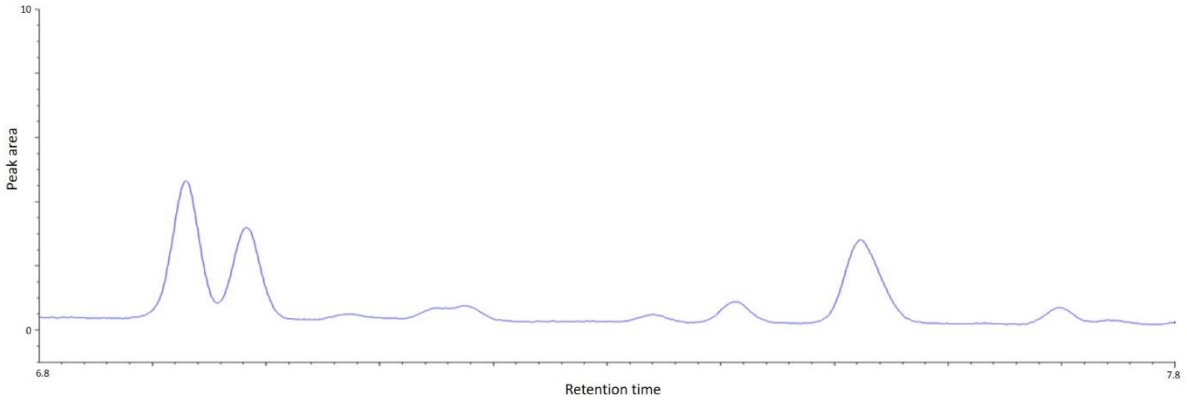


Figure A5. Gas Chromatography (GC) trace on a non-polar GC (HP-1) of a) air entrainment sample of *Triticum monococcum* MDR049 b) air entrainment sample of *T. monococcum* MDR049 co-injected with 2,4-dimethyl-1-pentene (25pg/µl). * denotes peak enhancement

(a)



(b)

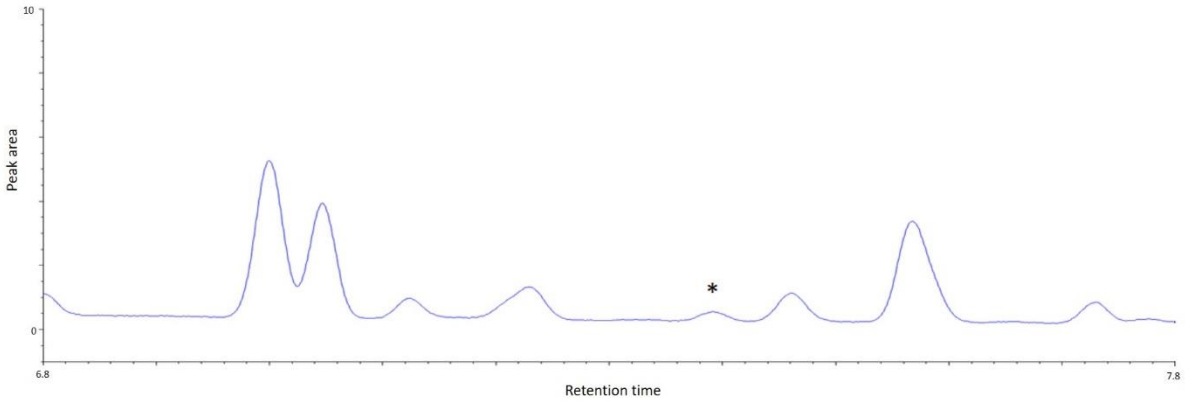
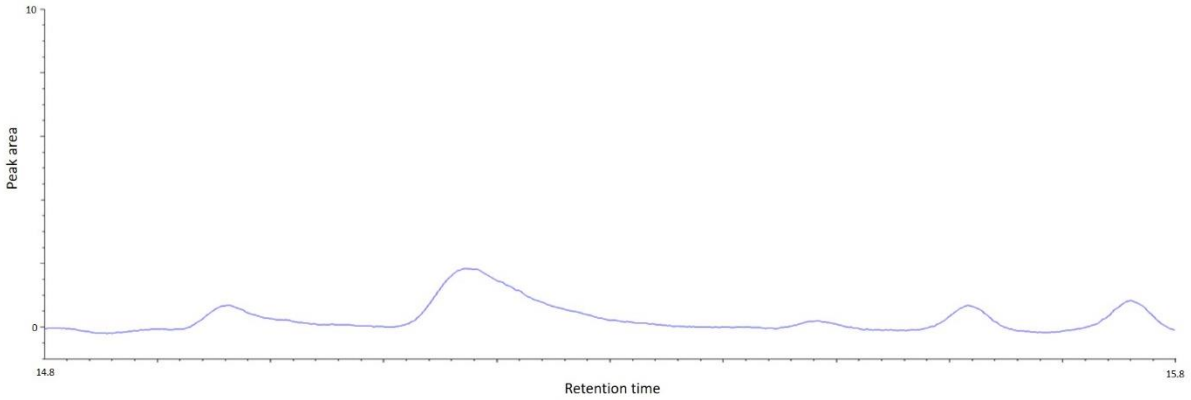


