Virus-induced effects on aphid-host interactions in Solanaceous plants

Warren Arinaitwe

Corpus Christi College

Department of Plant Sciences

University of Cambridge

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Warren Arinaitwe

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Abstract

Cucumber mosaic virus (CMV) has three genomic RNA segments, RNAs 1, 2 and 3, that encode five proteins. In some hosts, CMV induces biochemical or physiological changes that may influence virus transmission by aphids. In *Arabidopsis thaliana* ecotype Col-0 and cucurbits, CMV diminishes host suitability for aphids, inhibiting prolonged feeding and encouraging migration of viruliferous aphids from infected plants to healthy plants. In tomato (*Solanum lycopersicum*), a model species and economically important crop, viral modification of vector-host interactions for non-persistently transmitted viruses is understudied. I investigated CMV-induced effects on settling preference of two aphid species (*Myzus persicae*, a generalist, and *Macrosiphum euphorbiae*, a Solanaceous specialist) at 3, 9 and 21 days post-inoculation (dpi) and at 1 and 24 h following aphid release, using aphid free choice and trapping assays in light and dark conditions and under light of varying wavelengths. Further experiments were carried out to examine whether specific CMV viral proteins control aphid-tomato interactions. Finally, the role of plant signalling pathways in CMV-aphid interactions in tomato and tobacco was investigated.

In tomato, the effects of Fny-CMV infection on aphid settling varied as disease progressed. While pre-symptomatic (up to 3 dpi), infected tomato plants were more preferred by *Myzus persicae* than by *Macrosiphum euphorbiae*. As symptoms became apparent, between 9 and 21 dpi, more aphids settled on mock-inoculated plants than on virus-infected plants. In trapping assays in the dark, aphids did not prefer either type of plant, despite virus-infected plants emitting greater quantities of volatile organic compounds in the dark than in the light. In olfactometry assays, in which visual and contact cues were not available, aphids of both species were more attracted by odours emitted by virus-infected plants than to those of mock-inoculated plants. Thus, CMV stimulates multiple cues, but visual cues are prioritised in aphid-tomato interactions.

Specific CMV gene products, in particular the 1a, 2a and 2b proteins, contribute to virus-induced changes in plant-aphid interactions, sometimes in a host-specific fashion. The effects of CMV pseudorecombinant viruses on settling preference of *Myzus persicae* and *Macrosiphum euphorbiae* on tomato was assessed. Six pseudorecombinant viruses were made by systematic exchange of RNAs of LS-CMV, a mild strain on tomato, with those of Fny-CMV, a severe strain. It was found that RNA 2 regulates the ability of Fny-CMV to repel aphids in tomato. Further experiments with a recombinant CMV virus, where the Fny 2b protein was replaced with LS 2b, and the Fny-CMVΔ2b deletion mutant, suggested that the Fny-CMV 2b protein but not the LS-CMV 2b protein, influences settling of both aphid species on tomato.

I tested whether salicylic acid (SA) influences CMV-aphid-tomato interactions. *NahG*-transgenic tomato plants unable to accumulate SA were used. Fny-CMV infection of *NahG*-transgenic tomato plants affected the settling of *Myzus persicae* and *Macrosiphum euphorbiae* differently. *NahG*-transgenic plants infected with Fny-CMV attracted *Myzus persicae* but not *Macrosiphum euphorbiae*. More aphids of both species settled on mock-inoculated untransformed tomato plants than on Fny-CMV infected *Nah*G-transgenic plants. Thus, the effects of SA-dependent signalling on tomato-aphid interactions are limited, with only a small influence on the interaction with *Myzus persicae*.

Previous work showed that on tobacco plants infected with Fny-CMV and LS-CMV *Myzus persicae* survival and fecundity are enhanced but on plants infected with Fny-CMVΔ2b, aphid survival and fecundity are decreased. Using *CO11*-silenced transgenic tobacco plants I showed that the aphid resistance induced by Fny-CMVΔ2b infection is dependent upon defensive signalling mediated by jasmonic acid.

Dedicated to

Ms Irene Kikamweba (1959-2014)

for parenting me through difficult times

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Table of Contents

List of Figures

List of Tables

List of common abbreviations

AGO	Argonaute
AIN	*Acyrthosiphon*-induced necrosis
AOS	allene oxide synthase
bp	base pair
CaMV	cauliflower mosaic virus
CMV	cucumber mosaic virus
COI1	coronatine insensitive 1
Col-0	Columbia-0
CP	coat protein
DAS-ELISA	double antibody sandwich-enzyme linked immunosorbent assay
DCL	Dicer-like
df	degrees of freedom
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
dpi	days post-inoculation
dsDNA	double stranded DNA
DW	dry weight
EF1α	elongation factor 1α
ETI	effector-triggered immunity
Fny	Fast New York
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GC-MS	gas chromatography-mass spectrometry
GLVs	green leaf volatiles
JA	jasmonic acid
JAZ	jasmonate Zim-domain protein
LB	Luria-Bertani
LOX	lipoxygenase
LS	*Lactuca sativa*
MeJA	methyljasmonic acid
miRNAs	microRNAs
MP	movement protein
NBM	nutrient broth media
NPR1	nonexpressor of pathogenesis-related proteins 1
NT	non-transgenic/non-transformed

nt	nucleotide
NtCOI1	*Nicotiana tabacum* COI1
p	probability
PAMP	pathogen associated molecular pattern
PCR	polymerase chain reaction
PTI	PAMP-triggered immunity
PVX	potato virus X
PVY	potato virus Y
R-gene	Resistance gene (dominant)
RISC	RNA-induced silencing complex
RNA	ribonucleic acid
RT-PCR	reverse transcriptase-PCR
SA	salicylic acid
SEM	standard error of the mean
siRNA	short-interfering RNA
TFs	transcription factors
v/v	volume/volume
VOCs	volatile organic compounds
w/v	weight/volume

Chapter 1. General introduction

1.1 The importance of insect-transmitted plant viruses

In nature, the majority of plant viruses require insect vectors to move between plants (Bos, 1983; Kennedy, 1951; Nalam et al., 2019). Economically, yield losses associated with insect-transmitted viruses of crops have been estimated at more than 30 billion US dollars annually (Sastry & Zitter, 2014; Loxdale et al., 2020). The impact of plant viruses on agriculture may continue to increase as viruses also constitute the largest single group of novel and emerging diseases (Anderson et al., 2004; Lefeuvre et al., 2019). It is thought that the rise in novel viral pathogens and ability to thrive in new hosts and under new ecological conditions is due to their ability to evolve rapidly (Jones, 2009). Through enablers such as global trade, intensive agriculture and climate change, new viral strains and insect vector biotypes have emerged and spread to new farming landscapes (Pagán et al., 2012; Roossinck & García-Arenal, 2015).

Farmers struggle to manage viral pathogens on crops. For example, there are no equivalents to fungicides for the control of viral diseases (Jones & Naidu, 2019). Therefore, farmers heavily rely on preventive measures such as the use of host resistance (Carr et al., 2019; Palukaitis et al., 2013), accurate viral disease diagnostics (Boonham et al., 2014), and vector management using insecticides (Bragard et al., 2013) to monitor and control virus spread. Alternatively, induction of host resistance using exogenous application of natural resistance-inducing compounds like salicylic acid (SA) or various synthetic compounds have been tested but are not yet widely used (Faoro & Gozzo, 2015; Palukaitis et al., 2017). Some genetic resistance is dominant, relying on possession of a resistance (*R*)-gene that allows a plant to recognize a specific pathogen and trigger defence mechanisms (Section 1.6). However, some of the most effective and durable resistance (*r*) genes are recessive, where a host component essential for virus replication is absent or unable to interact with a cognate viral factor. The best understood *r* gene examples encode eukaryotic translation initiation factors required by potyviruses and some cucumoviruses to synthesise viral proteins and other aspects of the viral infection cycle, including intercellular movement (Truniger & Aranda, 2009). Studies in *Arabidopsis thaliana* (hereafter referred to as 'Arabidopsis') showed that a single recessive mutation, *cum1-1*, inhibited systemic movement of cucumber mosaic virus (CMV), resulting in delayed virus accumulation in newly emerged non-inoculated leaves (Yoshii et al., 1998, 2004). Compared to other pathogens such as fungi, viruses do not easily overcome genetic resistance, most likely because even a single mutation in the genome sequence is more likely to prove lethal rather than beneficial (García-Arenal & McDonald, 2003).

However, the use of pesticides to control insect vectors has remained the most common strategy used by farmers to limit viral diseases (Westwood & Stevens, 2010). Chemical control of vectors is costly and poses environmental and health problems (Köhler & Triebskorn, 2013; Carvalho, 2017; Ertl et al., 2018). Smallholder farmers in low-income countries often cannot afford classes of pesticides considered to be safe, and may not wear personal protective equipment (Nelson, 2020). Several studies have also indicated over-use of pesticides in many developing countries could contribute to environmental and food pollution and development of insecticide resistance (de Bon et al., 2014; Sheahan et al., 2017; Nelson, 2020). Therefore, adopting integrated pest/pathogen management strategies that minimise over-reliance on pesticides may be a way towards sustainable management of plant viruses, especially in low-income countries.

Integrated pest/pathogen management strategies may include the use of agricultural practices that manipulate vector behaviour to disrupt between-plants virus transmission, crop varieties with multiple viral disease resistance, and induction of host systemic resistance. To effectively develop and deploy such approaches, prior knowledge of virus-host-vector interactions is required (Jones et al., 2010; Dietzgen et al., 2016; Trivedi et al., 2016; Groen et al., 2017; Dáder et al., 2017).

This study aimed at contributing vital knowledge towards sustainable management of cucumber mosaic virus (CMV) by investigating CMV-induced effects on aphid behaviour using tomato (*Solanum lycopersicum* L.), a high value crop and widely used model, and tobacco (*Nicotiana tabacum* L.), another solanaceous plant widely used as a model for basic studies in plant pathology (Arie et al., 2007; Meissner et al., 1997; Schaeffer et al., 2012). The study drew inspiration not only from several past plant virus-insect interaction studies (see detailed review in Section 1.8), but also from a study by Groen *et al*. (2016) which showed that CMV-infected tomato plants emitted a blend of volatile organic compounds (VOCs) preferred by pollinators (buff-tailed bumblebees: *Bombus terrestris* subsp. *audax*) over the VOC blend emitted by non-infected tomato plants (Section 1.8.2). However, bees are not CMV vectors, and one of my aims was to determine if the same CMV-induced changes in emission of VOCs that influence bumblebees also affect aphid behaviour and thereby influence transmission.

1.2 Cucumber mosaic virus

Taxonomically, *Cucumber mosaic virus* is the type species of the viral genus *Cucumovirus* (Palukaitis & García-Arenal, 2003). The genus also includes *Tomato aspermy virus* (TAV) and *Peanut stunt virus* (PSV), and *Gayfeather mild mottle virus* (reviewed in Ouedraogo & Roossinck, 2019). CMV infects 1,071

species in 521 genera from 100 families of monocots and eudicots, including the solanaceous plants, tomato and tobacco (Yoon et al., 2019). Yoon and colleagues (2019) noted that new host species are discovered every year amongst weeds and ornamental plants, especially in the developing world, where taxonomic identification of plant species is still evolving.

CMV is a positive-sense single-stranded RNA virus. It has three genomic RNA segments, namely RNAs 1, 2 and 3, which encode five proteins (Jacquemond, 2012; Palukaitis & García-Arenal, 2003) (Fig. 1.1). The 1a and 2a proteins, encoded by RNAs 1 and 2, respectively, are viral components of the viral replicase complex. The 1a and 2a proteins can also influence viral movement (Carr et al., 1994; Gal-On et al., 1994). The 1a protein contains methyltransferase and helicase domains, which can influence the accumulation of the 2b protein and modulate its effects on host plants (Westwood et al., 2013). The 2a protein is the viral RNA-dependent RNA polymerase (Jacquemond, 2012; Palukaitis & García-Arenal, 2003). Westwood et al. (2013) showed that the 2a protein also stimulates PAMP (pathogen-associated molecular pattern)-triggered immunity (PTI), one of the layers of innate immunity. In Arabidopsis, this leads to several metabolic changes, including increased glucosinolate biosynthesis and consequent inhibition of aphid ingestion from the phloem of CMV-infected plants (Section 1.8). Recently, Rhee et al. (2020) characterised amino acid sequences within the 2a protein responsible for the induction of this CMV-induced feeding deterrence. RNA 2 also encodes the 2b protein, which is translated from subgenomic RNA4A (Cillo et al., 2002; Palukaitis & García-Arenal, 2003; Seo et al., 2008). The 2b protein is a viral suppressor of RNA silencing (Jacquemond, 2012) and this is discussed more in Section 1.4. The 2b protein also interferes with defence signalling mediated by the phytohormones SA (Ji & Ding, 2001) and jasmonic acid (JA) (Lewsey et al., 2010; Wu et al., 2017). RNA 3 is the translational template for the 3a movement protein (MP) and also encodes the viral coat protein (CP), which is translated from subgenomic RNA4 (Jacquemond, 2012; Palukaitis & García-Arenal, 2003). Both CP and MP are required for cell-to-cell viral movement through the plasmodesmata (Choi et al., 2005; Jacquemond, 2012), and long-distance movement of CMV via the phloem (Suzuki et al., 1991). The CMV CP is required for encapsidating viral RNA into virions but also determines the binding of virions in aphid stylets and the efficiency with which different aphid species transmit the virus (Perry et al., 1998; Liu et al., 2002) (Section 1.5.3).

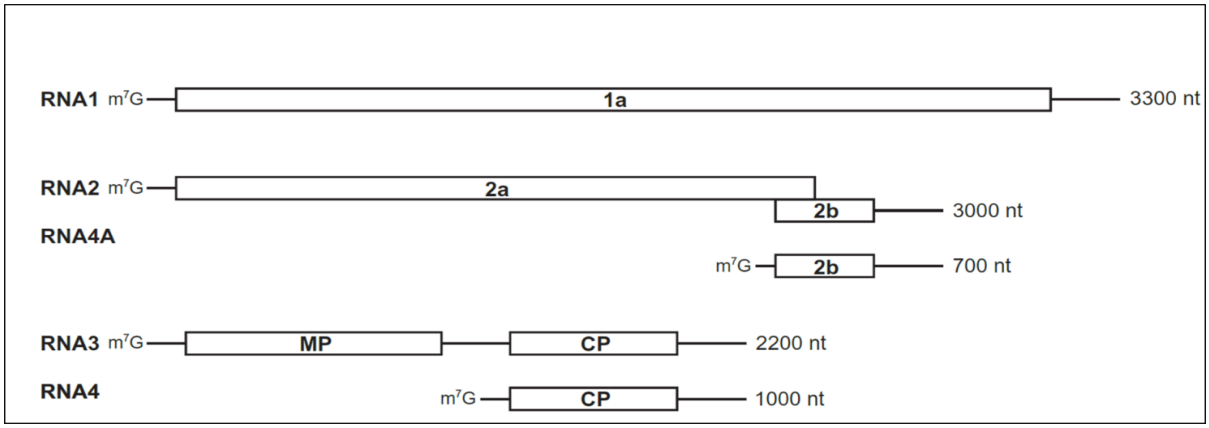


Figure 1.1 CMV genome organisation. Lines represent viral RNA, and boxes represent the open reading frames encoding the five proteins encoded by the three CMV genomic RNA segments (RNAs 1, 2 and 3). Genomes of other cucumoviruses resemble CMV. Protein 1a has methyltransferase and RNA helicase activity, whereas the 2a protein is the viral RNA-dependent RNA polymerase. The 2b protein is a counter-defence protein. The coat protein (CP) is required for encapsidation of the viral genomic RNAs into virus particles, and in addition to the movement protein (MP) is important for cell-to-cell movement and long-distance (systemic) movement. The MP and 2b proteins are translated from subgenomic RNAs 4 and 4a, respectively. CMV RNAs possess 5´methyl-G cap structures, and the 3´ termini have tRNA-like structures (not depicted). The approximate length of each RNA segment is given in nucleotides (nt). Adapted from Jacquemond (2012).

1.3 CMV strains

Most CMV strains can be placed into one of two Subgroups, i.e., Subgroup I or II. Strains were initially assigned to Subgroups based on host range and the serological properties of the CP, but nowadays, assignment is based on RNA sequence similarity (Owen & Palukaitis, 1988; Palukaitis et al., 1992; Roossinck et al., 1999). Subgroup I strain RNA sequences are more diverse than Subgroup II strains (Palukaitis & García-Arenal, 2003) and Subgroup I has been further divided into Subgroups IA and IB (Palukaitis & Zaitlin, 1997; Roossinck et al., 1999). Geographically, Subgroup IA and II strains occur worldwide, but Subgroup IB strains used to be most commonly found in Asia (Roossinck, 2002; Zitter & Murphy, 2009). However, Subgroup IB strains or reassortant (also called pseudorecombinant) strains containing genomic RNAs derived from different Subgroups, including IB, have been discovered elsewhere (for example, see Mutuku et al., 2018). Although most CMV strains can be placed into Subgroups IA, IB or II, others may be distinct enough to be placed in new Subgroups (Tepfer et al., 2016). In this study, focus was restricted to Fast New York (Fny), a Subgroup IA strain, and LS (named for lettuce, *Lactuca sativa*), a Subgroup II strain, which have both been sequenced and have been used for many years as experimental models (Palukaitis et al., 1992). Both strains were first described in the United States. Fny-CMV was isolated from *Cucumis melo* (Banik & Zitter, 1990), and LS-CMV from *Lactuca sativa* (Provvidenti et al., 1980), respectively. Both Fny-CMV and LS-CMV infect tomato (Palukaitis et al., 1992). The Fny-CMV strain induces various symptoms in tomato including stunting, leaf curling, chlorosis, fruit discolouration, and necrosis (Gallitelli, 1991; Jordá, 1992). In severe Fny-CMV infections, leaves of infected tomato plants can appear like 'shoestrings' (i.e., leaf lamina formation is suppressed). Contrastingly, LS-CMV-infected plants appear normal in size, but with leaf blades reduced in area and length (Cillo et al., 2009; Liao et al., 2015).

1.4 The CMV 2b protein

The 2b protein is the smallest (approx. 110 amino acids) of the five proteins encoded by CMV, TAV, and PSV. During infection, the 2b protein accumulates at low levels compared to the other viral proteins but powerfully influences vital aspects of the CMV-host relationships. As mentioned in Section 1.2, the 2b protein inhibits RNA silencing, an antiviral mechanism in plants (Csorba et al., 2015). In plants, RNA silencing is initiated by cleavage of virus-derived double-stranded RNAs (dsRNAs) by Dicer-like (DCL) proteins into 21-24 nt short-interfering RNAs (siRNAs) (Blevins et al., 2006). Cleaved siRNAs are shielded against enzymatic degradation via methylation by Hua enhancer 1 (HEN1) (Yang et al., 2006). RNA helicases unwind methylated siRNAs into a primary siRNA and a passenger strand. The primary siRNAs are then loaded into the RNA-induced silencing complex (RISC), where they direct cleavage of target RNA in a sequence-specific manner by endonucleases called Argonaute (AGO) proteins (Burgyán

& Havelda, 2011). As illustrated in Figure 1.2, the 2b protein inhibits RNA silencing by binding double-stranded siRNAs (González et al., 2012; Rashid et al., 2008; Shi et al., 2008).