Figure A6. Gas Chromatography (GC) trace on a non-polar GC (HP-1) of a) air entrainment sample of *Triticum monococcum* MDR049 b) air entrainment sample of *T. monococcum* MDR049 co-injected with 4,4-dimethyl-2-pentene (40pg/µl). * denotes peak enhancement

(a)



(b)

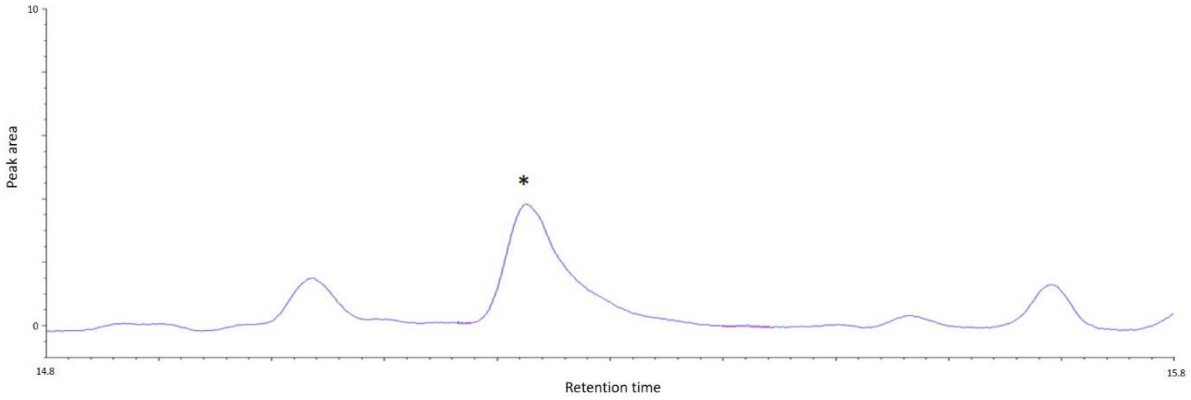
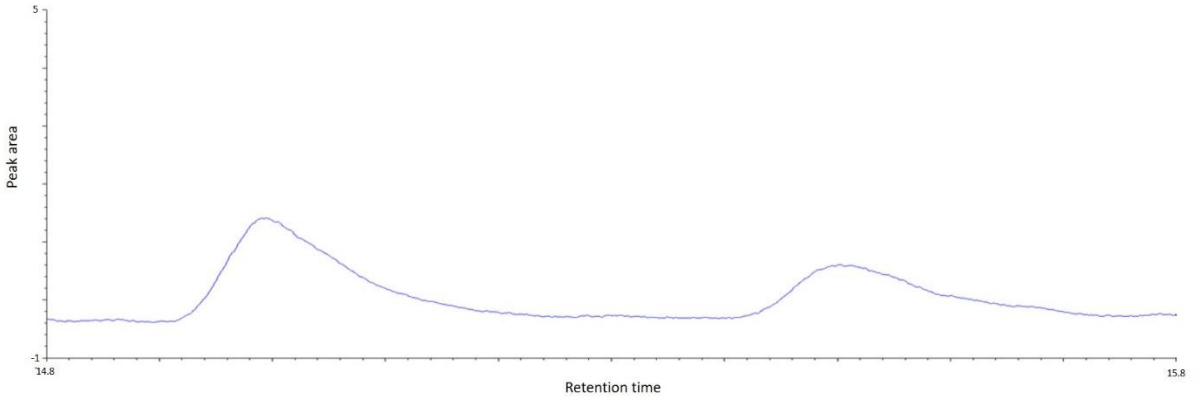


Figure A7. Gas Chromatography (GC) trace on a non-polar GC (HP-1) of a) air entrainment sample of *Triticum monococcum* MDR049 b) air entrainment sample of *T. monococcum* MDR049 co-injected with octanal (40pg/µl). * denotes peak enhancement

(a)



(b)