The 2b protein also binds to the AGO1 and AGO4 proteins (González et al., 2010; Hamera, 2012; Zhang et al., 2006). Inhibition of AGO1 can interfere with the activity of another group of small RNA molecules, called microRNAs (miRNAs), which are encoded in the plant genome and act as regulators of host mRNA stability and translational activity (Lewsey et al., 2007; Zhang et al., 2006). Plant miRNAs are 18-24 nucleotide long endogenous RNAs important in plant development (Kidner & Martienssen, 2005; Liu et al., 2017). All miRNAs are derived from non-coding stem-loop regions of primary transcripts called pri-miRNAs by DICER-LIKE1 (RNase III-like ribonucleases) in a sequence-specific manner (Park et al., 2002; Reinhart et al., 2002). Subsequently, mature miRNAs, together with siRNA, are loaded into RISCs for degradation or translation repression in a sequence-specific manner (Llave et al., 2002; Jones-Rhoades et al., 2006).

In Arabidopsis, the 2b proteins of Subgroup IA strains but not of Subgroup II strains influence symptom development via disruption of the miRNA-regulated host transcripts that encode factors controlling aspects of plant development (Du et al., 2014a,b; Lewsey et al., 2007; Zhang et al., 2006). The Fny-CMV 2b protein also influences symptoms through other, unknown effects in the nucleus (Du et al., 2014a). Du and colleagues (2014b) found a strong association between the effects of the 2b protein on miRNA159 activity and symptom induction by Fny-CMV in Arabidopsis. In contrast with its lack of effects on miRNA metabolism in Arabidopsis, infection with LS-CMV induced symptom development and altered miRNA-mediated gene regulation in tomato (Cillo et al., 2009). These studies suggest that the effect of 2b proteins on symptoms may be as dependent on plant species as on virus strain.

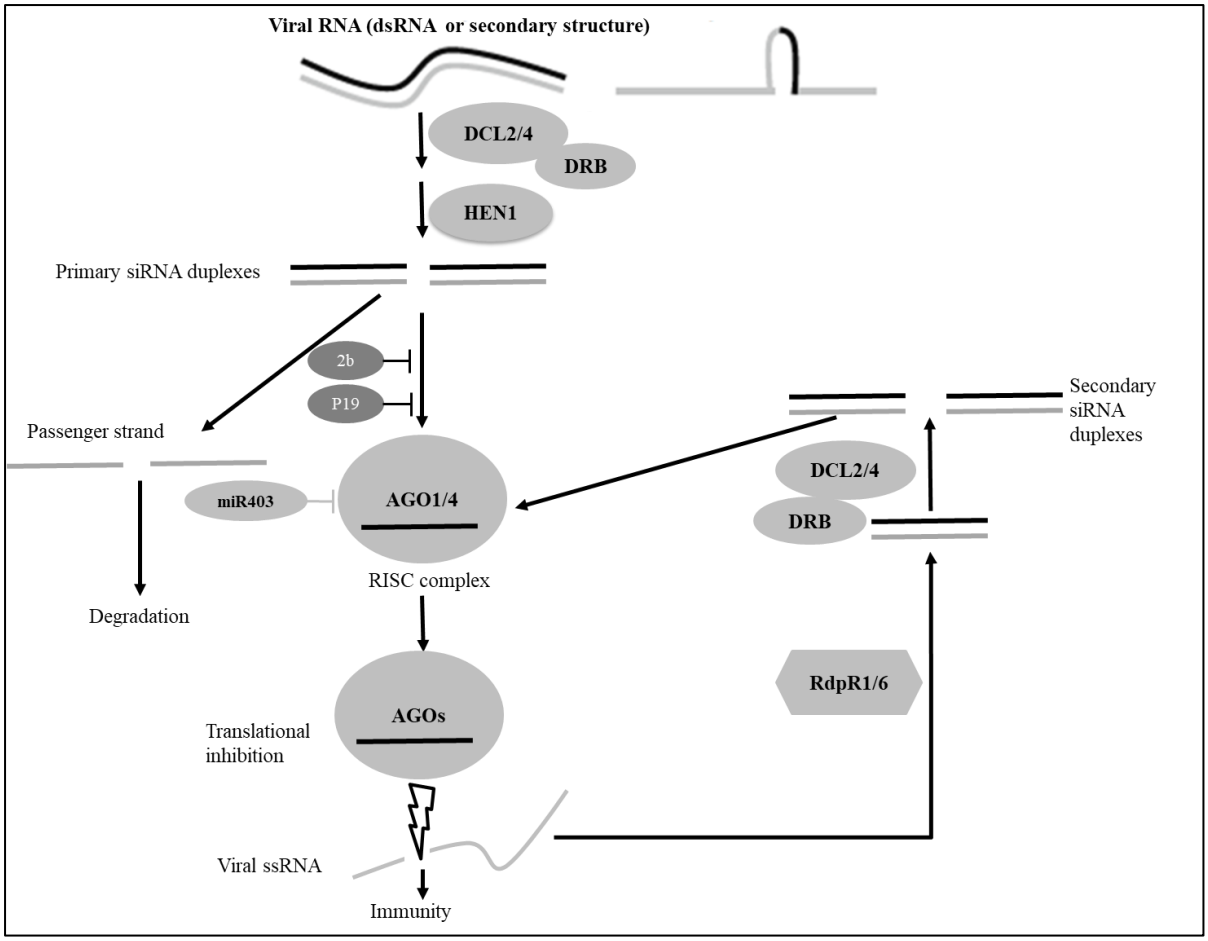


Figure 1.2. The antiviral RNA silencing pathway. Plant double-stranded RNA binding (DRB) proteins promote dicer-like (DCL) ribonucleases to cleave double-stranded regions within viral RNA molecules into primary siRNA duplexes. The passenger strand is degraded, and the primary siRNA is loaded to the RISC complex. AGO1 can inhibit AGO2 activity through miRNA, e.g. miR403 in tomato (Zhang et al., 2015). Host-encoded RNA-dependent RNA polymerases (labelled here RdpR1 or RdpR6) respectively generate further dsRNA to produce secondary siRNA duplexes. These siRNAs may then direct additional AGO-mediated cleavage against viral-derived RNAs. Silencing suppressors are shown in dark grey and host-encoded proteins in light grey. Adapted from Lewsey et al. (2009) and updated with information from Muhammad et al. (2019).

1.5 Transmission of viruses between plants

1.5.1 Horizontal transmission

Transmission is a critical process for viruses as it enables them to spread and survive over time and space. Viruses have evolved various strategies to increase their transmission efficiency between plant hosts within an ecosystem (Harris, 1977). One of the most important plant-to-plant transmission routes depends upon other organisms termed as vectors. Nematodes, parasitic slime moulds, previously classified as fungi, and plant-feeding arthropods constitute major virus vectors (Brault et al., 2010). Nematodes known to transmit viruses include *Xiphinema index,* which transmits the nepovirus grapevine fanleaf virus (Andret-Link et al., 2004; Nguyen et al., 2019), *X. diversicaudatum* that vectors the nepovirus Arabis mosaic virus in strawberry (Harrison & Cadman, 1959), and several trichodorid nematodes, which vector the tobravirus tobacco rattle virus (Ploeg et al., 1989).

Among parasitic slime moulds, specific Chytridiomycete and Plasmodiophoromycete species vector viruses in the genera *Tombusvirus*, *Dianthovirus*, *Furovirus*, and *Bymovirus* (Hull, 2014). Arthropods such as leafhoppers, mites, whiteflies and aphids frequently transmit viruses in plants, but several authors have indicated aphids as extraordinary propagators of viruses (Brault et al., 2010; Nalam et al., 2019). Aphids are efficient virus vectors because of their ability to: i) reproduce and multiply rapidly asexually and sexually; ii) produce winged variants necessary for long-distance dispersal and colonisation of a wide range of plants; iii) evade anti-insect host immunity, and iv) develop insecticide resistance (Brault et al., 2010; Vasquez, 1995; Powell et al., 2006).

1.5.2 Vertical transmission

Some viruses, including CMV in certain weeds (e.g. *Amaranthus* spp.) and cultivated crops (e.g. legumes), can be transmitted from virus-infected parents to offspring via pollen and seed (Hull, 2014; Jones, 2018). This channel, also known as vertical transmission, is exploited by about 20% of known plant viruses (Hull, 2014; Sastry, 2013). Epidemiologically, germinating infected seeds, volunteer plants (crop plants persisting from a previous growing season) and weeds may serve as primary inoculum for subsequent dissemination to other plants by aphids (Alexander et al., 2014; Makkouk et al., 2014). Seed transmission of CMV varies widely from 0.07% in zucchini (courgette: *Cucurbita pepo*) to 100 % in certain soybean (*Glycine max*) varieties (Takahashi et al., 1980). In some hosts, such as hulless oilseed pumpkin, CMV can remain infectious in seed for more than a year and in cowpea (*Vigna unguiculata*) for more than two (Salem et al., 2010; Tóbiás et al., 2008). With increasing global seed

trade, cross-continent spread of viral diseases, including CMV, has been noted in soybean, cowpea and mungbean (*Vigna radiata*) seed traded between Asia, Africa, and the Americas (Bashir & Hampton, 1996; Salem et al., 2010).

Seeds become infected with CMV in two main ways (Pagán, 2019). Firstly, infection may indirectly initiate from the megaspores (large egg-producing female cells), which further infect the ovule and finally the embryo and or seed coat. Secondly, it can occur from infected pollen to ovules and eventually the seed embryo during fertilisation. In pepper, a high incidence of CMV infection was detected in the seed coat (53-80%) and embryo (10-46%) (Ali & Kobayashi, 2010). Similarly, CMV was detected in seed coat cells and pollen grains of spinach (Yang et al., 1997). Seed transmission determinants for a few viruses, including specific CMV isolates such as Pg-CMV (a bean-infecting strain) has been mapped within RNA1, suggesting that the virus promotes seed transmission by controlling its replication within the embryo (Hampton & Francki, 1992). However, other specific viral and host proteins involved remain unidentified.

Additionally, abiotic factors such as plant age at infection and position of seeds on the plant affect CMV seed transmission. For instance, in lupins the transmission rate correlated with the age of the plant at infection (Geering & Randles, 1994). Lupin plants infected early at 58 days post emergence resulted in a high transmission rate compared to late infected plants at 114 days post-emergence. They also reported a high transmission rate in seeds harvested from the upper inflorescences compared to seeds obtained from the middle and primary inflorescences.

1.5.3 Aphid lifecycle

Aphids are small phloem sap-sucking insects in the superfamily Aphidoidea (order Hemiptera), and about 250 aphid species are known economic pests of cultivated crops. Several aphid species, including *Myzus persicae* (Sulzer), *Macrosiphum euphorbiae* (Thomas) and *Acyrthosiphon pisum* (Harris), have a complex lifecycle, which alternates between sexual and asexual reproduction in response to seasonal changes (Dixon, 1977; Moran, 1992) (Fig. 1.3). During warmer weather in spring and summer, sexual forms mate on primary hosts and subsequently lay cold-hardy winter eggs on herbaceous host plants (Leather, 1993). Overwintering of eggs is most frequent in temperate regions of northern Europe and North America but less likely in tropical climates. Aphids have a broad host range and can colonise any plant part (Nalam et al., 2019).

Aphids can be wingless or winged. Wing dimorphism in aphids is conditioned by environmental factors (mostly in parthenogenetically produced females) or can be genetically controlled, such as in male aphids. A recent study with *Acyrthosiphon pisum* showed that genetic dimorphism, also known as polyphenism, in aphids might be controlled by a 120 kb DNA insertion into the genome, which contains a duplicated *follistatin* gene in wingless males (Li et al., 2020). The *follistatin* gene encodes a glycoprotein that regulates wing development in female aphids.

Environmentally-induced polyphenism is dependent on factors such as aphid density, host nutrition, presence of natural enemies and temperature (reviewed in Braendle et al., 2006). In an aphid overcrowding experiment, Lees (1967) observed enhanced production of winged forms in *Megoura viciae* (Buckton). In another study, aphid crowding and reduced nutrition due to plant ageing induced the emergence of winged *Sitobion avenae* on wheat (Watt & Dixon, 1981). Furthermore, the presence of ladybirds, hoverfly larvae and lacewing, or aphid parasitoid wasps encouraged wing development in *Acyrthosiphon pisum* (Kunert & Weisser, 2005). Although winged aphids can migrate long distances (Van Emden et al., 1969), Pleydell *et al*. (2018) estimated that 50% of flying aphids might land within 90 metres, with only 10% of aphid flights extending beyond a kilometre.

These unique reproductive, morphological, and migratory features may facilitate quick aphid dispersal to broader areas and aid rapid virus transmission. Aphids transmit more than 50% of plant viral pathogens in at least eight known families (van Regenmortel et al., 2000). Using their stylets, aphids obtain virus particles from infected plants and can deliver virions to healthy plants with minimal damage to plant cells. This study focused on *Myzus persicae*, a generalist aphid and *Macrosiphum euphorbiae*, a specialist on solanaceous plants. Both of these aphids are among the most damaging insect pests of solanaceous plants, in large part because of their role in transmitting viruses (Blackman & Eastop, 2000; Kennedy et al., 1962).

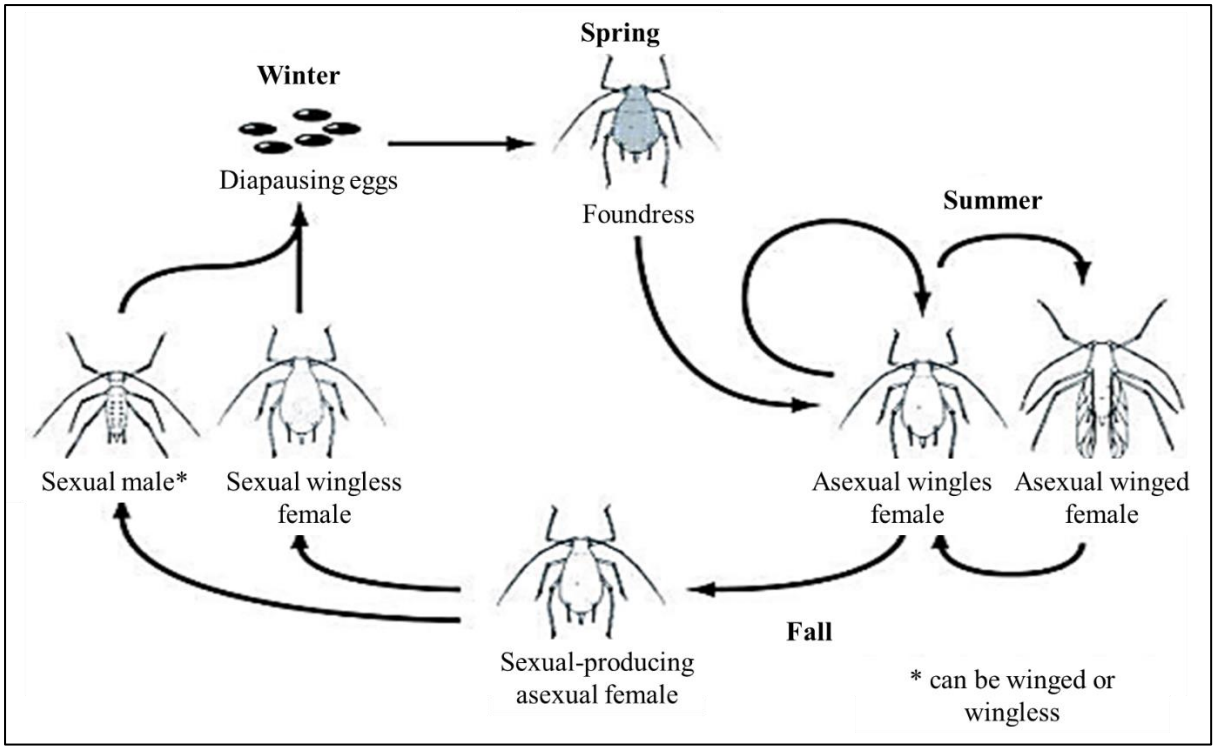


Figure 1.3 Generalist aphid lifecycle. The arrows indicate the direction of development. The lifecycle of most aphid species, especially in temperate regions with distinct seasons, is temperature- and day length-dependent (Trionnaire et al., 2008). During spring's warmer temperatures and extended photoperiods, aphid eggs laid by sexual morphs hatch into female nymphs, which mature into asexual adult females called foundresses. During summer, foundress aphids reproduce parthenogenetically and give rise to multiple generations of asexual offspring. Some of the morphs form wings and disperse. In autumn's shorter daylight and lower temperatures, sexual-producing asexual female aphids are born. These give rise to a mixture of egg-laying sexual wingless females and sexual males, which can mate and lay frost resistant eggs on winter hosts. The eggs remain dormant until warmer days in spring, and the cycle continues. Most aphids undergo four instars in about 23 days before they die (Horsfall, 1924). However, some species may live up to 41 days (MacGillivray & Anderson, 1958). This lifecycle diagram (not drawn to scale), typical of the pea aphid, *Acyrthosiphon pisum,* was adapted from Shingleton *et al*. (2003).

Myzus persicae and *Macrosiphum euphorbiae* can be distinguished by tubercles located between the base of the antennae and body shape (Fig. 1.4). *Myzus persicae* tubercles converge inwards while those of *Macrosiphum euphorbiae* bend outwards. Adult *Myzus persicae* have a small teardrop body shape (Fericean et al., 2011). On the contrary, *Macrosiphum euphorbiae* body is significantly more elongated (Fericean, 2015). Although both species undergo two stages, nymph and adult, the lifecycle of *Macrosiphum euphorbiae* is more plastic than that of *Myzus persicae* and other aphid species. *Macrosiphum euphorbiae* can complete its lifecycle on its primary host plants, thus avoiding alternating periods on secondary hosts like most *Myzus persicae* isolates (MacGillivray & Anderson, 1964). Furthermore, wingless *Macrosiphum euphorbiae* can reproduce sexually and asexually on secondary hosts, which is rare in *Myzus persicae* (Lamb & MacKay, 1997). This is possible because wingless asexual females can produce mating females and winged females and males on secondary hosts. A single female adult can produce 20 to 50 nymphs that mature in 14 to 21 days (D. G. Srinivasan & Brisson, 2012).

1.5.4 Insects as viral vectors

Pioneering transmission studies by Watson and Roberts (1939) measured time elapsed between virus acquisition from an infected plant to inoculation of a healthy host by insect vectors. These workers proposed three basic modes of viral transmission (persistent, semi-persistent, and non-persistent) discussed in detail in subsequent subsections. Well-characterised insect vectors that efficiently transmit plant viruses include aphids, whiteflies, thrips, leafhoppers and planthoppers. Some insect vectors may transmit more than one plant virus, while others are highly specialised (Whitfield et al., 2015). For example, aphids non-persistently transmit both tobacco etch virus, a potyvirus and CMV (Whitfield et al., 2015). Plant viruses transmitted by single insect species include maize mosaic virus by planthopper *Peregrinus maidis* and maize fine streak virus, which is vectored by leafhopper *Graminella nigrifrons* (Whitfield et al., 2018).

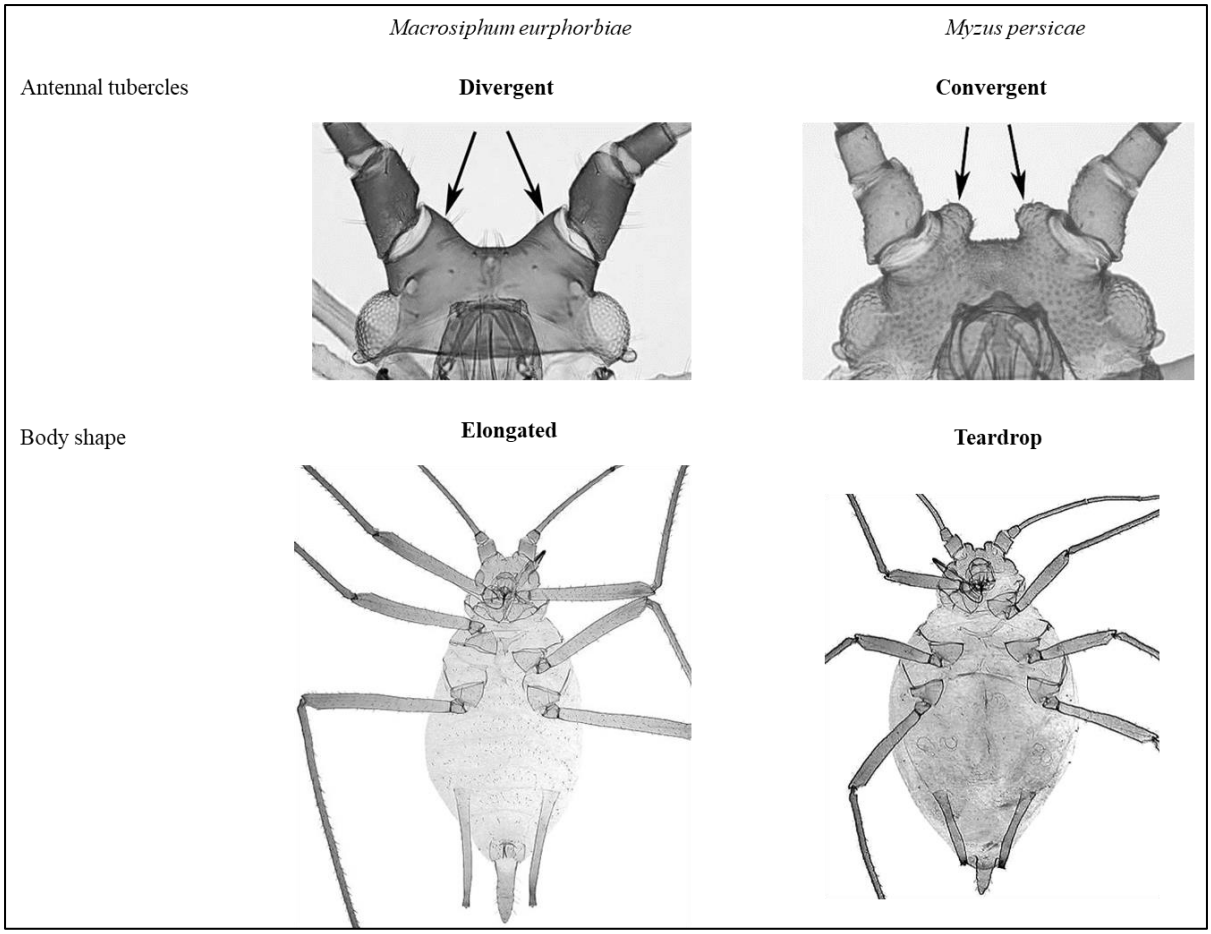


Figure 1.4. Micrographs of aphid antennal tubercles and body shape. The arrows indicate the location and form of tubercles. Tubercles are indentations at the base of each antenna. They are unique among different aphid species, e.g. convergent (form towards each other) in *Myzus persicae* or divergent (away from each other) in *Macrosiphum euphorbiae*. The drawings for tubercle formations and body shape were obtained from http://aphid.aphidnet.org/ and https://influentialpoints.com/, respectively.

1.5.4.1 Circulative non-propagative transmission

When viruses circulate in vector insects but do not replicate, this is called circulative non-propagative transmission. Viruses transmitted in this way include species in the families *Luteovidae*, *Nanoviridae* and *Geminiviridae*. Aphids solely transmit virus species in the first two families, while geminiviruses can be transmitted by whiteflies or leafhoppers (Hogenhout et al., 2008). Circulatively transmitted viruses require a third partner, the GroEL protein, which binds to the viral capsid protein to facilitate virion passage through the midgut and haemocoel without damage (Banerjee et al., 2004; Bouvaine et al., 2011). Endosymbiont bacteria (*Buchnera* spp.) that grow in specialised bacteriocyte cells in the insect abdomen produce the GroEL protein. It has been reported that virus particles of potato leafroll luteovirus (PLRV), which is ciculatively-transmitted by *Myzus persicae,* can move by endocytosis mechanism into the intestinal epithelial cells and haemocoel after 4 hours and 8 hours of acquisition, respectively (Garret et al., 1996). PLRV was still detectable in the aphid intestines for further 3 days and in the haemocoel for 8 days after removing the aphid from the virus-infected source.

Interestingly, at least one virus, tomato yellow leaf-curl virus, TYLCV (genus *Begomovirus* and family *Germiniviridae*), which was thought to be transmitted in a circulative, non-propagative fashion, may replicate in its whitefly (*Bemisia tabaci*) vector (Sinisterra et al., 2005). Ghanim and Czosnek (2000) showed that transmission of TYLCV among whiteflies was sex-dependent. Mating viruliferous male whiteflies transmitted TYLCV to females and vice versa but transmission was not possible between members of the same sex.

1.5.4.2 Circulative propagative transmission

Viruses transmitted in this manner replicate and systemically spread throughout the insect internal tissues before transmission via the salivary glands (Hogenhout et al., 2008). In this mode, the aphid-virus association is permanent. Also, infection of aphid offspring by viruliferous parents transovarially is possible. Plant viruses transmitted by aphids in this manner are few and restricted to the family *Rhabdoviridae* (Ng & Perry, 2004). Well documented rhabdoviruses, including lettuce necrotic yellows cytorhabdovirus (LNYV) and sonchus yellow net virus (SYNV), are transmitted in this mode (Ng & Perry, 2004). LNYV is transmitted by *Hyperomyzus lactucae* and *H. carduellinus* in *L. sativa*, *Datura stramonium*, *N. glutinosa* and certain tomato varieties. *Aphis coreopsidis* transmit SYNV in several plant species, including sowthistle (*Sonchus oleraceus*), *Lactuca sativa*, *N. glutinosa* and *Chenopodium quinoa* (Dietzgen et al., 2007). Boakye and Randle (1974) showed that LNYV persisted in at least two

generations of *H. lactucae,* and virus particles were detectable in salivary glands, brain, muscle, mycetomes, oesophagus and ovaries.

1.5.4.3 Noncirculative, stylet-borne transmission

Noncirculatively transmitted viruses exhibit a more superficial and transient relationship with their vectors, i.e., they remain in the stylet and foregut without entering insect cells or cycling between organs of the insect body (Fig. 1.5). Semi-persistent and non-persistent viruses fall in this category. Plant viruses transmitted in a semi-persistent manner are acquired within several minutes to hours, and virions can remain viable within the insect body for several hours (Sylvester, 1956). These viruses are internalised by aphids and stay bound to the foregut chitin lining. However, they do not replicate in the insect tissues. Non-persistently transmitted viruses require shorter acquisition, retention and inoculation time, usually seconds to minutes (Watson & Roberts, 1939). Non-persistent plant viruses remain loosely bound to virion-binding ligands in the stylet before transmission to new host plants. Many plant viruses classified in the genera *Potyvirus, Alfamovirus*, *Carlavirus*, *Fabavirus*, *Caulimovirus*, and *Cucumovirus* are transmitted by different aphid species in a non-persistent manner (Ng & Perry, 2004).

For efficient transmission, viruses that are non-persistently transmitted by aphids employ one of two mechanisms to facilitate binding of their virions to receptors within the stylet. Cucumoviruses, such as CMV, utilise the capsid mechanism in which a specific amino acid sequence in the CP interacts with the stylet receptors (Liu et al., 2002; Megahed & Pirone, 1966; Ng & Perry, 2004; Krenz et al., 2015). Potyviruses and caulimoviruses (e.g., cauliflower mosaic virus: CaMV) utilize a 'helper' mechanism in which one or more virally encoded non-structural proteins (helper components) form a molecular bridge between receptors in the stylet and sequences of the CP. Potyviruses encode a helper component-proteinase (HC-Pro) that includes, among its multiple functions, the ability to link potyviral CP molecules to a stylet receptor (Blanc et al., 1998). CaMV encodes two helper factors, the P2 and P3 proteins, which are mandatory for aphid transmission (Drucker et al., 2002; Hoh et al., 2010). The P2 protein is a non-viron HC-Pro whose N-terminus binds to the aphid receptor and whose C-terminus to the N-terminal region of P3. Recently, two stylet-localised virus-binding proteins, Stylin-01 and Stylin-02 in the pea aphid (*Acyrthosiphon pisum*) and *Myzus persicae,* were shown to interact with CaMV during transmission (Webster et al., 2018). These proteins were only present and accessible at particular comma-shaped regions in the stylet's internal surface in the stylet's common canal, now referred to as the 'acrostyle' (Uzest et al., 2007; Webster et al., 2018). However, it remains unknown whether similar stylet-localised binding proteins are vital in CMV transmission.

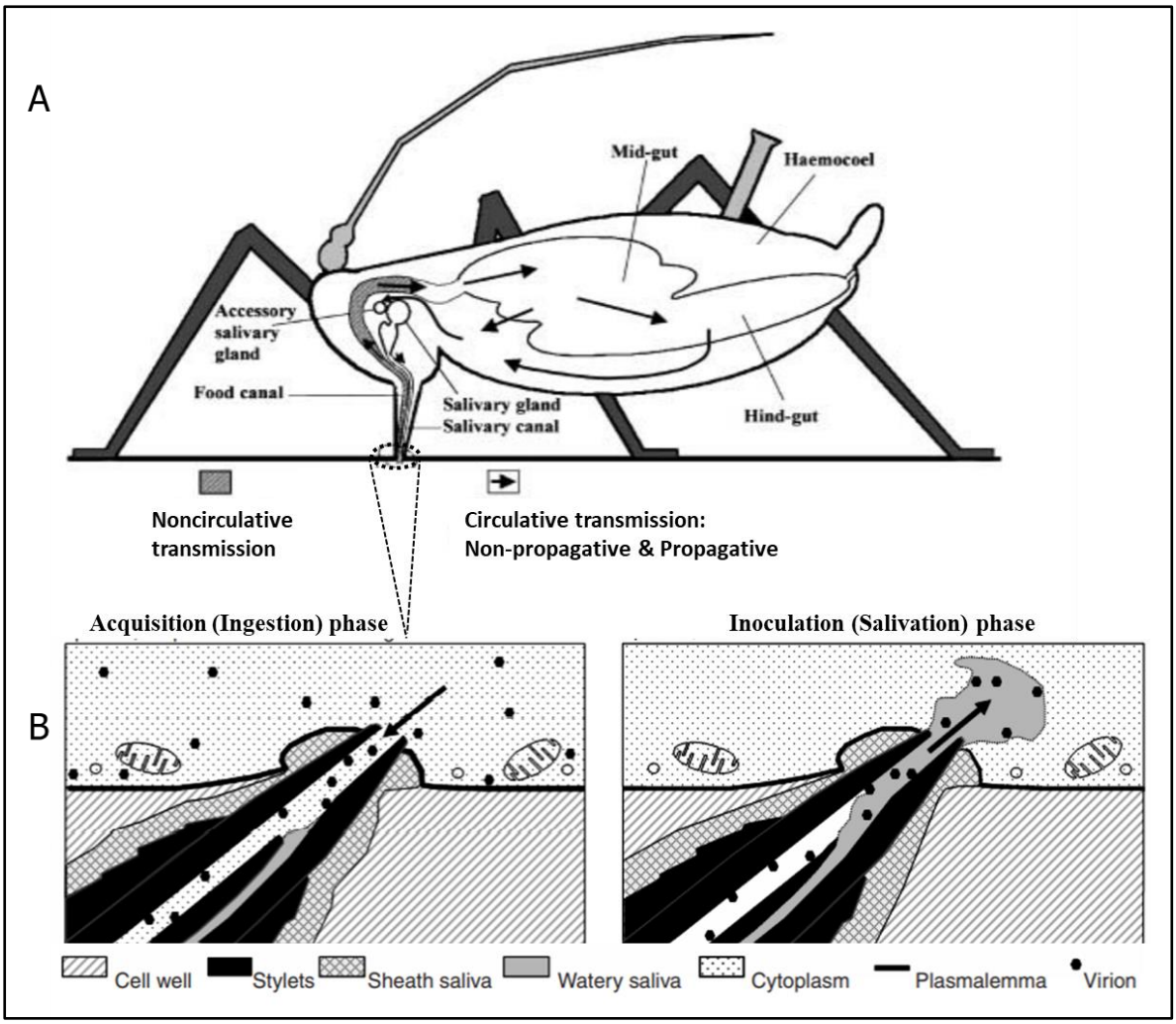


Figure 1.5. Modes of virus transmission and stylet activity during acquisition and inoculation phases. A. Noncirculative viruses are retained in the food canal and foregut before they are released during salivation. Circulatively transmitted viruses proceed from the food canal to the whole alimentary system, haemocoel and accessory gland before release through the salivary glands. Panel B shows an enlarged view of the stylet tip at the point where the salivary canal merges with the food canal to form the common canal, and the arrows indicate virus ingestion and inoculation phases, respectively. The diagrams were adapted from Ng & Perry (2004) (panel A) and Katis et al. (2007) (panel B).

1.5.4.4 Non-persistent transmission of CMV by aphids

Although CMV can be transmitted mechanically (the primary experimental method) and via seed in some hosts (Ali & Kobayashi, 2010), aphid vectors provide the main transmission route in nature (Gildow et al., 2008; Ng et al., 2005; Whitfield et al., 2015). More than 70 aphid species vector CMV (Jacquemond, 2012; Palukaitis et al., 1992). Among these aphid species, *Aphis gossypii* (Glover), *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas) are the most efficient and widespread vectors that transmit CMV in a non-persistent manner (Watson & Roberts, 1939; Hull, 2009). Aphids acquire virus particles during brief probes and ingestion from the epidermal cells of infected plants, and the initial aphid-virus interaction is determined by the CP (Kennedy et al.,1962). Different CMV strains utilise their CP alone to enhance their stylet-binding specificity and transmissibility (Gera et al., 1979; Chen & Francki, 1990). The significance of the CP in aphid transmission of CMV was demonstrated in mutagenesis studies where specific modifications in the CP did not affect virus infectivity or virion formation but negatively affected vector transmissibility (Liu et al., 2002). Other viral genera, which principally depend on their CP for transmission, include *Alfamovirus*, *Carlavirus* and *Crinivirus* (Ng et al., 2004; Weber, 1980).

1.6 Host recognition in pathogen and vector resistance

Plants respond to insect attack by activating defences which may include production of cuticle-associated surface lipids (Reina-Pinto & Yephremov, 2009), synthesis of olfactory cues such as trichome-released alarm pheromones (Kang et al., 2010), enhanced callose deposition (Verma & Hong, 2001), sieve element obstruction (Will & Van Bel, 2006), and anti-feeding deterrents (Westwood et al., 2013). These may reduce host plant attractiveness and accessibility to insects for colonisation, feeding and reproduction. This will also be detrimental to plant viruses that rely on insects for transmission.

Plant resistance to vector infestation can, in a small number of instances, follow the gene-for-gene hypothesis, proposed initially for plant resistance against pathogens, in which dominant *R* genes enable recognition of an invading pathogen or pest (Flor, 1955; Section 1.1). More recently, the co-evolution of plants and their pathogens and pests, which underpins the emergence of *R* genes, is visualised as the zig-zag model (Jones & Dangl, 2006). This model is two-layered. In the first phase, plants recognise conserved PAMPs, e.g. the bacterial flagellar protein-derived flg22 peptide, with transmembrane pattern recognition receptors, which initiates a form of resistance known as PAMP-triggered immunity (PTI) (Chinchilla et al., 2006). The second layer recognises more pathogen-specific molecules, which are usually pathogen effector molecules that have evolved to inhibit PTI. This

resistance response is called effector-trigger immunity (ETI). The emerging view for plant-insect interactions suggests that plants perceive herbivore-associated molecular patterns (e.g. chitin) to activate primary defence (PTI), and that host R proteins recognise more specific insect effector molecules resulting in ETI (Hogenhout & Bos, 2011; Kaloshian & Walling, 2016).

In plants, few durable anti-aphid *R* genes are known (Dogimont et al., 2010). *Vat*, a melon resistance gene controls a resistance mechanism that limits sieve element feeding by *Aphis gossypii* resulting in reduced insect fecundity (Boissot et al., 2016; Chikh-Rouhou et al., 2019). In barrel medic (*Medicago truncatula*), *AIN* (*Acyrthosiphon-induced necrosis*) confers resistance to *Acyrthosiphon kondoi* by mediating an ETI associated with localised plant cell death (a hypersensitive response)(Klingler et al., 2009). In soybean, *Rag1* conditions resistance against *Aphis glycines*, a soybean specialist (Hohenstein et al., 2019; Li et al., 2007). The tomato *Mi-1* gene confers resistance against *Macrosiphum euphorbiae* and infestation by root-knot nematodes (*Meloidogyne* spp.) (Kaloshian et al., 1997; Milligan et al., 1998). Kaloshian *et al*. (2000) later found that aphid feeding in the sieve element phase was markedly limited in *Mi-1* resistant plants compared to susceptible plants. In lettuce, the *Nr* gene introgressed from a wild relative *Lactuca virosa* hinders lettuce aphid (*Nasonovia ribisnigri*) reproduction by restricting phloem-feeding (ten Broeke et al., 2013). More recently, *Sieve element-lining chaperone 1* (*SLI1*), a homologue of the *Restricted Tobacco-etch virus Movement* (*RTM*) gene, which restricts long-range movement of certain potyviruses in Arabidopsis, was identified as a factor conferring resistance to *Myzus persicae* in this plant (Kloth et al. 2017). Kloth and colleagues (2017) observed prolonged aphid feeding and increased phloem ingestion rate on *sli1* mutants compared to wild-type plants. Activation of these defences trigger processes that fortify plant surface layers and intercellular spaces against insect feeding. Some of these defences are dependent upon signalling mediated by the phytohormones SA and JA.

1.6.1 Jasmonic acid

JA and its derivatives (jasmonates), including methyl jasmonate (MeJA) and the JA-isoleucine conjugate are involved in growth, development, metabolism, and responses to wounding as well as insect pest and microbial attack (Baldwin 2001; Browse 2009; Feussner &Wasternack 2002). JA biosynthesis begins in chloroplasts when α-linoleic (18:3) is converted to 12-oxophytodienoic acid (OPDA) by lipoxygenase, allene oxide synthase (AOS) and allene oxide cyclase. OPDA is converted to JA in the peroxisome.

JA is transported into the cytoplasm upon its formation, where the enzyme JASMONIC ACID RESISTANCE 1 (JAR1) conjugates it to its bioactive form, the JA-isoleucine conjugate (Fonseca et al., 2009). Other important derivatives are MeJA and *cis*-jasmone (Pauwels et al., 2008; Wasternack, 2007).

The JA-isoleucine conjugate is the key ligand in JA perception. It primes formation of the co-receptor complex of JASMONATE ZIM (JAZ) proteins with CORONATINE INSENSITIVE 1 (COI1) (Xie et al., 1998). COI1 is an E3 ubiquitin-ligase Skip-Cullin-F-box complex SCF^{COI1} that binds and degrades specific JAZ transcriptional repressors (Chini et al., 2007; Chung et al., 2009; Pauwels & Goossens, 2011; Fig. 1.6). In Arabidopsis, there are 12 JAZ proteins, and at least eight interact with COI1 in a JA-dependent manner (Chini et al., 2016). COI1-mediated degradation of JAZ proteins releases transcription factors (TFs), which triggers induction of JA-responsive gene expression. Different *jaz* knockout mutants result in JA insensitivity and compromised resistance to other pathogens and herbivores (Chini et al., 2016). Also, reduced expression of JA-mediated genes upon mechanical or insect damage has been reported in certain transgenic *COI1*-silenced *Nicotiana* plants compared to wild-type plants (Paschold et al., 2008; Shoji et al., 2008). These findings demonstrate the importance of JAZ-COI1 interactions in anti-insect JA-dependent gene expression.