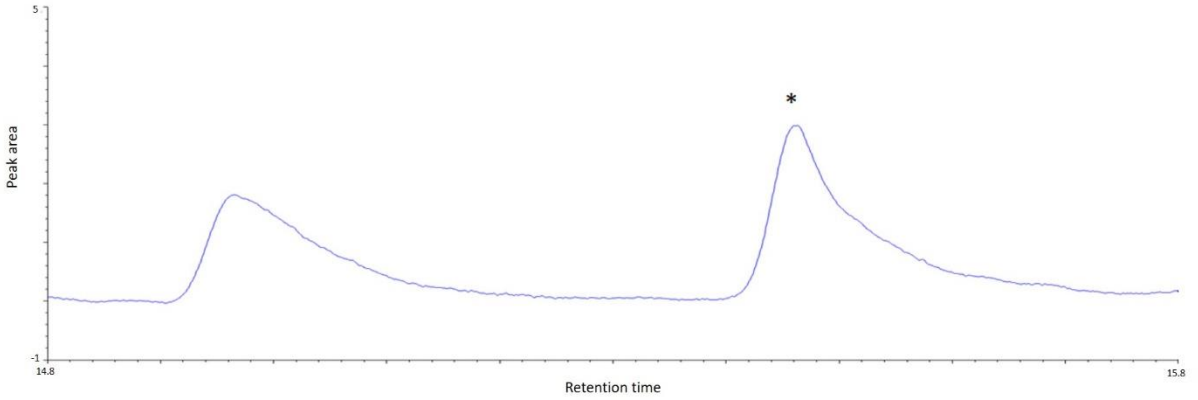
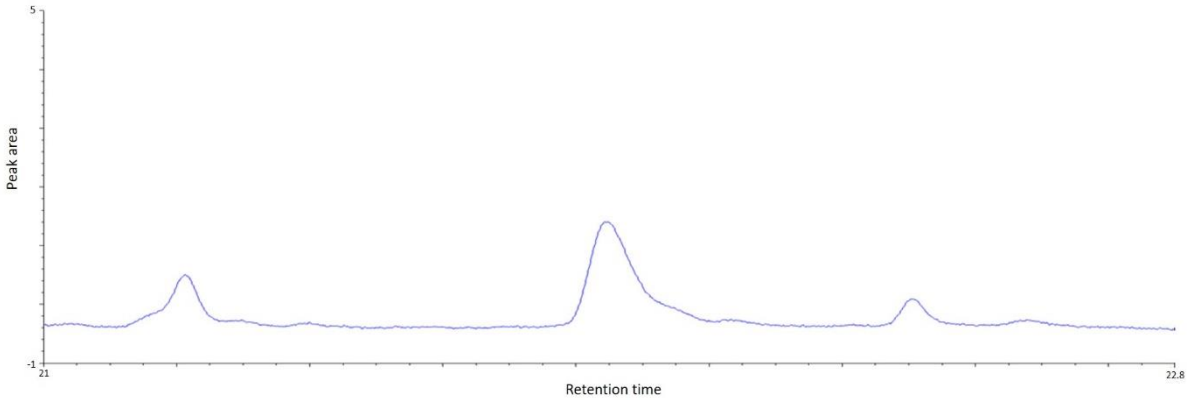


Figure A8. Gas Chromatography (GC) trace on a non-polar GC (HP-1) of a) air entrainment sample of *Triticum monococcum* MDR049 b) air entrainment sample of *T. monococcum* MDR049 co-injected with 4-ethylbenzaldehyde (125pg/µl). * denotes peak enhancement

(a)



(b)

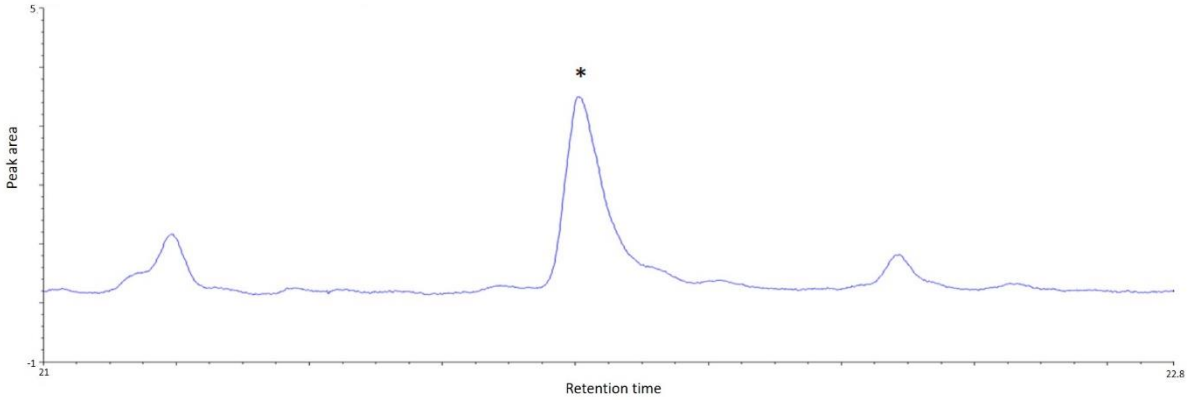
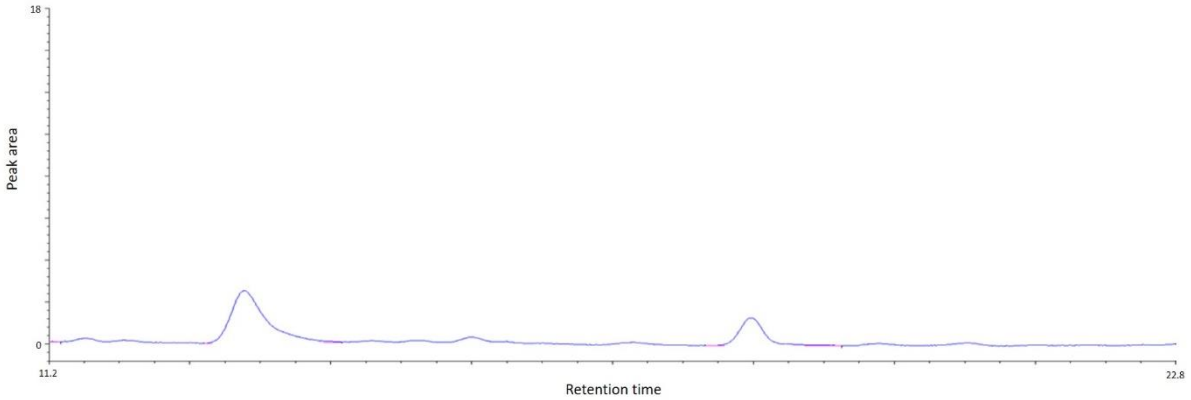