The best-studied TFs vital in plant-insect interactions include MYC2 and certain WRKY factors. In Arabidopsis, *AtMYC2* was found to negatively regulate indole glucosinolate biosynthesis (Dombrecht et al., 2007). They also found that MYC2 positively regulates JA-mediated insect resistance, possibly via the production of flavonoids. Similarly, MYC2 orchestrates JA-mediated activation of wounding- and pathogen-responsive genes in tomato (Du et al., 2017). Several WRKY TFs involved in insect-plant interactions have been identified in different plant species. For example, *NaWRKY3* and *NaWRK* regulate plant resistance against *Manduca sexta* and other native herbivores in *N. attenuata* (Skibbe et al., 2008), and *SlWRKY70*, a tomato orthologue of the Arabidopsis *WRKY70* gene, is required for *Mi-1*-mediated resistance against *Macrosiphum euphorbiae* (Atamian et al., 2012).

The genes induced by JA in plant-insect interactions include those encoding lipoxygenases, e.g. *LOX2* and *LOX5*; *OPDA reductases*; *terpene synthase* (*TPS*) genes, and glucosinolate biosynthesis genes. Lipoxygenases are key enzymes required in the early stages of JA biosynthesis. Several studies have shown that aphid infestation triggers the expression of different *LOX* genes. For example, increased levels of *4-hydroxyphenylacetaldehyde oxime monooxygenase* (CYP71E1), a cytochrome P450 *LOX*-encoding gene were reported in *Schizaphis graminum*-colonised sorghum (*Sorghum bicolor*) (Zhu-

Salzman et al., 2004). *Myzus persicae* infestation increased *LOX2* and *LOX5* expression levels in Arabidopsis and tomato (Nalam et al., 2012; Stout et al., 1999). In barley, *LOX2* was upregulated after *Rhopalosiphum padi* and *Myzus persicae* infestation (Delp et al., 2009; Losvik et al., 2017). *LOX2* accumulation was enhanced in *Rhopalosiphum padi*-infested resistant genotypes compared to their susceptible counterparts, and the level of *LOX2* accumulation correlated with the degree of genotype resistance (Delp et al., 2009). Aphid fecundity was reduced compared to control plants on transgenic barley plants overexpressing the *LOX2* gene (Losvik et al., 2017).

Genes involved in OPDA synthesis contribute to the defence against aphids. A recent study in maize showed that *12-oxophytodienoate reductase* (*OPR7* and *OPR8*), both genes involved in OPDA biosynthesis, were involved in defence against *Rhopalosiphum maidis* (Fitch) (Varsani et al., 2019). Exogenous application of OPDA followed by *Rhopalosiphum maidis* feeding on *opr7 opr8* double mutants increased callose accumulation in OPDA-treated and aphid-infested plants compare to *opr7 opr8* controls and untransformed maize plants with or without OPDA treatment (Varsani et al., 2019).

JA also mediates the production of VOCs that are significant in several aphid-plant interactions (see Section 1.8). For example, *TPS10*, an MYC2-regulated gene, promotes plant terpenoids that increase plant resistance to whiteflies (Li et al., 2014). However, the βC1 viral factor encoded by a TYLCV satellite DNA directly interacts with the host plant *TPS10* gene to subdue whitefly resistance and promote TYLCV transmission in tomato. *TPS03*, another MYC-controlled gene that encodes *(E, E)-α-farnesene synthase,* alters aphid-parasitoid interactions in Arabidopsis (Kroes et al., 2017). Kroes and colleagues (2017) reported that *Brevicoryne brassicae* infestation enhanced the expression of *TPS03*, which in turn encouraged colonisation of aphid-infested plants by *Diadegma semiclausum*, a parasitoid of the Diamondback moth (*Plutella xylostella*).

JA also regulates glucosinolate biosynthesis (Mewis et al., 2005). Glucosinolates are nitrogen- and sulphur-containing secondary metabolites important in anti-insect plant defence, especially in the *Brassicaceae* plant family (Winde & Wittstock, 2011). In Arabidopsis, the application of MeJA increased the amount of indole glucosinolates by 3- to 4-fold (Michael Dalgaard Mikkelsen et al., 2003). They also reported increased levels of *CYP79B2* and *CYP79B3* genes in MeJA-treated plants. These genes catalyse indole-3-acetaldoxime (IAOx) production, an intermediate substrate in glucosinolate biosynthesis (Hull et al., 1999; Mikkelsen., 2000). In *Brassica* plants, specific glucosinolates act as aphid feeding deterrents, and their effect on aphids may be heightened under CMV infection (see Section 1.8).

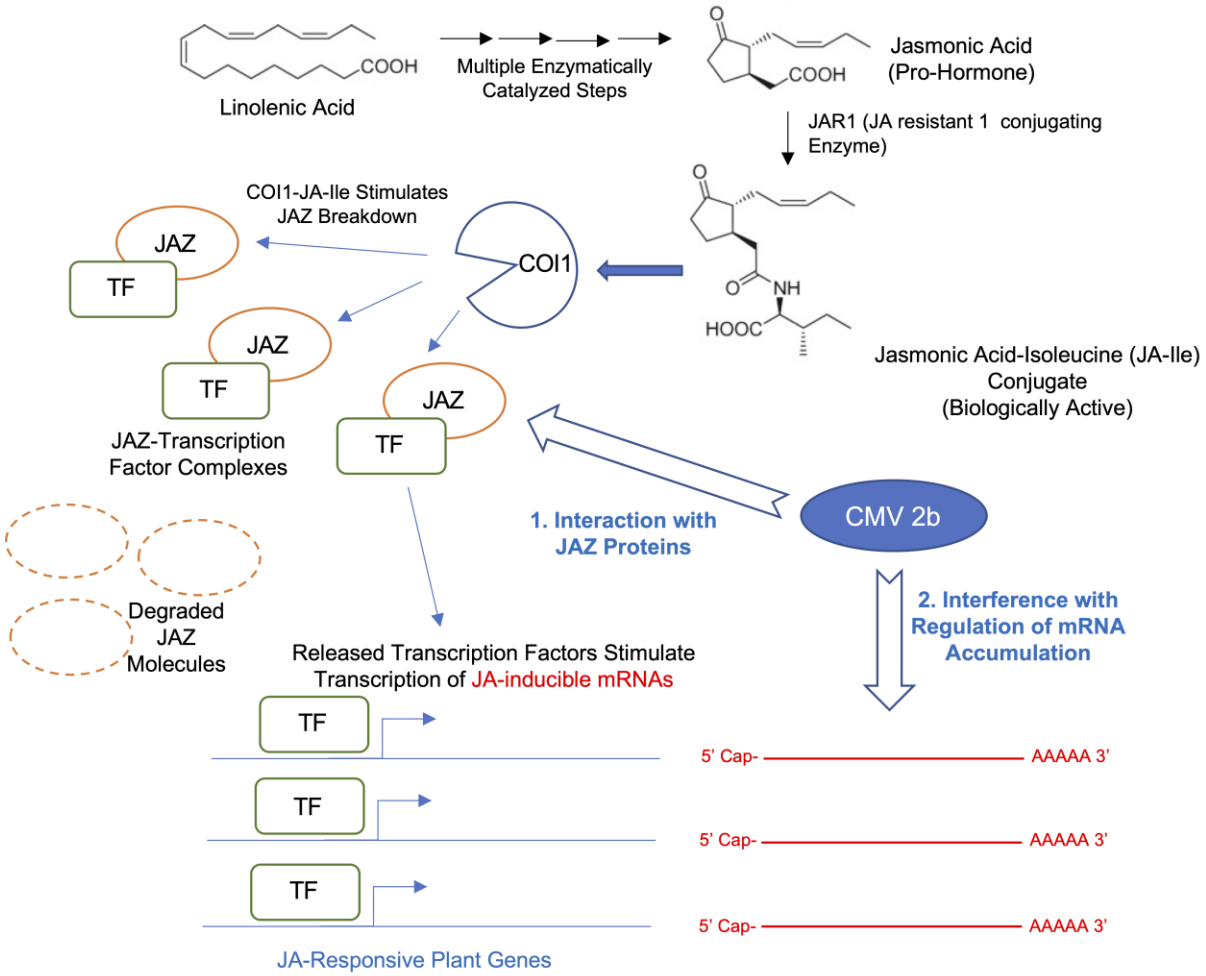


Figure 1.6. A simplified model of jasmonate biosynthesis and perception, and the points at which the cucumber mosaic virus 2b protein may interfere with responses to jasmonates. JA is synthesized from the fatty acid linolenic acid. Among the enzymes involved is LOX2, expression of which is positively regulated by JA-mediated signalling. JA is a pro-hormone that is converted by JAR1 to JA-Ile, in which an amide bond links JA to an isoleucine moiety. The active hormone JA-Ile binds COI1 and strengthens its association with JAZ repressor factors, and this activated COI1 catalyses ubiquitination of JAZ factors leading to their proteolytic degradation. JAZ proteins bind to certain TFs and repress their activity. Destruction of JAZ factors allows TFs to interact with cognate promoters and stimulate transcription of mRNAs encoding JA-inducible proteins. The CMV 2b protein inhibits transcriptional responses to JA. Two mutually compatible mechanisms have been proposed to explain the mode of action, which are: (1) a direct interaction with JAZ proteins (Wu et al., 2017), and (2) interference with the regulation of mRNA stability by small RNAs (Lewsey et al., 2010). The outline of JA biosynthesis and signal transduction is based upon: Chini et al. (2007, 2016); Farmer (2007); Fonseca et al. (2009); Ma & Ma (2016), and Thines et al. (2007).

1.6.2 Salicylic acid

SA (2-hydroxybenzoic acid) regulates various developmental processes and defence against viruses and non-viral pathogens in plants (Malamy & Klessig, 1992; Murphy et al., 2020). In the tobacco mosaic virus (TMV)-tobacco pathosystem, SA is required to prevent the escape of TMV from HR lesions in tobacco cultivars possessing the *N* resistance gene (reviewed by Palukaitis & Carr, 2008). The first evidence that SA is an endogenous defence signal was discovered in tobacco by White (1979). He showed that injection of tobacco plant leaves with SA or aspirin (acetylsalicylic acid) enhances resistance to TMV in tobacco *NN* genotype plants. Subsequent work showed that SA biosynthesis increases following ETI/HR and confirmed that SA is a key defence hormone (reviewed by Murphy et al., 2020).

Expression of many SA-induced genes to pathogens requires the factor Nonexpressor of Pathogenesis-Related Proteins 1 (NPR1) (Cao et al., 1994). NPRI acts as a transcription co-activator of SA-dependent resistance by interacting with TFs such as TGACG-BINDING FACTORs (TGAs) (Zhang et al., 2003). The interaction between NPR1 and TGAs activates *pathogenesis-related* (*PR*) and other defence genes (Carr et al., 2010). Although NPR1-triggered resistance to bacterial and fungal pathogens is common and well understood (J. M. Zhou et al., 2000), it is less certain that all examples of SA-induced resistance to viruses depend upon NPR1 (reviewed in Murphy et al., 2020). Liu and others (2002) showed that the *NPR1*-like gene in *N. benthamiana* is required in *N*-mediated resistance against TMV (Liu et al., 2002). More recently, it was demonstrated that the *NPR1* gene is necessary to activate chemically-induced defence against Plantago asiatica mosaic virus in Arabidopsis (Matsuo et al., 2019). However, several other studies found no evidence for a role for NPR1 in induced resistance to viruses (reviewed in Murphy et al., 2020).

NPR1 plays a unique role in SA-JA crosstalk in different insect-plant interactions (see examples in Thaler et al., 2012). It acts as a negative regulator of hormonal crosstalk in the presence of foraging herbivores by preventing suppression by SA of JA-dependent defence signalling (Rayapuram & Baldwin, 2007). For example, in Arabidopsis, NPR1 regulates synthesis of GRX480 (a redox regulation protein), which represses transcription of the JA-responsive *Defensin 1.2* (*PDF1.2*) gene in an SA-dependent manner (Ndamukong et al., 2007). In lima bean (*Phaseolus lunatus*) and cotton (*Gossypium hirsutum*) plants, increased SA accumulation triggered by whiteflies and mealybugs decreased sensitivity to JA (Zhang et al., 2009, 2011).

1.6.3 The role of JA and SA in aphid-host interactions

In most cases, the SA- and JA-dependent signalling pathways act collaboratively rather than independently against foraging insects (Cipollini et al., 2004; Koornneef et al., 2008). Several groups have reported that aphids deceptively induce SA biosynthesis to inhibit JA-dependent defence responses (see Åhman et al., 2019 and reference therein). The interplay between SA and JA regulates several aphid-plant interactions. For example, in a susceptible genotype of *Medicago truncatula*, SA-inducible genes were upregulated while JA-responsive genes were down-regulated upon feeding by *Acyrthosiphon pisum* under elevated atmospheric CO_2 (Sun et al., 2018). In *Solanum tuberosum*, *Myzus persicae* attack enhanced the expression of SA-regulated *PR* genes. However, JA-responsive genes remained unaffected, confirming that aphids exploit SA and JA antagonism to improve their colonisation success (Alvarez et al., 2014). Coppola et al. (2013) showed that *Macrosiphum euphorbiae* predominantly activated SA-dependent responses compared to JA-dependent defence responses in tomato. In another study where an aphid-resistant tomato variety was compromised in SA accumulation by expressing a *NahG* transgene (derived from a bacterial gene encoding SA hydroxylase), resistance against *Myzus persicae* aphid was lost (Kaloshian, 2004). Similar results were reported in *NahG*-transgenic Arabidopsis plants (Mewis et al., 2005); population growth of *Myzus persicae* and *Brevicoryne brassicae* was severely compromised on *NahG*-transgenic plants compared to non-transgenic plants. Mewis et al. (2005) also observed reduced *Myzus persicae* performance on *npr1* mutants. But contrary to Mewis and colleagues' (2005) findings, no effect on *Myzus persicae* reproduction was observed on wild-type plants versus *eds5* and *eds9* mutants, which are also compromised in SA signalling (Moran & Thompson, 2001). These findings, especially those with *npr1* mutants and *NahG*-transgenic plants, concur with the hypothesis that aphids indirectly antagonise JA-related defence by amplifying SA accumulation to foster infestation (Zhu-Salzman et al., 2004, 2005). Although SA-dependent crosstalk is a widely known contributor in various anti-aphid immune responses, independent activation of JA has been reported in specific aphid-host interactions. For example, in Arabidopsis Col-0, JA-associated defence genes such as *OPR2, OPR3, LOX3* and *LOX4* were downregulated by *Myzus persicae* within the first 5 hours of infestation with no indication of SA signalling (Bricchi et al., 2012). A similar finding was reported with *Macrosiphum euphorbiae* in a susceptible tomato genotype (Rodriguez et al., 2010).

SA-JA crosstalk has also been implicated in long-distance movement of plant viruses. For instance, tomato plants infected with tomato spotted wilt virus (TSWV), which is vectored by western flower thrips, were found to have high levels of SA but decreased JA levels (Abe et al., 2012).

Western flower thrips preferentially fed on TSWV-infected plants compared to healthy plants. It has not yet been determined if TSWV targets SA and JA pathways to promote its transmission by thrips.

1.7 Sensory cues in aphid-host interactions

Herbivorous insects rely on several visual, olfactory, tactile, and taste cues to find, accept or reject, plant hosts suitable for feeding or reproduction, or both (Reeves, 2011).

1.7.1 Visual cues

Light reflected by different surfaces influences various insect species' preference and settling behaviour, including aphids and whiteflies (Coombe, 1982; Döring & Röhrig, 2016; Döring, 2014; Prokopy & Owens, 1983). Several phototactic studies have shown that aphids preferentially orient towards green and yellow surfaces (Kennedy et al., 1961; Prokopy & Owens, 1983). Aphid vision depends on photoreceptors' sensitivity in their compound eyes, but photoreceptor complements differ between aphids. For instance, *Myzus persicae* has three photoreceptors [ultraviolet (UV), blue and green] with maximum sensitivities at 320–330 nm (UV), 440–480 nm (blue) and 530 nm (green) (Kirchner et al., 2005; Yang et al., 2015). In contrast to *Myzus persicae*, the visual system of *Brevicoryne brassicae*, the cabbage aphid, is dichromatic with peak sensitivities at 350 nm (UV) and 520–530 nm (green) (Doring & Kirchner, 2007). Both aphid species showed overall spectral sensitivity to green, which suggests that the cabbage aphid is most responsive to green despite having a distinct photoreceptor suite to *Myzus persicae*.

The plant-insect co-evolution theorists Archetti and Brown (2004) hypothesised that visual cues are the principal drivers of aphid migratory behaviour to green-leaved primary host plants in winter. In contrast, it was noted that bright autumn colours, such as red, act as a deterrent to herbivore infestation (Archetti, 2000). Döring and colleagues (2009) developed a colour preference model that showed aphids could distinguish between red (625-740 nm) and green (500-565 nm) and innately possess the capacity to avoid red plant surfaces. In autumn, *Rhopalosiphum padi* preferentially colonised bird cherry trees (*Prunus padus*) with green leaves rather than trees with yellow leaves (Archetti & Leather, 2005). This observation supported the autumn co-evolution model by Archetti & Brown (2004), and the Döring *et al*. (2009) colour choice model.

1.7.2 Volatile cues

VOCs are aromatic, fat-soluble compounds with low boiling points; chemical properties that enable VOCs to easily evaporate from plant leaves, flowers, fruits, stems and roots (Insam & Seewald, 2010; Picazo-Aragonés et al., 2020; Vespermann et al., 2007). Common microbe-inducible VOCs include alcohols, alkenes, ketones, benzenoids, pyrazines, thioesters and terpenes (Zhou & Pichersky, 2020). VOCs that influence insect behaviour are sometimes called 'semiochemicals', i.e. signalling chemicals (Pickett et al., 2012). Aphids use plant-emitted VOCs as semiochemicals to locate host plants (Pickett et al., 2012; Powell & Hardie, 2001). The first attempt to understand the role of VOCs in aphid-host interactions tested the olfactory responses of *Aphis fabae* to spindle tree (*Euolzymus europaeus*), dock (*Rumex obtusifolius*) and broad bean (*Vicia faba*) using an olfactometer (Jones, 1944). Although *Aphis fabae* did not show a significant olfactory response, these aphids preferred volatiles emitted by primary hosts (spindle trees and beans) compared to dock plants. A blend of 15 specific VOCs rather than a single compound was later identified as responsible for this behaviour (Webster et al., 2008). In addition to aphids using VOCs as species-specific host cues, they can also use them to distinguish between virus-infected and uninfected individuals (reviewed in Webster, 2012). Examples of odour-mediated aphid behaviour are discussed in Section 1.8.

Herbivore-induced plant volatiles (HIPVs) are important in recruiting natural enemies (Turlings & Erb, 2018). HIPVs produced by infested plants vary depending on the plant species, the attacking insect species, infestation level, and morph type (Agbogba & Powell, 2007; Norkute et al., 2019; Xiu et al., 2019). Common HIPVs associated with insect predators include terpenoids, methyl salicylate, MeJA, and other GLVs (Arimura et al., 2005; Snoeren et al., 2010). In barley (*Hordeum vulgare* L), ladybird beetles (*Coccinella septempunctata*) were significantly attracted to VOCs by *Rhopalosiphum padi*-infected plants. Their findings suggested that HIPVs cues are utilised by aphid predators in prey finding (Norkute et al., 2019).

1.7.3 Tactile cues

Plants may respond to mechanical or insect contact by altering their physiology, morphology and biochemistry (Braam, 2004). Such changes may provide reliable cues to insects, including host plant nutritional quality and suitability for their growth, reproduction, and survival. For example, caterpillar movement on tomato leaves elicits anti-herbivore defence responses resulting in increased glandular trichome density in emerging leaves and enhanced VOCs emissions in emerging leaves (Peiffer et al., 2009).

Trichomes (leaf hairs) act as contact sensors or barriers against insects (Peiffer et al., 2009; Shepherd et al., 2005). Glandular trichomes are a vital source of terpenes (Li et al., 2004), which may repel herbivores or enhance recruitment of natural enemies to infested plants (Kant et al., 2004; Schnee et al., 2006). Tomato varieties possess various combinations of seven trichome types. Types VI and VII are glandular trichomes comprising four- to eight-celled heads (Thipyapong et al., 1997). Insect movement on tomato surfaces may rupture these heads, releasing their cellular contents, which polymerise into a sticky exudate (Peter et al., 1995; Tian et al., 2012). The exudates may induce deterrence against aphids (Sarria et al., 2010). For example, high-density trichomes and exudates in *Cucumis melo* negatively affected the settling behaviour of *Aphis gossypii*. Aphids reject leaf discs of a hairy variety compared to a hairless genotype, but this was reversed by washing the leaf discs (Sarria et al., 2010). Similarly, *Myzus persicae* markedly preferred hairless tomato genotypes compared to hairy genotypes. In glass Y-tube olfactory assays, aphids preferred VOCs emitted by hairy tomato plants compared to hairless mutants, suggesting a relationship between trichome density and volatile production (Dr Lewis Watt, *Unpublished*).

1.7.4 Taste cues

After landing, aphids use their stylets to puncture plant cells and evaluate the plant sieve-tube sap contents for essential nutrients (Schröder et al., 2017). During the evaluation process, they rely on taste signals along the stylet pathway such as sugar content, amino acid levels, vitamins, and pH to select host plants for survival, growth and reproduction (J.L Auclair, 1969). It has been reported that among different sugar sources, sucrose is an important clue for the orientation behaviour of several aphid species (Hewer et al., 2010). In choice-chamber experiments, *Myzus persicae*, *Myzus viciae*, *Macrosiphum euphorbiae* and *Rhopalosiphum maidis* showed a marked preference for sucrose-containing artificial diets compared to other sugars. Hewer and colleagues (2010) further showed that in the absence of sucrose, these aphid species favoured raffinose. In the same study, they observed that aphid preference began to decline beyond a sucrose concentration of 15%, suggesting that sucrose viscosity may also play a key sensory role in aphid behaviour by hindering ingestion. This result was corroborated by Mittler (1967) who reported constrained uptake of highly viscous fluids (diets with ≥ 20% sucrose concentration) by *Myzus persicae*.

An imbalance in plant amino acid content hinders aphid performance (Ponder et al., 2000; Retnakaran & Beck, 1968; Simpson et al., 1995). *Acyrthosiphon pisum* requires ten essential amino acids, i.e. arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are required for aphid survival and growth (Retnakaran & Beck, 1968). Using artificial diets, it

was shown that the omission of half of these amino acids (especially methionine) resulted in dramatic aphid weight loss and impaired reproduction. *Rhopalosiphum padi* phloem ingestion on barley was reduced on nitrogen-deficient plants, which contained lower concentrations of non-essential amino acid than nitrogen-supplemented plants (Ponder et al., 2000). Amino acid nutrition is also involved in wing polymorphism. Omission of methionine and histidine from diets induced wing formation, whereas arginine, leucine, lysine, and proline omission induced alatae formation in *Aphis fabae* (Leckstein & Llewellyn, 1973).

Vitamins in the plant sap influence aphid preference and settling behaviour. Treating plants with thiamine (vitamin B1) elicits host defence against pathogens and insects (Ahn et al., 2007). Recently, it was reported that plants germinating from barley or pea seeds pre-soaked in thiamine solution were less acceptable to *Rhopalosiphum padi* on treated plants compared to control plants: 70% of aphids migrated and settled on water-treated plants (Hamada & Jonsson, 2013). Additionally, thiamine-treated plants exhibited antibiosis against aphids leading to reduced aphid reproduction and survival.

During stylet probing, aphids may also utilise pH sensing to discriminate between xylem vessels and sieve-tube. Several studies have reported a strong association between pH and aphid behaviour (J.L Auclair, 1969; Hewer et al., 2010). Findings presented in a review by Auclair (1969) showed that slightly alkaline chemically defined diets (pH 7.3-7.4) enhanced aphid performance. In contrast, highly acidic and alkaline diets hinder feeding leading to increased aphid mortality rates. For instance, using electronic penetration graph (EPG: an electric circuit-based system used by biologists to study insect-plant interactions by examining insect feeding activity), several aphid species, including *Myzus persicae* and *Macrosiphum euphorbiae,* showed sustained feeding on artificial diets of pH 7.2, which approximates the phloem sap pH (Hewer et al., 2010).

1.8 Virus-induced changes in plant-aphid interactions

1.8.1 CMV-induced changes in interactions of aphids with Arabidopsis, cucurbits and tobacco

Viruses appear to be able to enhance their transmission and spread by inducing changes in VOC emission, plant defence and biosynthesis of insect feeding deterrents (reviewed in Carr et al., 2020). Virus-induced metabolic changes differ between hosts, and the changes may influence the behaviour of insect vectors in various ways (Carmo-Sousa et al., 2014; Mauck et al., 2010a; Westwood et al., 2013). For example, Westwood et al. (2013) showed that *Myzus persicae* migrated away from CMV (strain Fny) infected *Arabidopsis* (ecotype Col-0) plants. In the same study, aphids confined on infected

plants gained less weight compared to those on mock-inoculated plants. EPG results showed limited phloem sap ingestion rates by aphids on CMV-infected Arabidopsis plants. This reluctance of aphids to feed from the phloem was attributed to increased biosynthesis of 4-methoxy-indol-3-yl-methylglucosinolate since the mean relative growth rate of *Myzus persicae* was not decreased on CMV-infected mutant plants that were unable to accumulate this metabolite (Westwood et al., 2013). 4-methoxy-indol-3-yl-methylglucosinolate is a glucosinolate with antixenotic effects, i.e., it deters aphid feeding (Kim & Jander, 2007; Kim et al., 2008; Westwood et al., 2013). Westwood et al. (2013) observed strain-specific differences in the induction of aphid resistance. LS-CMV induced no aphid resistance in infected plants. This result was also noted by Hily et al. (2014). Rhee et al. (2020) exploited differences between the amino acid sequences of the Fny-CMV and LS-CMV 2a proteins to identify a specific residue in the Fny-CMV 2a protein (valine 237) needed for induction of antixenosis in Arabidopsis. The corresponding residue in the amino acid sequence of the LS-CMV 2a protein is isoleucine. Rhee et al. (2020) also showed that *Myzus persicae* confined on Arabidopsis plants infected with Fny-CMV displayed decreased fecundity as well as decreased growth.

Myzus persicae and *Aphis gossypii* also reproduced less abundantly on CMV-infected squash (*Cucurbita pepo*) plants when compared to mock-inoculated plants (Mauck et al., 2010a). However, in the same study, aphids of both species were more attracted to these poor-quality host plants than mock-inoculated plants. This behaviour was attributed to increased emission of plant VOCs. In greenhouse experiments, it was found that despite the initial attraction of aphids to CMV-infected plants, they were more likely to migrate to healthy plants subsequently (Mauck et al., 2010a). Similar results were reported in CMV-infected cucumber (*Cucumis sativus*) (Carmo-Sousa et al., 2014). Experiments with Arabidopsis indicated that CMV infection discourages settling of aphids, which may increase the chance of CMV transmission (Bravo-Cazar, 2019; Westwood et al., 2013;). Recently, Donnelly et al. (2019) demonstrated with mathematical modelling that that feeding deterrence could enhance localised plant-to-plant spread by wingless aphids. However, this effect on transmission is localised and self-limiting, and winged aphids are more efficient at initiating epidemic spread. However, feeding deterrence is not induced in all CMV hosts. Bravo-Cazar (2018) observed arrestment of *Myzus persicae* on CMV-infected plants of Arabidopsis accession Ei-2, and by mixing Col-0 and Ei-2 plants in microcosms, it was possible to disrupt CMV transmission.

In *N. tabacum* cv. Xanthi, CMV infection fostered aphid survival, whereas CMVΔ2b infection-induced strong aphid resistance (Ziebell et al., 2011a). *Myzus persicae* confined on CMVΔ2b-infected tobacco showed decreased fecundity and increased mortality compared to those confined on CMV-infected

tobacco plants. Ziebell and colleagues (2011) found that the CMV 2b protein encourages aphid infestation in tobacco by masking the effects of another virus factor, thus inhibiting plant resistance in virus-infected hosts. More recently, Tungadi and others (2020) identified this factor, which has the potential (in the absence of the 2b protein) to induce aphid resistance in tobacco as being the CMV 1a protein (see Section 1.3). Surprisingly, another study by Tungadi and colleagues (2017) found that although CMV induced changes in VOC emission, aphids showed a marginal preference for mock-inoculated plants over CMV-infected plants. They concluded that virus-induced qualitative and quantitative modifications of VOC emission were partly due to the CMV 2b counter-defence protein, but that the changes induced in tobacco by CMV did not influence aphid settling.

CMV and its 2b protein repress expression of JA-regulated genes in Arabidopsis (Lewsey et al., 2010; Westwood et al., 2014; Wu et al., 2017; Fig. 1.6). Approximately 90% of JA-related genes were significantly inhibited in JA-treated plants (Lewsey et al., 2010). Considering that the JA-dependent defence signalling pathway is vital in defence against insects, these findings suggest a possible mechanism by which CMV infection may influence host-vector interactions. Similar observations of suppression of JA-induced transcriptional responses were reported in tobacco and *N. benthamiana* (Westwood et al., 2014; Ziebell et al., 2011). CMV-induced effects on the JA-dependent defence signalling pathway are thought to result either from disruptions in small RNA pathways as first noted in *N. attenuata* (Pandey & Baldwin, 2007) or inhibition of COI1-JAZ protein interactions (see Fig. 1.6).

1.8.2 Changes induced by potyviruses in interactions between host plants and aphids

In tic beans (*Vicia faba* L.), *Acyrthosiphon pisum* preferentially settled on bean yellow mosaic virus (BYMV)-infected plants compared to their mock-inoculated counterparts (Hodge & Powell, 2008). The attraction of aphids to BCMV-infected plants was due to visual cues arising from yellowing leaf symptoms. However, BYMV infection did not confer enhanced reproduction or survival on *Acyrthosiphon pisum*. The poor fitness of *Acyrthosiphon pisum* on BYMV-infected plants was due to plant tissue dieback. It was proposed that the initial attraction of pea aphids to infected plants followed by poor aphid survival encourages BYMV acquisition and onward migration of viruliferous aphids.

Aphis gossypii reproduction and lifespan is enhanced on papaya ringspot virus-infected squash due to enhanced host free amino acid and soluble carbohydrate nutrient content in virus-infected compared to mock-inoculated plants (Gadhave et al., 2019). Previous studies had shown that higher amounts of free essential amino acids such as threonine, arginine, and lysine (Auclair, 1963; Wilkinson & Douglas,

2003) and soluble carbohydrates such as raffinose encourage prolonged aphid feeding, which enhances aphid performance (Hewer et al., 2010).

In soybean, *Myzus persicae* and *Rhopalosiphum maidis* showed no preference between the potyvirus soybean mosaic virus (SMV)-infected plants and mock-inoculated plants (Fereres et al., 1999). However, *Rhopalosiphum maidis* remained longer on uninfected than on SMV-infected soybean plants before take-off. Fereres and colleagues (1999) adduced that short stays on SMV-infected soybean plants by *Rhopalosiphum maidis* could increase the chance of SMV transmission to healthy plants. Thus, SMV can induce different changes in vector-host interactions depending on the aphid species.

Infection with the potyvirus potato virus Y (PVY) altered the settling behaviour of *Myzus persicae* and *Macrosiphum euphorbiae* on potato (Boquel et al., 2011). EPG showed that *Myzus persicae* phloem sap ingestion was enhanced and probing duration at the epidermis reduced. The reverse effect on sap ingestion was noted for *Macrosiphum euphorbiae*. Boquel et al. (2011) also reported higher PVY transmission rates by *Myzus persicae* compared to *Macrosiphum euphorbiae*. Subsequently, the same group discovered that PVY infection affects aphid settling behaviour differentially (Boquel et al., 2012). While *Myzus persicae* probes its host on the first landing, *Aphis fabae, Sitobion avenae* and *Brevicoryne brassicae* do not. Instead, they migrated randomly to different plants before settling (Boquel et al., 2012). Similarly, CMV induced distinct feeding behaviour between aphid species. *Aphis fabae*, a bean specialist, showed reduced phloem ingestion on infected plants, whereas *Myzus persicae*, a generalist aphid experienced stylet difficulty during phloem probing on virus-infected plants (Wamonje et al., 2020).

1.8.3 CMV infection induces changes in interactions between host plants and beneficial, non-vector insects

CMV infection altered the foraging behaviour of bumblebees on tomato, even though these insects do not vector the virus (Groen et al., 2016). This was due to the induction of quantitative and qualitative changes in VOC emission. Experiments with the CMVΔ2b mutant in tomato and Arabidopsis suggested the involvement of the 2b protein in disrupting the production of pollinator-perceivable VOCs (Groen et al., 2016). Groen *et al*. (2016) attributed the effects of 2b on volatile emission to disruption of miRNA regulation of JA-regulated gene expression (Lewsey et al., 2010; Pandey & Baldwin, 2007).

However, Wu et al. (2017), after investigating the effects of the 2b protein on interactions of aphids with detached Arabidopsis leaves, contended that 2b influences volatiles by binding JAZ proteins (Section 1.6.1).

Bumblebee-pollinated CMV-infected tomato plants yielded more seeds than their mock-inoculated counterparts. CMV infection also enhances pollination services by bees in tomato (Groen et al., 2016; Platoni et al., *Unpublished*) and common bean plants (infected with CMV or two potyviruses: Mhlanga, 2019). In both plants, bee pollination rescued seed production in infected plants. Evolutionary mathematical modelling suggests an advantage for susceptible genotypes over resistant in natural communities of pollinated plants but only if pollinators favour pathogen-infected susceptible plants (Groen et al., 2016).

CMV-infected squash plants produced VOCs, which reduced the survival and performance of *Aphis gossypii* but increased that of *Chrysopidae* (*Neuroptera*), an aphid predator (Mauck et al., 2015). This CMV-conferred benefit to predators of aphid vectors may enhance the chances of survival of the infected plants and its potential as primary sources of inoculum for subsequent infections. Thus, the increased fitness of predators on plants infected with CMV may be one of the indirect ways through which the virus preserves itself in the ecosystem.

1.9 The potential epidemiological consequences of virally modified plant phenotypes

Taking CMV as an example of a virus that can modify aphid-host interactions in several ways (Section 1.8.1), the Type 1 outcome is where CMV infection induces plant mechanisms that deter aphids from prolonged feeding from phloem tissues and encourage probing of epidermal cells. Since CMV is a non-persistently transmitted virus, this may encourage virus-bearing aphids to move to healthy plants. Hosts that interact with Fny-CMV resulting in a Type 1 outcome include Arabidopsis (ecotype Col-0), squash and cucumber (Section 1.8.1). In Type 2 hosts, aphids preferentially settle on CMV infected plants and exhibit increased survival and reproduction rates (i.e. in a Type 2 outcome, the plant is exhibiting virus-induced susceptibility to aphid infestation) (Fig. 1.7). Examples of hosts that exhibit Type 2 outcomes when infected by CMV include tobacco (Ziebell et al., 2011; Tungadi et al., 2020) and accession Ei-2 of Arabidopsis (Bravo-Cazar, 2019). For another virus, the potyvirus TuMV, a Type 2 outcome occurs in Arabidopsis Col-0 (Casteel et al., 2014), showing that a plant can respond in more than one way, depending upon the virus. Work by Bravo-Cazar (2019), using a range of Arabidopsis

accessions, suggested that Type 1 and Type 2 outcomes are extreme ends of a continuum that includes neutral effects on aphid performance.

A Type 2 outcome was considered unlikely to foster onward transmission of the virus, and Mauck and colleagues (2014) suggested that when virus-infected plants were more susceptible to aphid infestation, this represented an instance in which host and virus are poorly adapted to each other. However, others suggested that although such plants would not be good foci for rapid spread of the virus to neighbouring hosts, they would act as useful hosts for aphids during stressful conditions of cold or drought. Thus Type 2 outcomes would benefit vector and virus by aiding persistence during difficult conditions or inclement seasons (Westwood et al., 2013; Groen et al., 2017).

Donnelly et al. (2019) mathematically modelled the effects of virus-induced changes on aphid-host interactions, which they termed 'virally modified plant phenotypes'. Where virus infection made plants attractive to aphids but distasteful (a virally modified plant phenotype equivalent to a Type 1 outcome they termed 'attract and deter'), this would accelerate the dispersal of virus-bearing aphids to neighbouring plants, in line with the conceptual models of Mauck et al. (2010a) and Westwood et al. (2013) (Fig. 1.7). However, a consequence of forcing aphids to migrate from infected plants without obtaining nourishment, the opportunity to settle and reproduce, or avoid predation is that enhanced transmission, especially by wingless aphids, will be rapid but of limited extent (Donnelly et al., 2019). Counter-intuitively, the model predicts that Type 2 outcomes will enhance epidemics since aphids will be crowded on infected plants, engendering production of winged aphids (Section 1.5.1) that disperse viral inoculum over greater distances (Donnelly et al., 2019).

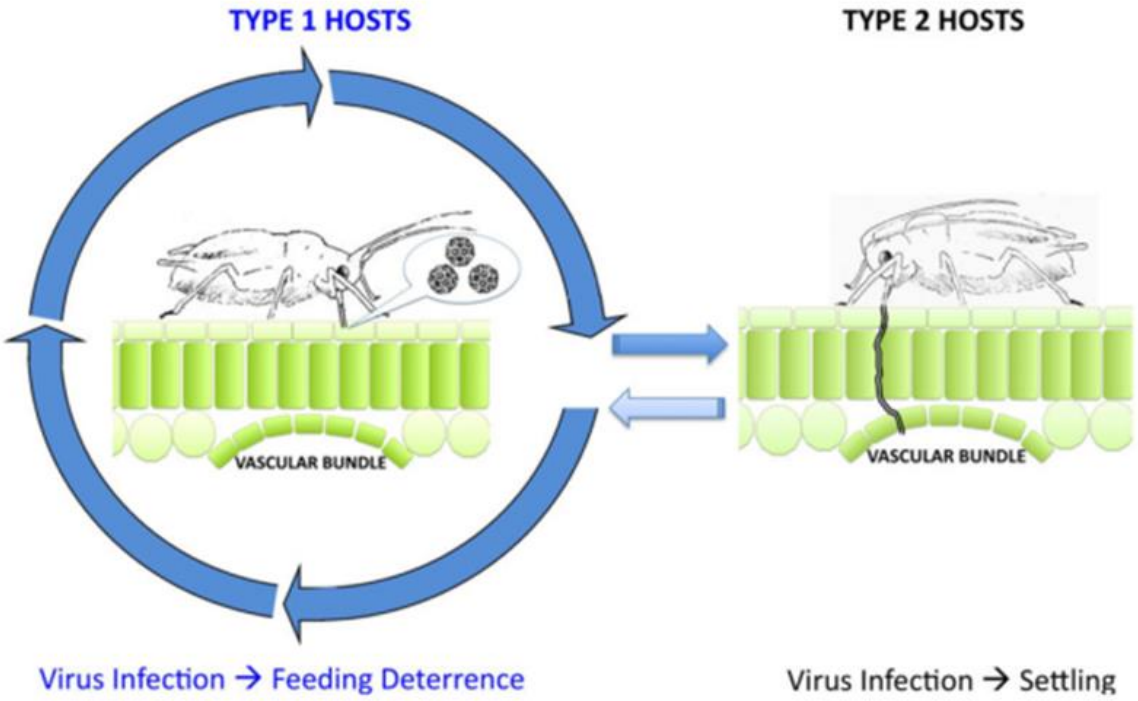


Figure 1.7. Working model of virus-mediated aphid-host interactions. Type 1 hosts promote migration of viruliferous aphids, which may encourage virus transmission, whereas Type 2 hosts foster aphid settling and reproduction on infected plants. Such hosts (Type 2) may act as refuges to aphid vectors during stressful periods of cold and drought. Recent modelling by Donnelly et al. (2019) suggests that such hosts could promote the development of winged morphs and contribute to longer-range transmission. The illustration was taken from Westwood et al. (2013).