Figure A9. Gas Chromatography (GC) trace on a non-polar GC (HP-1) of a) air entrainment sample of *Triticum monococcum* MDR049 b) air entrainment sample of *T. monococcum* MDR049 co-injected with decanal (200pg/µl). * denotes peak enhancement

(a)



(b)

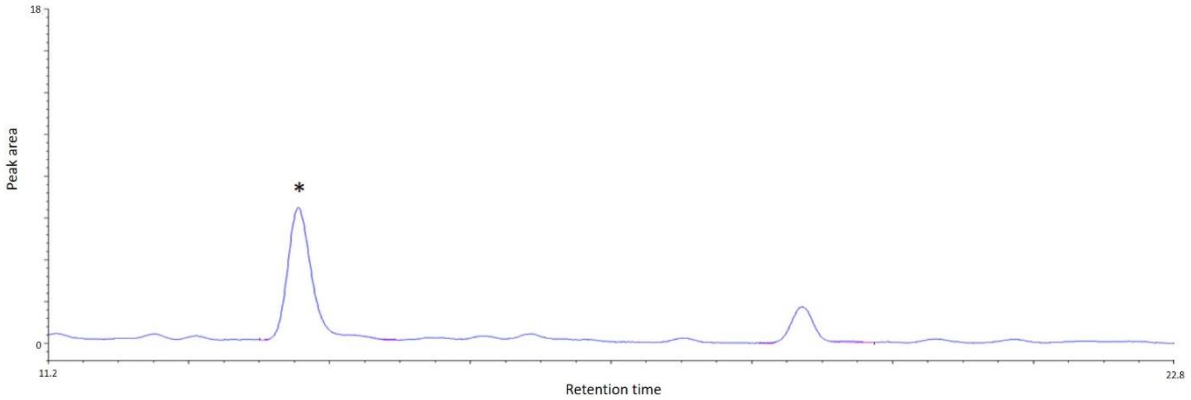
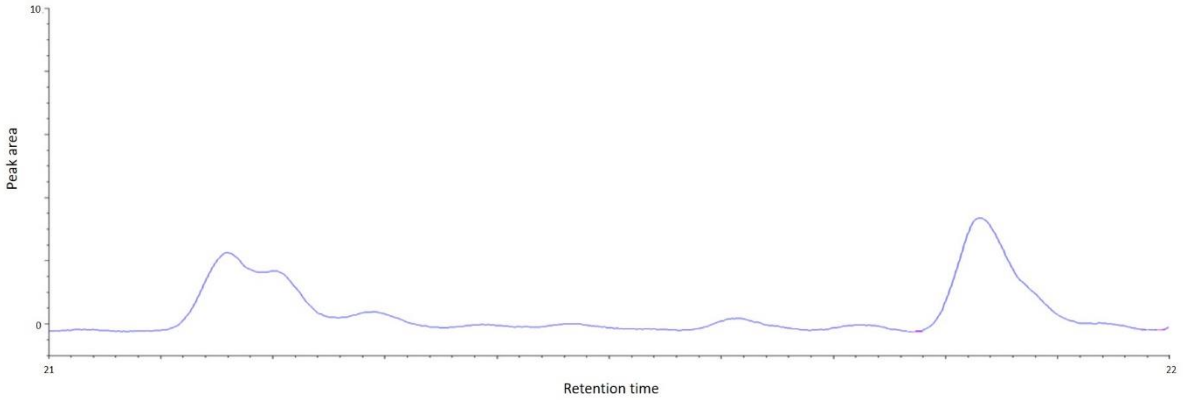


Figure A10. Gas Chromatography (GC) trace on a non-polar GC (HP-1) of a) air entrainment sample of *Rhopalosiphum padi* infested *Triticum monococcum* MDR049 b) air entrainment sample of *R. padi* infested *T. monococcum* MDR049 co-injected with heptanal (325pg/µl). * denotes peak enhancement

(a)