1.10 Research Objectives

Based on current epidemiological modelling (Section 1.9) and other findings on manipulation by CMV of insect-host interactions reviewed in this Chapter, I formulated four main research hypotheses, listed below.

1) That CMV induces aphid species-specific effects on aphid behaviour on tomato, particularly concerning differences in the behaviour of generalists compared to specialist aphids.

2) That visual cues, volatile cues, or both are involved in CMV-induced aphid preference and settling responses on tomato.

3) That one or more specific CMV gene products are vital in conditioning CMV-induced changes in aphid-host interactions in tomato.

4) The SA and JA signalling pathways are involved in CMV induction of virally modified plant phenotypes affecting aphids on tomato and tobacco plants.

I tested these hypotheses through the specific objectives listed below.

1. Using aphid free-choice experiments, I determined if CMV-infection alters the preference and settling properties of *Myzus persicae* and *Macrosiphum euphorbiae* in tomato. I examined if aphid behaviour is changed at different time intervals post-infection. (Chapter 3).

2. I evaluated if visual cues, volatile cues or both are involved in CMV-aphid interaction in tomato (Chapter 4).

3. Following on from investigations of objectives 1 & 2, I investigated whether specific CMV gene products are involved in tomato-aphid interactions (Chapter 5).

4. I also investigated how SA and JA influence CMV-induced changes in host-aphid interactions on Solanaceous plants. Firstly, *NahG*-transgenic tomato plants (Sections 1.10.2 & 2.2) were used to examine if SA is involved in Fny-CMV-induced changes in *Myzus persicae* and *Macrosiphum euphorbiae* settling and preference responses (see results in Chapter 6). Secondly, the role of JA on *Myzus persicae* reproductive fitness was examined on *COI1*-silenced transgenic tobacco plants (Section 2.7) (Chapter 7).

Chapter 2. Materials and Methods

2.1 General chemicals, glass, metal, and plasticware, and sterilization procedures

Most laboratory chemicals were obtained from Sigma-Aldrich (Gillingham, UK), Fisher Scientific (Loughborough, UK), or Duchefa (Melford Laboratories, Ipswich, UK) unless indicated otherwise. All glass flasks, bottles and plastic equipment were first autoclaved for 15 minutes at 121°C at 15 pounds per square inch pressure before use. Other glassware, ceramics (including mortars and pestles), and metal items such as semi-circular aluminium plates (Section 2.11) were soaked in 3.0 % (w/v) sodium hypochlorite solution for a minimum of thirty minutes, rinsed with deionised water and baked at 180°C for 2 hours. Single-use sterile plastic equipment, such as 50 ml Greiner centrifuge tubes, Petri dishes, and syringes, was obtained from BD Biosciences, Oxford, UK. Forceps, blades and cork borers were heat-sterilised: they were briefly soaked in 70 % (w/v) ethanol, and excess ethanol burnt off with a Bunsen flame. All stock solutions were prepared with deionised water and most sterilized by autoclaving. Solutions of heat-labile substances (e.g., antibiotics) were filter-sterilized through 0.2µm pore filters (Schleicher and Schuell, Dassel, Germany). The ultra-pure water was produced using a Milli-Q ultrapure water purification system (Millipore, Billerica, MA, USA).

2.2 Plants and aphids

Plants were grown on Levington M3 compost (Scotts, Chilliworth, Ipswich, UK) in a 16-hour photoperiod (200 $\mu E.m^2.s^{-1}$ of photosynthetically active radiation), at 22°C and 60 % relative humidity in a controlled environment room (Conviron, Manitoba, Canada) at the Plant Growth Facility (University of Cambridge Botanic Garden), unless stated otherwise. Chinese cabbage (*Brassica rapa* subspecies *pekinensis*) cv. Green Rocket F1 (Kings Seeds, Essex, UK) and potato (*Solanum tuberosum* L.) cv. Desiree (Berrycroft Stores Ltd, Cambridge, UK) were used to multiply and maintain colonies of the aphids *Myzus persicae* Sulz. clone US1L (Devonshire & Sawicki, 1979) and *Macrosiphum euphorbia*e (Thomas, 1878), respectively. Both clones of aphids were originally obtained from Rothamsted Research.

Most experiments with aphids were carried out with non-transgenic tomato (*Solanum lycopersicum* L.) plants and plants of a *NahG*-transgenic tomato line, both in the Moneymaker cultivar background. The *NahG*-transgenic line, SLJ 7321, was described in Brading *et al*. (2000) and generously provided by Prof. Jonathan Jones (The Sainsbury Laboratory, John Innes Centre, Norwick, UK). *NahG*-transgenic plants cannot accumulate normal levels of SA due to constitutive expression of SA hydroxylase. Tomato

seeds were germinated on moist filter paper placed in sterile plastic Petri dishes. The seeds were incubated at 28°C for five days, then transferred to a mixture of Levington M3 compost and sand in a 3:1 ratio. Tomato plants were used in aphid free-choice bioassays examining virus-induced effects on aphid behaviour. *Nicotiana benthamiana* Domin., *RDR6i*-transgenic *N. benthamiana*, and tobacco (*Nicotiana tabacum* L.) cv. Xanthi plants were used for virus propagation (Section 2.3). The wild-type *N. benthamiana* is the highly virus-susceptible lab accession (Wylie et al., 2015). *RDR6i*-transgenic *N. benthamiana* plants have been silenced for expression of the antiviral RNA silencing factor RNA-dependent RNA polymerase 6 and show even greater susceptibility to many viruses than wild-type *N. benthamiana* plants (Schwach et al., 2005). Tobacco was also used for the generation of transgenic plants silenced for *NtCOI1* expression (Section 2.7). Tobacco and *N. benthamiana* seeds were sown in 10 mm plastic saucers for seven days before transferring to 9 cm square pots containing Levington M3 compost. To grow plants for virus propagation or purification, seeds were germinated on compost supplemented with Intercept 70WG, which contains the systemic insecticide imidacloprid, at a rate of 0.002 % (w/v).

2.3 Cucumber mosaic virus (CMV) mutants and pseudorecombinants

For agroinfection I used lines of *Agrobacterium tumefaciens* cells carrying various plasmids containing infectious cDNA clones of Fny-CMV [pCB301-Fny109, pCB301-Fny209, pCB301-Fny309 each representing the three RNAs (Dr Zhiyou Du, *Unpublished*), and LS-CMV [pCB301-LS109, pCB301-LS209 pCB301-LS309] (Liao et al., 2015). For the Fny-CMVΔ2b mutant, a clone of Fny209.Δ2b (Ryabov *et al*., 2001) subcloned into the T-DNA vector pCB301 with pCB301-Fny109 and pCB301-Fny309 was used. All infectious Fny-CMV clones for agroinfection were generated by Dr Zhiyou Du and colleagues by subcloning of infectious clones described by Rizzo & Palukaitis (1990) and Zhang et al. (1994) into pCB301 (Liao et al., 2015) to make them suitable for agroinfection.

2.3.1 Inoculation of *Nicotiana benthamiana* plants by agroinfiltration

A. tumefaciens carrying plasmids containing infectious cDNA clones of RNA segments used to reconstitute Fny-CMV, LS-CMV and Fny-CMVΔ2b were grown on kanamycin (100 µg/ml)-containing solid Luria-Bertani (LB) growth media and incubated at 28°C overnight. A colony from each plate was transferred to 5 ml of liquid media containing kanamycin (100 µg/ml). The liquid cultures were incubated overnight at 28°C in an orbital shaker. Overnight bacterial cultures were centrifuged at 6500 rpm (Beckman JA-20 rotor) for 5 minutes. Carefully, the supernatant was discarded. Pellets were resuspended in 2 ml of agro-infiltration buffer (10 mM $MgCl_2$, 10 mM MES pH 5.6 and 10 mM

acetosyringone). Using a spectrophotometer (Unicam Helios Gamma), optical densities at 600 nm (OD_{600}) for each infectious clone were determined and standardized to 0.4.

I made Fny-CMV, LS-CMV, Fny-CMVΔ2b and infectious CMV pseudorecombinants by mixing in equal volumes cultures of *A. tumefaciens* cells carrying cloned viral RNA segments of interest. Several *N. benthamiana* leaves were infiltrated with 2 ml of the *A. tumefaciens* mixtures with a disposable syringe. Agroinfiltrated plants were maintained in controlled growth rooms (see Section 2.2). At 14 days post-infiltration, about 30-40 g of infected leaves were harvested for virion preparation.

2.3.2 Mechanical inoculation

Fny-CMV, LS-CMV and CMV pseudorecombinant viruses were propagated in *N. benthamiana* plants, and Fny-CMVΔ2b in *RDR6i*-transgenic *N. benthamiana* plants because Fny-CMVΔ2b replicates poorly in wild type *N. benthamiana*. For inoculum production, 2-week-old non-transgenic or *RDR6i*-transgenic *N. benthamiana* plants were mechanically inoculated with either sap from a previously infected plant, with purified virions (4 µl of 500 ng/µl purified virus) (Section 2.3.4) or with *in vitro*-synthesised viral RNA (Section 2.3). Control plants were mock inoculated with sterile water. For virus or mock inoculation, two cotyledons per plant were first dusted with Carborundum (silicon carbide) powder, an abrasive that wounds the plant surface, allowing virus particle penetration (Rawlins & Tompkins, 1934), before applying inoculum or sterile water (mock inoculum) with a gloved finger. Following inoculation, plants were covered with plastic propagator lids to prevent wilting over the next 24 hours. For aphid experiments, tomato or tobacco plants were inoculated when 10-14 days-old and used for experiments at 10-14 days post-inoculation (dpi).

2.3.3 Confirmation of CMV infection by ELISA

Infection with Fny-CMV, LS-CMV, Fny-CMVΔ2b, or CMV pseudorecombinants was confirmed using the double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) technique (Clark & Adams, 1977). The DAS-ELISA kit was sourced from Lynchwood Diagnostics (Grantham, UK). Following the manufacturer's instructions, 96-well ELISA plates (Immuno Plate F96 MaxiSorp, Scientific Laboratory Supplies, East Riding, Yorkshire, UK) were coated with 100 µl of CMV coat protein-specific antibody (anti-CMV IgG) solution diluted 1000x in the provided coating buffer. Plates were coated with Saran wrap (Dow Chemical) and incubated in a sandwich box to maintain humidity during overnight incubation at 4°C. Leaf samples from infected plants were collected in 1.5 ml microcentrifuge tubes and immediately homogenised in the provided extraction buffer at a ratio of 1:20 (w/v) using sterile

plastic micropestles. The extraction buffer was made of 20 mM Tris buffer pH 7.4, 137 mM sodium chloride (NaCl), 3 mM potassium chloride (KCl), 2 % (w/v) polyvinylpyrrolidone (PVP) 24kD, 0.05 % (v/v) Tween-20, and 0.02 % (w/v) sodium azide (NaN_3) preservative. The samples were centrifuged at 13,000 rpm for two minutes and kept on ice. Antibody-coated plates were rinsed three times with phosphate-buffered saline [PBS: 3.2 mM Na_2HPO_4 and 0.5 mM KH_2PO_4 pH 7.4, 1.3 mM KCl, 135 mM NaCl, 0.05 % (v/v) Tween-20]. Washed plates were dried by tapping on paper towels several times to remove excess wash buffer before 100 µl of the plant extract, positive control, mock-inoculated plant as a negative control and extraction buffer as blank control were loaded in a duplex to washed, coated plates. Plates were enclosed in Saran wrap, placed in a sandwich box and incubated at 4°C overnight. Following incubation, plates were washed three times with PBS followed by drying, as previously described. 100 µl of diluted (1:1000) secondary antibody conjugated to alkaline phosphatase were dispensed into wells of the washed plates. The plates were enclosed as before to maintain humidity and incubated at 28°C for 3.5 hours. After incubation, plates were washed with PBS and dried as described previously. The substrate was prepared by dissolving *para*-nitrophenylphosphate in substrate buffer (1 M diethanolamine pH 9.8, 0.02 % (w/v) NaN_3) at 1 mg/ml, and 100 µl added to each well. The plate was incubated at room temperature for 30-60 minutes to allow colour development. Optical absorbance readings at 405 nm were taken in a Titertek Multiscan PLUS MKII (Hunstville, AL, USA) ELISA reader. Test results were considered valid if the positive control was positive, and the blank control wells remained colourless. Samples with absorbance values greater than twice the mean values of the negative controls were considered positive (Hu et al., 1995).

2.3.4 Virion purification

CMV virions were purified using a procedure described by Ng *et al.* (2005). Fresh leaf samples were harvested from virus-infected *N. benthamiana* or *RDR6i* plants (for Fny-CMVΔ2b). The leaves were weighed and blended in a pre-chilled blender (Magimix, Farnham, UK) containing ice-cold Buffer A [0.5 M sodium citrate pH 6.5, 5 mM disodium EDTA, 0.5 % (v/v) thioglycolic acid], and chloroform, in a ratio of 1g plant tissue: 2ml Buffer A: 2ml chloroform. The homogenate was filtered through one layer of muslin pre-soaked with distilled water. The homogenate was then centrifuged at 10,000 rpm (Beckman JA-20 rotor) for 15 minutes at 4°C. The aqueous phase was recovered and divided equally into ultracentrifuge tubes cushioned with 5 ml of Buffer A plus 10 % (w/v) sucrose. The solutions were centrifuged at 40,000 rpm (Beckman Ti 70 rotor) for 1 hour 15 minutes at 4°C. The pellet obtained was resuspended in 5 ml of Buffer B [5 mM sodium borate pH 9.0, 0.5 mM disodium EDTA, 2 % (v/v) Triton-X 100] and left shaking overnight at 4°C. On the following day, the virion suspension was centrifuged at 6,000 rpm (Beckman JA-20 rotor) for 10 minutes at 4°C. The supernatant was centrifuged at 40,000

rpm (Beckman Ti 70 rotor) for 1 hour 15 minutes at 4°C on a 5 ml cushion of Buffer C (5 mM sodium borate pH 9.0, 0.5 mM disodium EDTA). The pellet was resuspended in 200 µl of Buffer C with 0.02 % (w/v) NaN_3 as a preservative. The concentration of the virus particles (mg/ml) was determined by measuring the absorbance at 260 nm and dividing this value by the 1 $mg.ml^{-1}$ extinction coefficient (5) (Lot & Kaper, 1976). Virion suspensions were stored at 4°C and remained infectious for at least three months.

2.4 RNA manipulations

2.4.1 Total RNA extraction

Total RNA from virus-infected plants was extracted using a phenol-chloroform based method (Chomczynski & Sacchi, 1987). All procedures were performed on ice or at 4°C to avoid degradation of nucleic acids by ubiquitous nucleases. Fresh samples collected from young, infected leaves were ground into powder in liquid nitrogen in chilled ceramic mortars and pestles. Ground leaf samples were transferred into labelled sterile microcentrifuge tubes. 1ml of a TRIzol-based extraction buffer [38% (v/v) Tris-buffered phenol, 0.8 M guanidine thiocyanate, 0.4 M ammonium thiocyanate, 0.1 M sodium acetate pH 5.0, 5% (v/v) glycerol] was added in each microcentrifuge tube. They were centrifuged at 13,000 rpm (Hermle Z 400K, 220.88 V01 rotor) for 10 minutes at 4°C. Supernatants were transferred into fresh 1.5 ml microcentrifuge tubes. In each tube, 400 µl of chloroform: isoamyl alcohol in a ratio of 24:1 were added, followed by vigorous shaking. After that, they were centrifuged at 13,000 rpm (Hermle Z 400K, 220.88 V01 rotor) for 15 minutes at 4°C. The mixture separated into a lower, phenol/chloroform phase, an interphase, and a colourless upper aqueous phase following centrifugation. RNA was contained in the aqueous phase. About 200 µl of the upper aqueous phases were transferred to 1.5 ml tubes. Equal volumes of -20°C chilled isopropanol were added to each aqueous layer and gently mixed by inverting the tubes several times. The tubes were immediately incubated at -20°C for 1 hour and centrifuged again at 13,000 rpm (Hermle Z 400K, 220.88 V01 rotor) for 10 minutes at 4°C to pellet precipitated nucleic acids. Supernatants from each tube were discarded, and pellets were washed with 75 % (v/v) ethanol, then centrifuged at 4°C at 13,000 rpm (Hermle Z 400K, 220.88 V01 rotor) for 4 minutes. The 75 % (v/v) ethanol was discarded, and the tubes were vacuum-dried for 15 minutes. For routine RT-PCR, the pelleted nucleic acids were dissolved in 50 µl of autoclaved ultrapure water and stored at -80°C.

For gene expression studies, the total RNA extraction protocol was modified to improve the quality of nucleic acids. After the 75 % (v/v) washing step, pellets were resuspended in 400 µl autoclaved

ultrapure water. An equal volume of 4 M lithium chloride was added to precipitate single-stranded nucleic acids. The samples were incubated at -20°C for at least 2 hours. After incubation, they were centrifuged at 4°C, 13,000 rpm for 15 minutes (Eppendorf, 5415R rotor). The supernatant was discarded. The pellets were resuspended in 400 µl autoclaved ultrapure water. An equal volume of acidic phenol, chloroform, isoamyl alcohol mixed in the ratio 25:24:1 was added to each sample. The samples were then centrifuged at 13,000 rpm (Eppendorf, 5415R rotor) at 4°C for 10 minutes. The aqueous layers were transferred to fresh 1.5 tubes. To re-precipitate total RNA, 100 % (v/v) ethanol and 1/10 volume of 3 M sodium acetate were added to each sample. The samples were vortexed and incubated at -20°C for 1 hour. After incubation, they were centrifuged at 4°C, 13,000 rpm (Eppendorf, 5415R rotor) for 15 minutes. The pellets were washed by adding 1 ml of 75 % (v/v) ethanol to each tube followed by centrifuging at 13,000 rpm (Eppendorf, 5415R rotor) for 5 minutes at 4°C. Ethanol was pipetted out, and the final pellets air-dried for 15 minutes in a flow hood. The pellets were eluted in 30 µl autoclaved ultrapure water. The quantity and integrity of each total RNA were determined by spectrometry (Section 2.4.2) and agarose gel electrophoresis (Section 2.6), respectively.

2.4.2 Determination of total RNA concentration

With the aid of a Nanodrop spectrophotometer (Thermo Scientific, Walton, MA), the concentration (ng/µl) of 1 µl of each extracted total RNA sample was determined, using sterile water as a blank. The 260/280 absorbance ratio of every sample was measured. Samples with 260/280 ratios ranging between 1.8-2.0 were considered to be of high purity. Total RNA samples with 260/280 ratios below 1.8 were deemed to be contaminated with protein or carried-over phenol.