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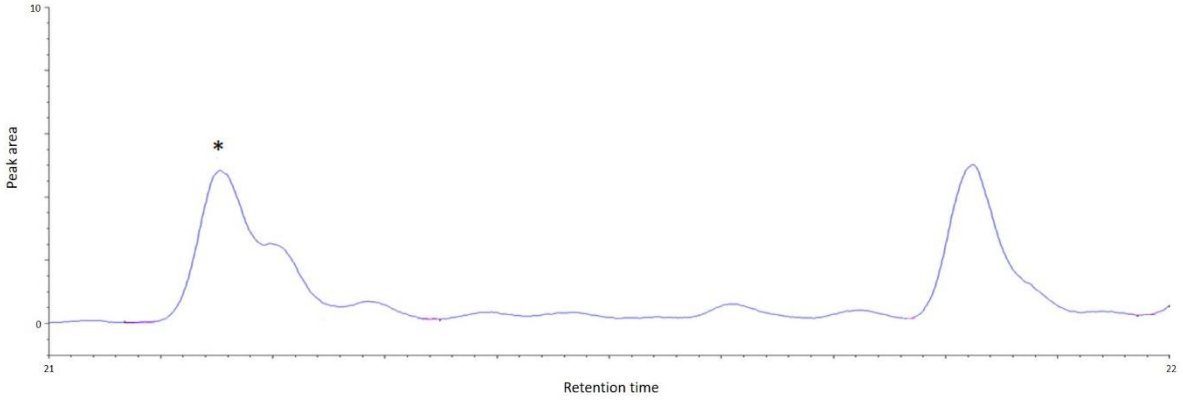
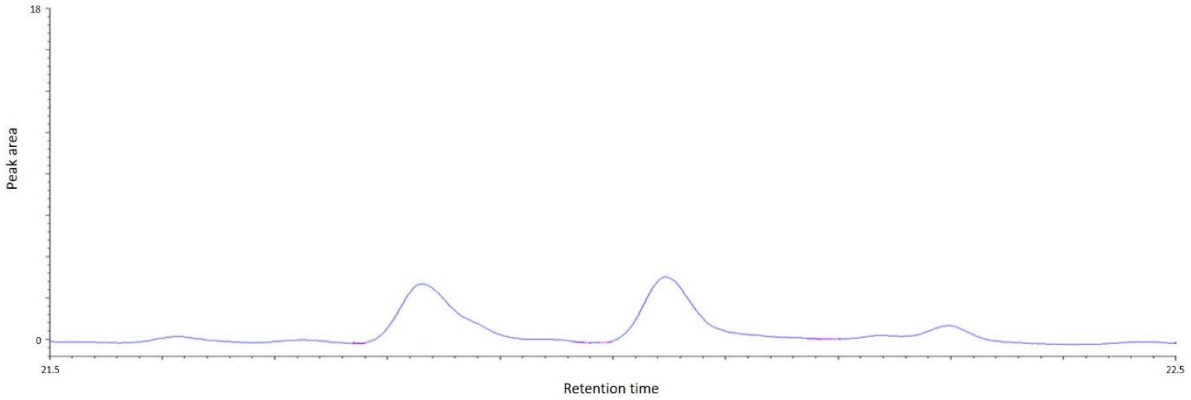


Figure A11. Gas Chromatography (GC) trace on a non-polar GC (HP-1) of a) air entrainment sample of *Rhopalosiphum padi* infested *Triticum monococcum* MDR049 b) air entrainment sample of *R. padi* infested *T. monococcum* MDR049 co-injected with naphthalene (75pg/µl). * denotes peak enhancement

(a)



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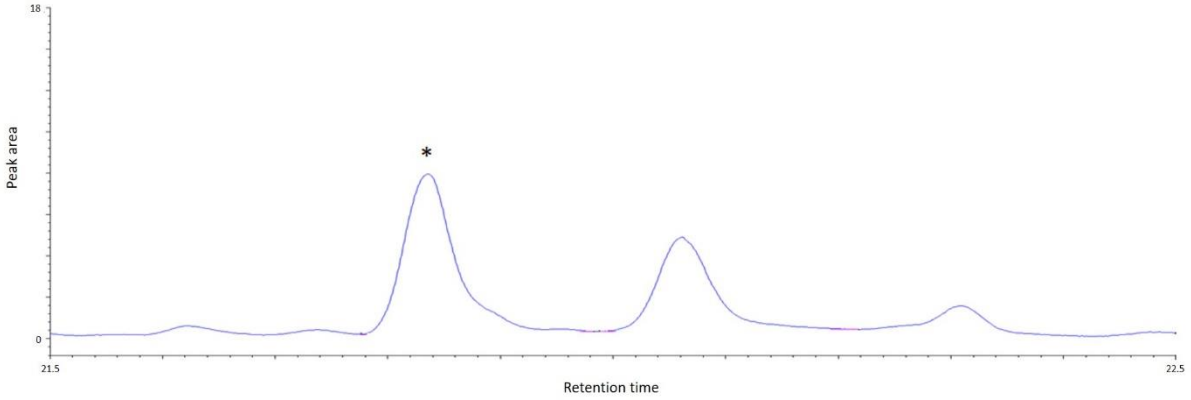
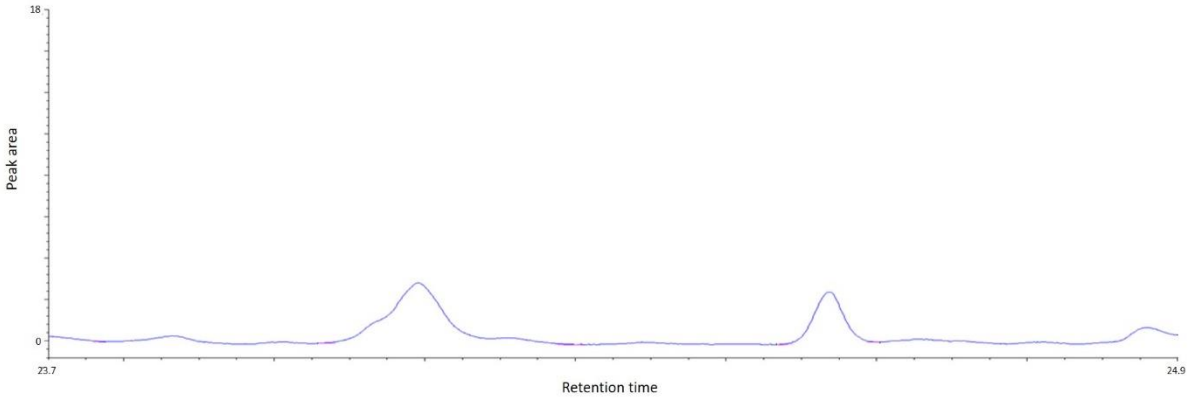


Figure A12. Gas Chromatography (GC) trace on a non-polar GC (HP-1) of a) air entrainment sample of *Rhopalosiphum padi* infested *Triticum monococcum* MDR049 b) air entrainment sample of *R. padi* infested *T. monococcum* MDR049 co-injected with decanal (500pg/µl). * denotes peak enhancement

(a)



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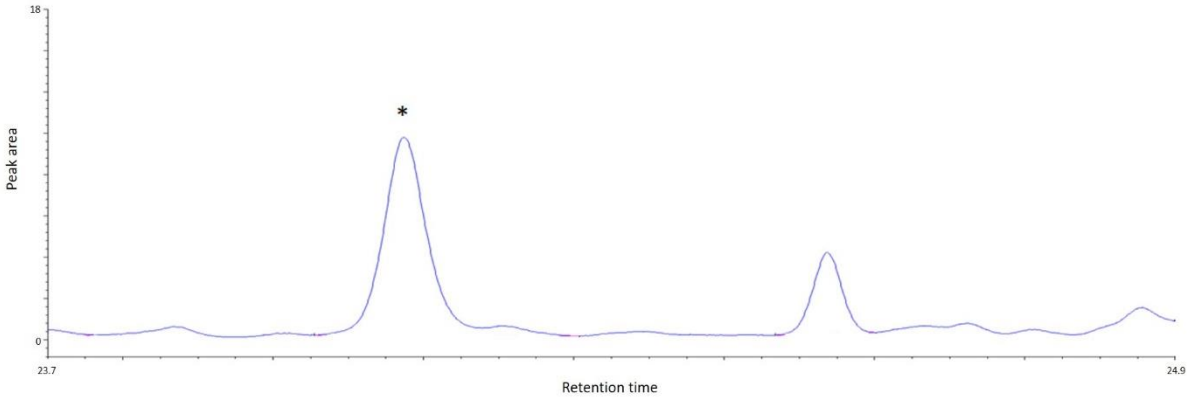

Figure A13. Gas Chromatography (GC) trace on a non-polar GC (HP-1) of a) air entrainment sample of *Rhopalosiphum padi* infested *Triticum monococcum* MDR049 b) air entrainment sample of *R. padi* infested *T. monococcum* MDR049 co-injected with 4-ethylacetophenone (725pg/µl). * denotes peak enhancement

Appendix B

The gene expression of genes in MDR037 and MDR037 infested for 3, 6, and 24hours were compared to the gene expression of their respective un-infested plants.

The highest number of differentially expressed genes were present in 24hours post infestation (hpi) for both genotypes across all three significance levels.

Table B1. Number of upregulated and downregulated genes in *Triticum monococcum* MDR037 and MDR049 at 3, 6 and 24 hours post *R. padi* infestation in comparison to un-infested *T. monococcum* MDR037 and MDR049 respectively, at three significance levels <0.05, <0.01 and <0.001

Genotype	**Hours post infestation**	**P-value significance level**					
		<0.05		**<0.01**		**<0.001**	
		Up regulated	**Down regulated**	**Up regulated**	**Down regulated**	**Up regulated**	**Down regulated**
MDR037	3h	622	1541	261	1092	112	734
MDR037	6h	76	373	28	226	13	135
MDR037	24h	3086	3310	1843	2137	961	1360
MDR049	3h	690	1564	331	1085	129	677
MDR049	6h	678	960	215	274	68	74
MDR049	24h	2006	2743	1202	1867	627	1164

Appendix C

A locomotion study was carried out where 5 alate *R. padi* were place of GS12 *T. aestivum* Solstice, *T. monococcum* MDR037, MDR045 and MDR049 for 20minute. The position of the aphid off or on the plant was recorded every 2 minutes. The position of the alate aphid antennae was recorded for aphids which were on the plant.

Alate aphids spent the least amount of time on Solstice and MDR049, of alate aphid on the plants, aphids on MDR045 and MDR049 spent the least proportion of time with their antennae backwards which indicates aphid stylet penetration, salivation and feeding (Fig C1).