2.4.3 Removal of genomic DNA from plant total RNA preparations

The total RNA extraction method used in this study did not eliminate genomic DNA. I removed genomic DNA by treating every sample with TURBO DNase (Ambion, Austin TX, USA). The TURBO DNase kit was used following the manufacturer's protocol. A maximum concentration of 10 µg of each sample was used for each DNase treatment. In 44 µl of total RNA, 5 µl of 10x DNase buffer and 1 µl of DNase enzyme were added. The resultant reaction mix was incubated at 37°C for 30 minutes in a Stuart SBH130D block heater. The reaction was inactivated by adding 5 µl of inactivation reagent in each treated and incubated sample. The reaction was mixed by tapping on the tubes followed by incubation at room temperature for 5 minutes. They were then centrifuged at 13,000 rpm (Eppendorf, 5415R rotor) for 2 minutes at room temperature in a benchtop centrifuge. The supernatant was transferred into sterilized fresh 1.5 ml microfuge tubes. The concentrations of each sample were re-determined,

as previously described in section 2.4.2. The integrity of DNase-treated total RNA was assessed on a denaturing agarose gel (Section 2.4.4).

2.4.4 Denaturing agarose gel electrophoresis of total RNA

Using a method described by Sambrook *et al*. (1989), DNase-treated RNA samples were separated by denaturing agarose gel electrophoresis and analysed for degradation. In each sample, a denaturation RNA loading dye was added in a ratio of 1:1. The dye contained 64 % (v/v) formamide, 8.2 % (v/v) formaldehyde, 0.625x 3-(N-morpholino) propanesulphonic acid (MOPS) buffer pH 7.0 (5x MOPS buffer contains 0.2 M MOPS pH 7.0, 50 mM sodium acetate, and 5 mM EDTA), 0.05 µg/ml ethidium bromide, 0.25 % (w/v) bromophenol blue and 0.25 % (w/v) xylene cyanol FF. The sample/loading dye mixture was heated at 65°C for 15 minutes in a Stuart SBH130D block heater. After heating, samples were electrophoresed on a 1.5 % (w/v) agarose gel in 1x TBE buffer (for 1000 ml TBE: 10.8 g Tris pH 8.0, 5.5 g boric acid, 4 ml 0.5 M EDTA) at 100 V for 40 minutes in a Power Pac 3000 unit (Biorad, Hemel Hempstead, Hertfordshire, UK). Intact total RNA stained with ethidium bromide showed clear ribosomal RNA bands when placed on a UV transilluminator for imaging (Biorad, Hemel Hempstead, Hertfordshire, UK). Degraded RNA samples were not used for further analysis.

2.4.5 Synthesis of cDNA

First-strand cDNA synthesis was performed using a Promega GoScript Reverse Transcription Kit (Promega, Madison, WI, USA). A protocol provided by the manufacturer was adopted with minor alterations. For each cDNA reaction, 1-5 µg of plant total RNA was used. Per reaction, 4.5 µl of total RNA extract, 0.5 µg random hexamers for synthesising CMV-derived cDNAs or oligo$(dT)_{15}$ for reverse transcription of plant mRNAs and 0.5 µl of autoclaved ultrapure water were mixed in a 0.2 ml PCR tube. The mixture was incubated in a heating block at 70°C for 5 minutes to unfold RNA, and samples were immediately placed on ice for 5 minutes, followed by centrifuging for 10 seconds in a microcentrifuge. A reverse transcription mixture [5x GoScript reaction buffer (4 µl), 1.5 mM $MgCl_2$ (3 µl), 0.5 mM dNTP mixture (1 µl), RNase OUT (1 µl), GoScript Reverse Transcriptase (1 µl) and sterile water (5 µl)] was added to the initial 5 µl of total RNA and primer mix, making a final reaction volume of 20 µl. In a PE Biosystems GeneAmp 9700 PCR machine, reactions were annealed at 25°C for 5 minutes, followed by extension at 42°C for 1 hour. Reverse transcriptase was inactivated by incubation at 70°C for 15 minutes. The resultant cDNA was stored at -20°C for further use.

2.5 Polymerase chain reaction

Polymerase chain reactions (PCRs) were performed using Biomix red which contained *Taq* DNA polymerase (Bioline Reagents Ltd, UK). For viral detection, cDNA was diluted ten times with autoclaved ultrapure water. Plant DNA used was undiluted. A 10 µl volume reaction containing 5 µl Biomix Red, 0.2 µl forward primer, 0.2 µl reverse primer, 3.6 µl autoclaved ultrapure water and 1 µl of DNA template was adopted for each sample. Appropriate forward and reverse primers for viral and plant DNA detection were selected from Table 2.1. In a PE Biosystems GeneAmp 9700 PCR machine, the samples were denatured at 94°C for 3 minutes followed by 30 cycles of 94°C, 55°C (annealing), 72°C (extension) each temperature step for 30 seconds and a final extension step at 72°C for 1 minute. The reactions were kept at 4°C if not immediately analysed by agarose gel electrophoresis and UV visualisation (Section 2.4.7).

For gene expression studies on *COI1*-silenced transgenic tobacco plants (Section 2.7.1), reverse transcription-coupled quantitative PCR (RT-qPCR) with SensiMix™ SYBR® (Bioline Reagents Ltd, UK) was used. For each assay, a 25 µl volume reaction of 2x SensiMix™ SYBR® No-ROX (12.5 µl), 25 µM specific forward and reverse primer (0.5 µl each), sterile water (9.5 µl), and 2 µl of diluted cDNA templates (synthesized using total plant RNA templates: Section 2.4.5) was constituted. Samples, including water controls, were loaded onto a 96-well qPCR plate (Bio-Rad, Hemel Hempstead, Herts, UK). Each sample/control was loaded in triplicates. The plates were sealed with sealing film (Bio-Rad) and briefly pulsated at low speed to collect reaction contents to the bottom of each well. Samples were run on a CFX96 Real-Time PCR detection system logged to a PC running CFX manager software. The following conditions were used: 95°C, 2 minutes (initial denaturation); 44 cycles of 95°C, 2 seconds; 57°C, 30 second and 72°C, 20 seconds. At the end of each qPCR run, a melting curve analysis between 65°C and 95°C was performed. Generated raw data was exported and processed in LinRegPCR software which computed baselines, threshold cycle numbers and amplification efficiency.

Using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001; Yuan et al., 2008), mean relative changes in gene expression were computed. The expression levels of the transcripts of interest were normalised using the expression level of the transcripts of two housekeeping genes, *glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*) and *elongation factor 1 α* (*EF1α*) (Table 2.1). These housekeeping genes were preferred because they were previously shown to be unresponsive to MeJA treatment and stable PCR internal controls (Westwood et al., 2014).

2.6 Agarose gel electrophoresis of DNA

PCR amplicons were separated by electrophoresis on ethidium bromide-containing 1.2 % (w/v) agarose gels in TAE buffer [40 mM Tris(hydroxymethyl) aminomethane hydrochloride (Tris-HCl) pH 8.0, 1 mM EDTA and 0.1142 % (v/v) glacial acetic acid]. Gels were submerged in TAE buffer and ran in an MHU-1010 gel rig (Flowgen). Adjacent lanes of 1kb size marker were loaded in every gel run. Electrophoreses were performed at 90-100 V for 45 minutes. Ethidium bromide-stained DNA fragments were visualised using a UV-based gel analysis system (Section 2.4.4).

2.7 Generation of *COI1*-silenced transgenic tobacco plants

A fragment of the tobacco *NtCOI1* gene cloned into the pBI121 vector (Shoji et al., 2008) (a kind gift from Dr Tsubasa Shoji, Nara Institute of Science and Technology, Japan) was used for transformation of tobacco using *Agrobacterium tumefaciens* strain GV 3101 (C58-C1 Rif) (Holsters et al., 1980). One ml of *A. tumefaciens* culture harbouring the plasmid pBI-NtCOI1 (Shoji et al., 2008) was grown to an optical density at 600 nm (OD_{600}) of 1.0 and transferred into 100 ml of 2 x YT media [1.6 % (w/v) bacto-tryptone, 1 % (w/v) bacto-yeast extract and 5 % (w/v) NaCl, adjusted to pH 7.0] supplemented with kanamycin (50 µg/ml). The culture was grown overnight at 28°C in a G24 shaking incubator (New Brunswick Scientific, Edison NJ, USA). After 24 hours, cells were pelleted by centrifuging at 6500 rpm (Hermle Z 400K, 221.08 V01 rotor) for 20 minutes at 4°C. The supernatant was discarded and the cells resuspended in 10 ml 0.46 % (w/v) Murashige and Skoog (MS) medium (Duchefa Ltd, Haarlem, The Netherlands) plus 3 % (w/v) sucrose. Leaves from 6-week-old tobacco cv. Xanthi plants (Section 2.2) were surface sterilised in 5 % (v/v) sodium hypochlorite solution and rinsed four times in distilled water. Sterile glass bowls (Section 2.1) were re-sterilised using a UV Stratalinker 2400 (Stratagene, La Jolla, California, USA) in auto-cross-link mode. Sterilised leaf disks were excised with a cork borer and immersed in the earlier prepared culture containing MS and 3 % (w/v) sucrose. They were shaken gently for 30 minutes and later transferred on their adaxial side to Nutrient Broth Media (NBM) made of 0.46 % (w/v) MS salt, 3 % (w/v) sucrose and 0.8 % (w/v) Phytoagar (Melford Biolaboratories Ltd, Ipswich, UK). After 48 hours, the leaf discs were turned over onto new NBM media plates. This nutrient media contained α-naphthalene-acetic-acid (0.1 µg/ml), benzylaminopurine (1 µg/ml), carbenicillin (50 µg/ml) and kanamycin (50 µg/ml). Plates were then incubated in a tissue culture room maintained at 23°C and 16 h photoperiod. Every week, leaf discs were transferred to fresh NBM media supplemented with antibiotics until callus development, which occurred 4-5 weeks after transformation. Each callus was excised onto new NBM media with antibiotics and hormones in sterile Phytatrays (Sigma-Aldrich) and monitored for 2-4 weeks until shoots developed fully.

Table 2.1. Primers used in this study

Target	Primer name	Primer sequence (5' – 3') [1]	Size (bp)	Purpose	Source
CMV RNA1	SJ3	CCACCCGAACTCATTCGACATT	465	RT-PCR detection of L_1 F_2 L_3 infection	Dr Sun-Ju Rhee
	SJ4	TTTCCTCGCGGSGTCTCAAAG			
CMV RNA2	SJ5	GAAGCTAAGGTGATGGAACCTTGC	311	RT-PCR detection of L_1 F_2 L_3 infection	Dr Sun-Ju Rhee
	SJ6	AGCGGTRTACTTCTTAAASGCGC			
CMV RNA3	SJ7	CGCAGGTGGTTAACGGTCTTT	643	RT-PCR detection of L_1 F_2 L_3 infection	Dr Sun-Ju Rhee
	SJ8	TTGAATGCGCGAAACAAGCTT			
EF1α	JHW_NT_001F	GCATGCGTCAAACTGTTGCTGT	89	RT-qPCR	Westwood et al., 2014
	JHW_NT_001 R	TTCTTCTGAGCAGCCTTGGTGA			
GAPDH	JHW_NT_002 F	ATGTGGTGTCCACAGACTTCGT	140	RT-qPCR	Westwood et al., 2014
	JHW_NT_002 R	AAGCAATTCCAGCCTTGGCATC			
LOX2	MGL_NT_019 F	AGGCAGGACAGGCCGCAAAC	99	RT-qPCR	Westwood et al., 2014
	MGL_NT_019 R	CCAAATCGCTCGTCCCTTGGCA			
COI1	NtCOI15a	YTIAAYTAYATGACIGA	850	Validation of tobacco transformants by RT-PCR	Dr Trisna Tungadi
	NtCOI15b	GCICKYTCISWRAARCARCA			

[1] In the primers for detection of CMV RNA1, RNA 2, and *NtCOI1* transcript accumulation, the non-standard nucleotides Y = C or T, I = Inosine, R = A or G, K = G or T, and W = A or T.

Individual plant shoots were excised onto a rooting media [0.23 % (w/v) MS salt, 1 % (w/v) sucrose and 0.8 % (w/v) Phytoagar adjusted to pH 5.8] in 250 ml metallic cap containers (Thermo Scientific, Newport, UK). Rooting media was supplemented with 50 µg/ml carbenicillin and 250 µg/ml of cefotaxime only. Cefotaxime was used to complement carbenicillin activity in suppressing *A. tumefaciens* growth. No hormones were added. After 2-3 weeks on rooting media, shoots developed roots. They were left to grow strong roots and leaves for extra four weeks before they were transferred on Levington M3 soil (Section 2.2), supplemented with plant food (Miracle-Gro, Scotts Company, Surrey, UK). Pots containing individual plantlets were initially covered with plastic cups to maintain constant moisture and humidity around the young plants. Soil mixtures used to transition plantlets from rooting media to soil-based conditions were sterilised by microwaving in an autoclave bag for 5 minutes.

Plantlets were hardened off by gradual repositioning of plastic cups until they were completely removed after five days. To quickly identify primary *COI1*-silenced tobacco transformants (generation T_0), about 0.1 mg of newly emerged tobacco leaves were collected into 1.5 ml microcentrifuge tubes. With a plastic micro pestle, leaf samples were ground without extraction buffer. In each 1.5 ml tube of sample powder, 400 µl of extraction buffer [200 mM Tris HCL, pH 7.0; 250 mM NaCl, 25 mM EDTA and 0.5 % (v/v) SDS] were added. The sample mixtures were vortexed for 5 seconds, followed by centrifuging at 13,000 rpm for 1 minute. All centrifuging steps were done at room temperature in a benchtop centrifuge (Eppendorf, 5415R rotor). The supernatants were transferred to fresh 1.5 ml microcentrifuge tubes. Equal volumes of isopropanol were added to the supernatants. The mixture was incubated at room temperature for three minutes and centrifuged at 13000 rpm for five minutes. The supernatant was discarded, and the pellets were air-dried for 20 minutes at room temperature. The DNA pellets were dissolved in 100 µl of 1x TE buffer (10 mM Tris pH 8.0, 1 mM EDTA), and used in PCR assays to identify potential *COI1*-silenced tobacco lines using appropriate primers (Table 2.2). T_0 plants were allowed to grow until flowering and seeds harvested. Subsequent generations up to T_3 were obtained by sowing seeds on antibiotic selection media.

Seedlings that survived on selection media were transferred to soil and allowed to grow until maturity. Generation T_3 seeds were used in aphid survival and colony size experiments (Section 2.8.4).

2.7.1 Methyljasmonate treatment of tobacco plants

Successfully *COI1*-silenced tobacco lines were expected to show a diminished response to exogenously applied JA. Transformed tobacco plants and untransformed tobacco plants were sprayed with 250 µM methyl jasmonate until surface runoff. Control plants were sprayed with tap water. The plants were kept in a controlled environment growth room for 6 hours before extraction of RNA for gene expression quantification by RT-qPCR (Section 2.5).

2.8 Aphid experiments

2.8.1 Aphid free-choice bioassays for preference and settling studies

Aphid free-choice experiments (Westwood et al., 2013) were performed with wingless *Myzus persicae* and *Macrosiphum euphorbiae* on virus-infected or mock-inoculated tomato plants. Two tomato seedlings grown as described in Section 2.2 were transferred to plastic pots measuring 13 square cm in different treatment pairs. At 10 dpi, using a paintbrush, 20 aphids were transferred into 1.5 ml microcentrifuge tubes a day before the experiment and kept at 4°C overnight. Before use, aphids were revived by placing them under light for 30 minutes. They were then placed equidistantly in each treatment pair (Fig. 2.1A). The lid of each tube was opened to release aphids. The number of aphids that settled on either plant was counted at 1 and 24 hours post-release. Each pot containing a pair of plants was wrapped in a micro-perforated bread bag (Seal Packaging, Bedfordshire, UK) and kept in an insect-proof cage to avoid aphids from escaping (Insect Cage Net, Carmarthen, Dyfed, UK). This experiment was replicated four times under growth room conditions in Section 2.2.

In experiments aimed at tracking the initial choice of aphids on virus-infected or mock-inoculated plants in the dark and light conditions, the aphid free-choice assay was used with modifications (Fig. 2.1B). Double-sided adhesive tape (Q-connect, Sheffield, UK) was placed near each plant in the treatment pair to trap aphids, thus indicating their initial direction of travel. Trapped aphids were counted at 1 hour post-release. Experiments were carried out at least three times for each aphid species.

2.8.2 Innate aphid preference under different light filters

Different wavelengths of light may modify the visual response of insects, including aphids (Chittka, 2001). Many insects prefer blue or ultraviolet, and others can be attracted to green or yellow (Coombe, 1981; Zhang et al., 2016). Some insects can respond to more than one wavelength band. The effect of

light on CMV-aphid-tomato interactions is unknown. I examined the innate choice of *Myzus persicae* and *Macrosiphum euphorbiae* under light of different wavelengths.

Transparent cellophane papers in green, yellow, blue, red and clear colour were used. A spectrophotometer (Unicam Helios Gamma) was used to determine the light transmittance of each cellophane paper. The experiment was conducted at the plant growth facility under controlled light, temperature and humidity (Section 2.2). Pots containing pairs of virus-infected/mock-inoculated plants and mock-inoculated controls were placed in plastic boxes measuring 60 cm long x 20 cm high (Fig. 2.1C). The boxes were grey inside, which helped diffuse light. As previously described, double-sided adhesive tape was placed near each plant in the treatment pair (Section 2.8.1; Fig. 2.2). The boxes were then sealed on top with a specific cellophane paper. A clear cellophane paper was used as a control. Boxes were closed in the dark to avoid any possible light-induced visual cues outside the experimental environment and moved to a controlled environment room (Section 2.2). Aphid preferences were recorded after 1 hour. Each cellophane paper treatment was repeated three times.

2.8.3 Adult aphid survival and colony size experiments

To evaluate if aphid resistance induced by the viral mutant Fny-CMVΔ2b in tobacco is JA-dependent, aphid survival and reproduction was monitored on plants of selected *COI1*-silenced transgenic tobacco lines (Section 2.7). Unless otherwise stated, all aphids used in this experiment were 1-day-old nymphs. Survival and colony size of *Myzus persicae* on virus-infected and water-inoculated three-weeks-old transformed and non-transformed tobacco plants were tested.

On each plant, two adult aphids were placed on the abaxial surface of the fourth true leaf. Using clip cages, the movement of aphids was restricted. The adults were left to reproduce overnight. After 24 hours, all adult aphids and nymphs produced were removed except for one nymph left on the plant. The retained nymph in each clip cage, also called 'founder aphid', was left to reproduce for a further 14 days. The numbers of surviving founder aphids and offspring produced per founder were counted on the fifteenth day post-placement. All experiments were performed in a controlled environment room (Section 2.2) and carried out at least three times.