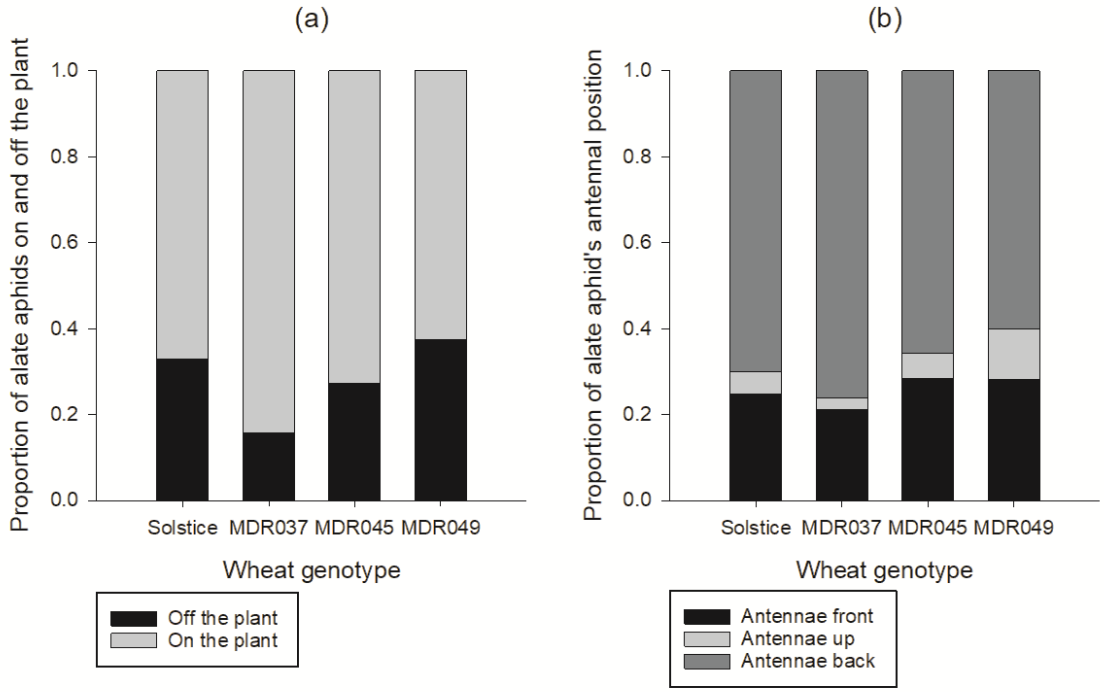

Figure C1. The proportion of time alate aphids spent **a)** on and off plants **b)** with their antenna positions to the front, upwards and backwards when placed on *Triticum aestivum* Solstice, *Triticum monococcum* MDR037, MDR045 and MDR049

A Pearsons correlation was done to determine whether the differences in numbers of aphids and natural enemy presence between years and weeks within years was correlated with meteorological factors. Aphid numbers were not correlated with any of the meteroloigcal parameters tested. These results are present in the appendix. maximum temperature (R = -0.09, P = 0.63), minimum temperature (R = -0.05, P = 0.81), rainfall (mm) (R = -0.03, P = 0.88) or rainfall duration (hr) (R = -0.06, P = 0.75) (Fig C2). The natural enemy presence was not correlated with maximum temperature (R = 0.16, P = 0.39), minimum temperature (R = 0.45, P = 0.06), rainfall (mm) (R = 0.10, P = 0.60) or rainfall duration (hr) (R = 0.03, P = 0.89). (Fig C3)

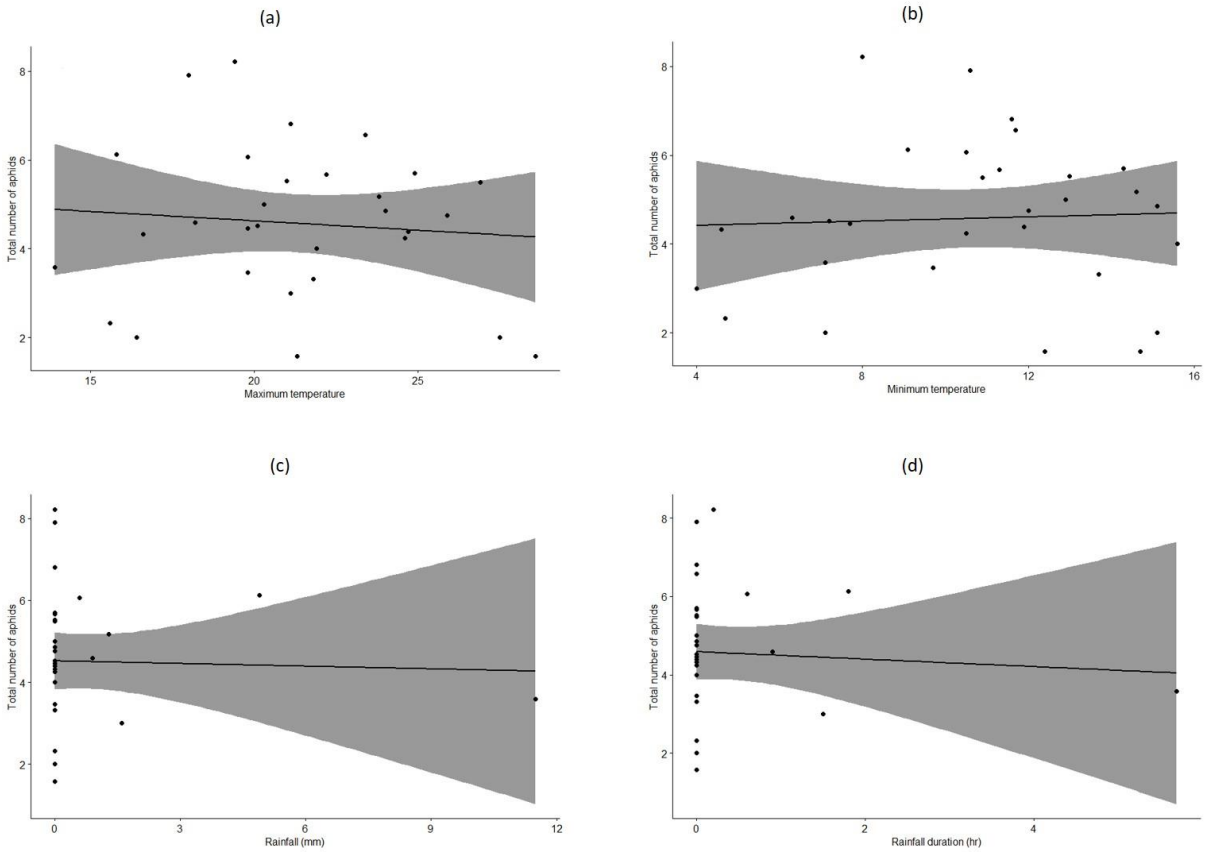

Figure C2. Correlation between number of aphids observed and the **a)** maximum temperature, **b)** minimum temperature, **c)** rainfall (mm), **d)** rainfall duration (hr) across three field trial years 2017, 2018 and 2019. Shading represents confidence intervals.

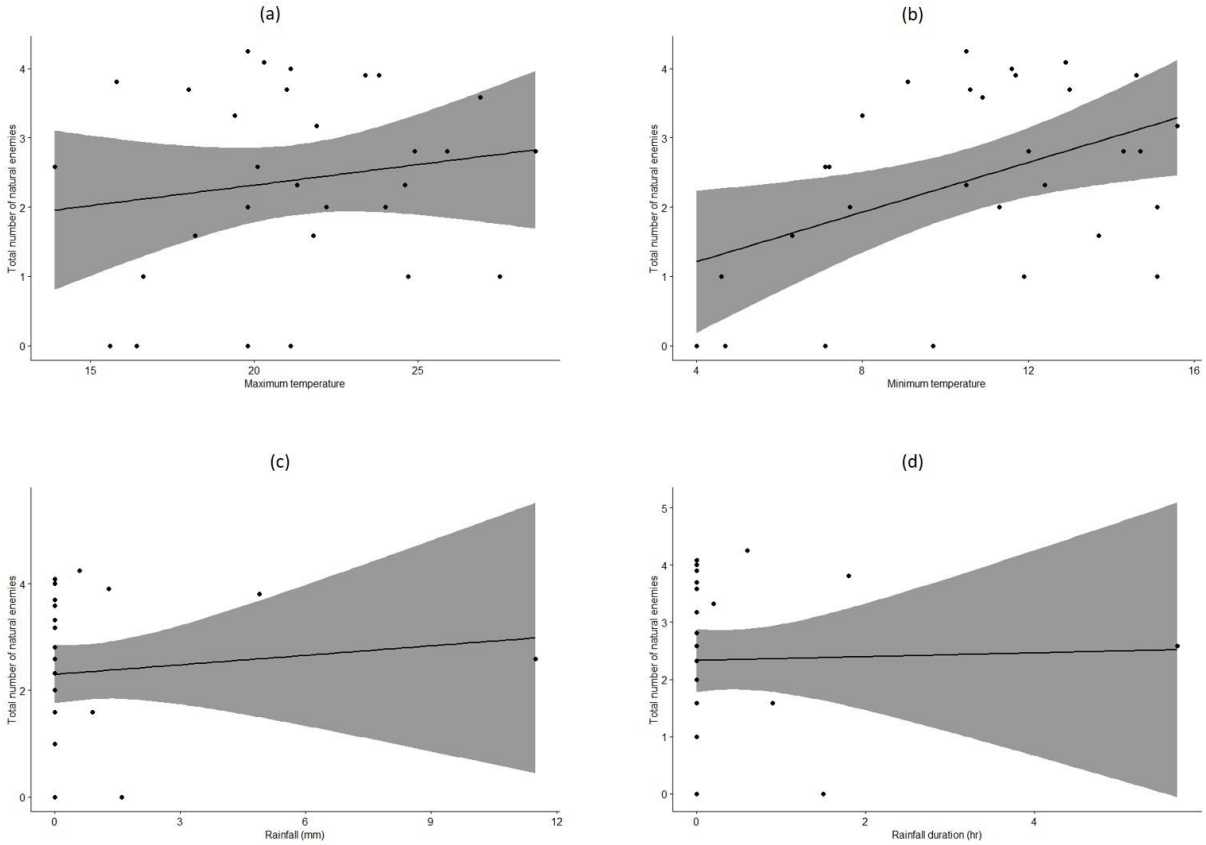

Figure C3. Correlation between total number of natural enemies observed and the **a)** maximum temperature, **b)** minimum temperature, **c)** rainfall (mm), **d)** rainfall duration (hr) across three field trial years 2017, 2018 and 2019. Shading represents confidence intervals.