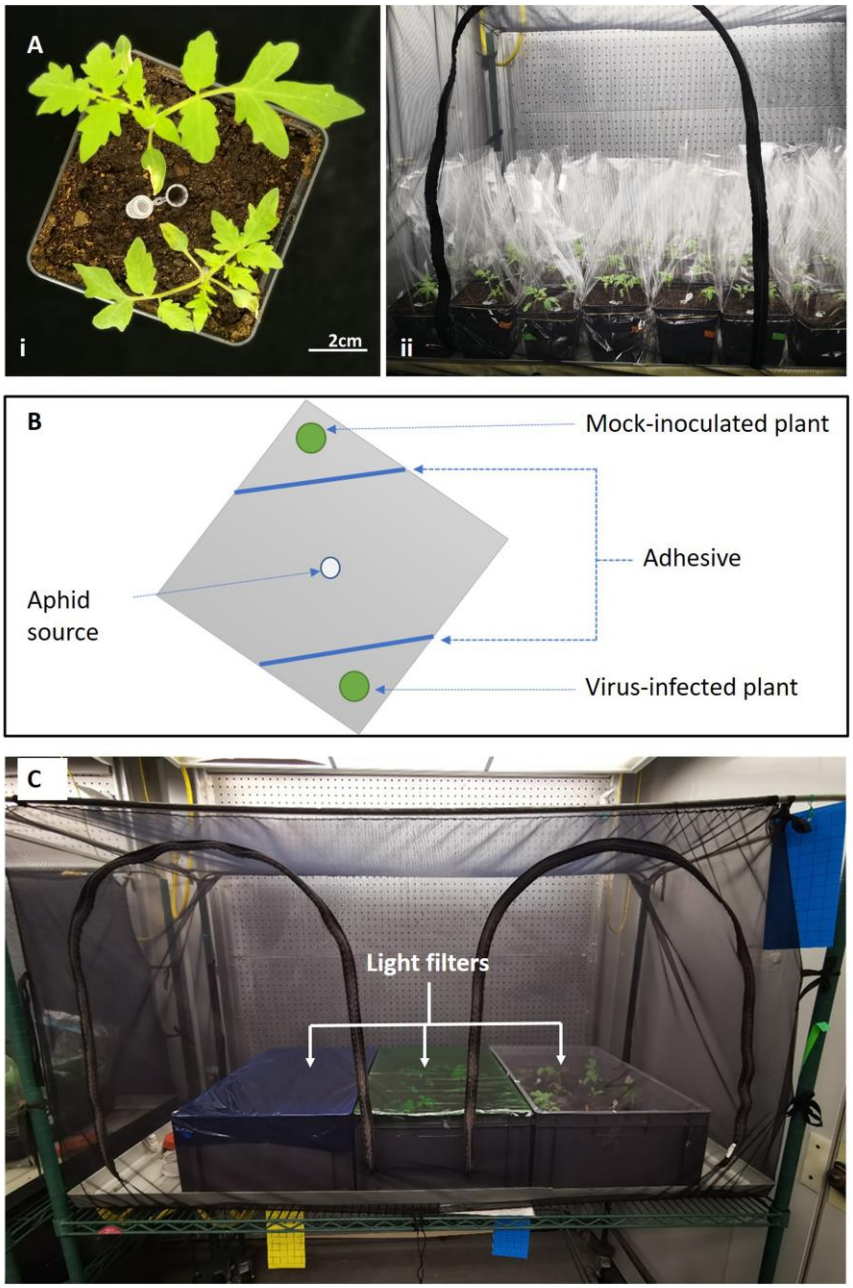


Figure 2.1. Aphid preference bioassays. A indicates the settling assay. Panel **i** shows a typical treatment pair of plants with a microcentrifuge tube containing aphids placed at midway between the plants, and panel **ii** shows how aphids in treatment pots were contained. Plants were wrapped in micro-perforated bags in an insect-proof cage within a growth room at the Plant Growth Facility (University of Cambridge Botanic Garden, Cambridge, UK). Aphids were allowed to choose between either plant being compared, and the number of aphids settling on each plant was counted at 1 h and 24 h post-release.

B Illustrates the adhesive tape trap assay, a system used to determine the innate aphid choices on virus-infected or mock-inoculated tomato plants. In each treatment pair, 20 wingless aphids were released, and the number of aphids trapped on either tape were counted after 1h post-release.

C shows the setup for investigating initial aphid preference under lights of different wavelengths described in section 2.8.2. Test plants (combinations of mock-inoculated versus virus-infected and mock-inoculated plants versus mock-inoculated plants) were placed in grey plastic boxes. Then the Innate preference of *Myzus persicae* and *Macrosiphum euphorbiae* under a given light filter was monitored after 1 hour aphid post-release.

2.9 Y-tube olfactory assays

The responses of aphids to VOCs emitted by virus-infected tomato plants and mock-inoculated tomato plants were tested using a glass Y-tube olfactometer assay (Fig. 2.2). All Y-tube olfactometry experiments were conducted in the virology laboratory at the Department of Plant Sciences, University of Cambridge. Room temperature and relative humidity were maintained at 22°C and 60 %, respectively. Normal laboratory light was unadjusted, but direct light was diffused by plain white paper placed above the Y-tube olfactometer. All glassware used was washed with acetone and baked to remove residual organic materials (Section 2.1). Test and control plants were placed at equal distances from the Y-tube, which was clamped vertically. Plants were each enclosed in glass bell jars connected by silicone tubing to an air pump and the Y-tube from the sides and top, respectively. Air supplied to the experimental unit was first purified before reaching the plants by passing through an activated charcoal column in a glass tube. The silicone tubes leading to both arms of the Y-tube were fitted tightly with glass adapters. The adapters were covered with a muslin cloth to avoid aphid escape during the experiment. The distance from the base of the Y-tube to the bifurcation measured 5 cm.

To stabilise airflow before the start of the experiment, air was pumped through the bell jars into the olfactometer arms for 5 minutes before aphid release. Seven-day-old *Myzus persicae* and 14-day-old *Macrosiphum euphorbiae* were used (see Section 2.2). The aphids were collected into a 1.5 ml microcentrifuge tube and kept at 4°C overnight before use. With a paintbrush, individual aphids were released at the base of the Y-tube, downstream of the air sources. Aphids were observed as they moved inside the Y-tube. Each aphid was observed for at least 20 minutes. A choice for either air source was recorded when an aphid passed a 2 cm mark up one of the olfactometer arms. The time taken for each aphid to make a decision was recorded. A no-choice result was noted when a particular aphid had made no choice after 20 minutes. Between experiments, the position of air sources was regularly swapped to control for any directional biases in the setup. All parts of the apparatus, including platinum-cured rubber tubes, were cleaned with acetone between experiments.

2.10 Dynamic headspace entrainment of tomato plant volatiles

VOCs from virus-infected and mock-inoculated tomato plants were captured using the dynamic headspace air entrainment method (Beale et al., 2006). VOCs were collected from inoculated plants at 9 dpi and in the dark. This experiment was done to demonstrate if the VOC signatures differed between plants under illumination versus plants placed in darkness. Dark conditions were created by wrapping glass bell jars containing plants with aluminium foil. Volatile entrainment was done over 24 hours and

repeated three times using fresh plants and cleaned equipment between replicates. Virus-infected and mock-inoculated tomato plants were separately placed under glass bell jars (Fig. 2.3). Glass bell jars were sterilised by soaking in 0.2 % (v/v) Teepol (Teepol Products Ltd., Kent, UK) for 30 minutes to remove organic residues. They were rinsed with deionised water, followed by 100 % acetone (to remove ionic residues), then baked as described in Section 2.1. The spaces between the glass bell jars and soil containing plastic pots were closed off with two previously baked semi-circular metallic plates with a hole in the centre to accommodate the stem. Bulldog clips firmly secured the metal plates to exclude unwanted soil volatiles. Open space around the stem was sealed with polytetrafluoroethylene tape (Gibbs & Dandy, Luton, UK).

By the aid of an air pump, air was streamed into the glass jar-containing plants at a rate of 800 ml/min. Air was first passed through an activated charcoal filter for purification before reaching the plants. Air containing plant VOCs was drawn out of each plant-containing glass jars at a 700 ml/min rate through Porapak Q filters fitted at the top. Internally, the Porapak Q filter (50 mg, 60/80 mesh size) had small glass beads between two plugs of silanised glass wool, which prevented air contamination from the outer chamber. VOCs were trapped in the Porapak Q filters over 24 hours. Entrained volatiles were eluted from the columns using 700 µl of diethyl ether. They were kept at -80°C until analysis by coupled gas chromatography-mass spectrometry (GC-MS). Leaf fresh and dry weight were measured to enable normalization of the volatile abundance. After every VOCs entrainment, the Porapak Q filters were washed three times with 1 ml of diethyl ether and air-dried in a fume hood for 2 hours.

2.11 Coupled Gas Chromatography-Mass Spectrometry

VOCs were separated on the Thermo Scientific TG-SQC capillary column (Thermo Scientific, UK) by gas chromatography (GC). The injection volume (splitless) was 1µl, injector temperature was 200°C, and helium was used as the carrier gas at a constant flow rate of 2.6 $ml.min^{-1}$ in an oven maintained at 30°C for 5 minutes and then set to increase the temperature at 15°C/min to 230°C. The total run time per sample was 18.33 minutes. The column was directly coupled to a mass spectrometer (ISQ LT, Thermo Scientific, UK) with an MS transfer line temperature of 240°C. Ionization was by electron impact at an ionic temperature of 250°C. Mass ions were detected between 30 and 650 m/z. Data were collected using Xcalibur software (Thermo Scientific, UK). Generated mass spectra were used to compare VOCs by principal component analysis in MetaboAnalyst 4.0 (Chong et al., 2019) using binned m/z and percentage total ion count (%TIC) values. Individual VOCs emitted by the virus-infected and mock-inoculated plants were compared with those in the National Institute of Standards and Technology (NIST) spectral databases (http://www.nist.gov).

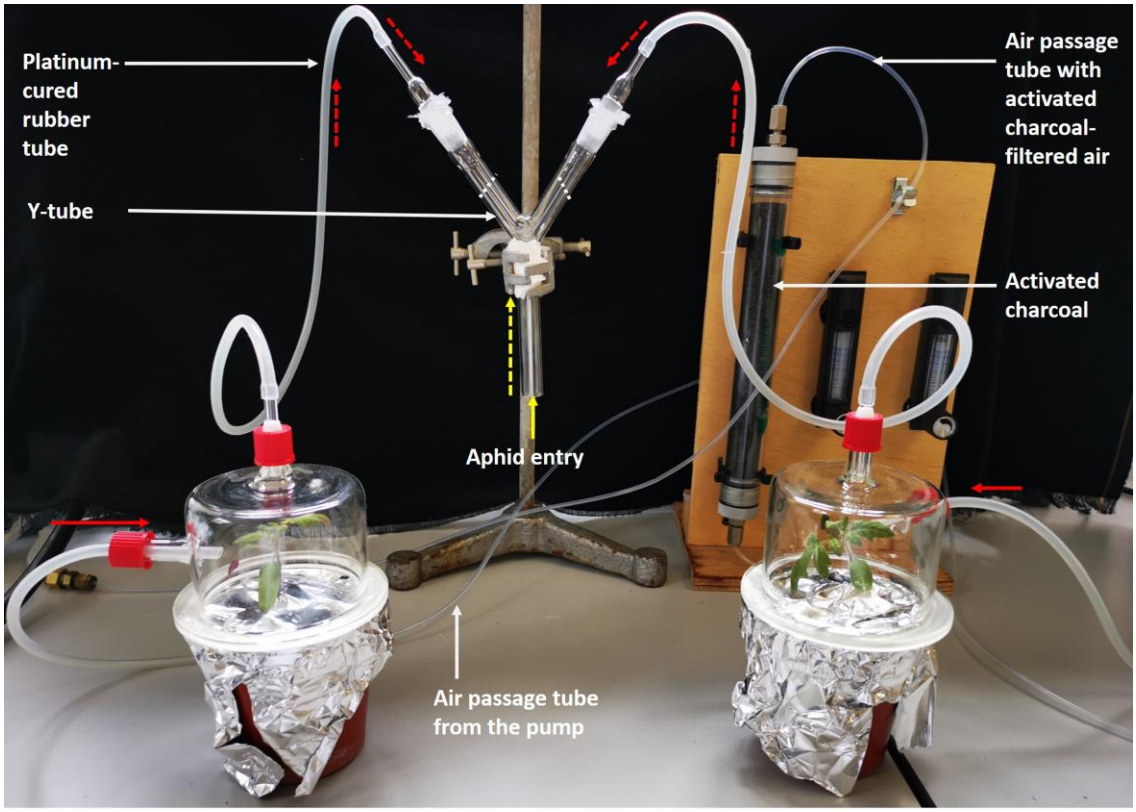


Figure 2.2. Y-tube olfactory assay examining innate preference of aphids to odours emitted by virus-infected plants or mock-inoculated plants. Wingless 7-day old *Myzus persicae* and *Macrosiphum euphorbiae* aphids were allowed to choose between separate odours of tomato plants infected with Fny-CMV and mock-inoculated plants present in air previously purified by pumping it through activated charcoal. Two or three aphids were released at the base of the Y-tube, and the time they took to move upwards to the 2cm mark in either Y-tube arm (white dotted lines) was monitored with a stop clock. Aphids that did not make any choice in 20 minutes were replaced with new aphids and timed afresh. The solid red arrows represent air inflow; red dotted arrow: flow of plant odours and a yellow dotted arrow indicate the direction of aphid movement after release from the Y-tube base.

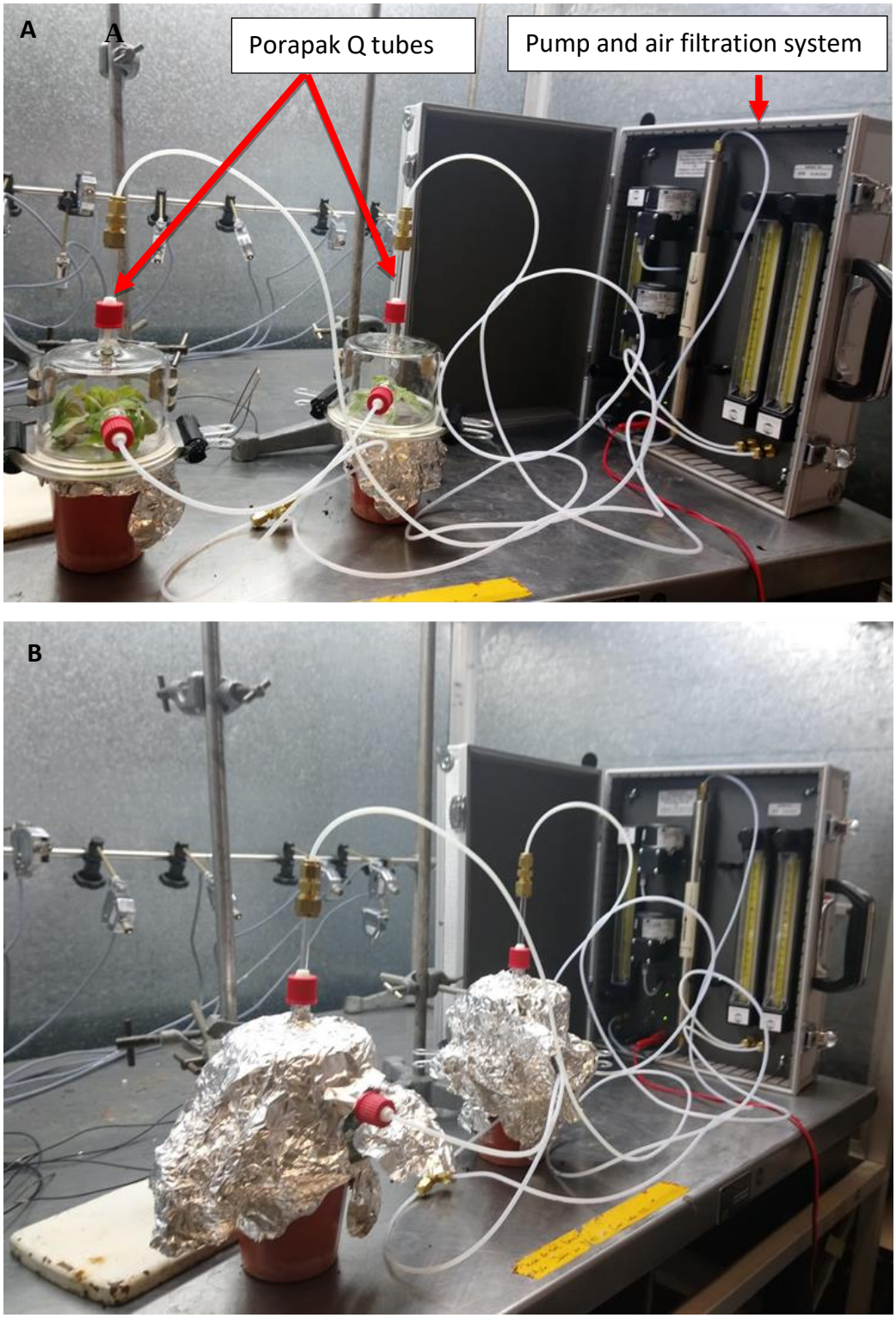


Figure 2.3. Headspace entrainment for VOC collection from virus-infected and mock-inoculated plants under illumination or in darkness. Plant-containing glass jars were covered with aluminium foil as seen in B (and with room lights off) to obtain VOCs emitted in the dark instead of under illumination (A). The VOC collection period used for tomato was 24 h.

2.12 Statistical analysis

All aphid preference count data obtained under assays in Section 2.8.1 and Section 2.8.2 were first checked for normality using the Shapiro-Wilk test (Shapiro & Wilk, 1965). Data that did comply with the normality test were transformed using the method of Johnson (1978). Normalised data were subjected to a binomial test to determine if aphid responses differed significantly from a predicted frequency distribution of 100:0 for either mock-inoculated or virus-infected plants. Where appropriate, Tukey's HSD test was used to compare sample means, as you will notice in subsequent chapters. All data analyses were performed in R, and the probability (p) threshold was maintained at 0.05 (R statistics, 2015). Differences in aphid preference and settling response were considered significant if p was < 0.05. Aphid preference and settling plots with standard error of mean bars were plotted in Microsoft Office Excel 2016.

Categorical data obtained from olfactory assays (Section 2.9) and aphid survival (Section 2.8.3) was analysed using Chi-square test. Kruskal-Wallis test (Kruskal & Wallis, 1952) were used to tease apart aphid fecundity on transformed and untransformed tobacco plants infected with Fny-CMV, Fny-CMVΔ2b or mock-inoculated (Section 2.8.3). Data for VOC emission rates and quantitative changes (Section 2.11) were subjected to ANOVA and Tukey's HSD post hoc test separated treatment means. Histograms and boxplots were drawn in Microsoft Office Excel 2016 and R statistical software, respectively.

Chapter 3. The effects of cucumber mosaic virus on the interaction between aphids and tomato plants

3.1 Introduction

Viruses induce physiological and metabolic changes in susceptible host plants that can influence their transmission and spread by vectors (Boquel et al., 2012; Castle & Berger, 1993; Srinivasan & Alvarez, 2007) (Section1.8) . The precise nature of the changes depend upon the combinations of virus, insect vector, and plant species involved. For instance, potato virus Y (PVY) differentially affects the settling behaviour of two aphid species on potato plants. *Myzus persicae* settled and reproduced better on PVY-infected plants compared to healthy plants, while the behaviour of *Macrosiphum euphorbiae* was unchanged (Srinivasan & Alvarez, 2007). Time-course data from aphid free-choice assays of Arabidopsis infected with CMV showed that compared to mock-inoculated plants, virus-infected plants progressively became repellent to *Myzus persicae* as the infection developed (Murphy et al., unpublished; Westwood et al., 2013)

CMV infection also modifies the relationship between tomato plants and pollinators by attracting bumblebees, which may in part be due to virus-induced changes in VOC emission (Groen et al., 2016). However, in tomato the effect of CMV infection on aphids (the vectors of CMV) remains unknown, and until this study, it was not known if CMV-infected plants would be attractive or repellent to aphids, if there would be aphid species-specific differences, or if any changes in aphid-tomato interactions would be mediated by VOCs or by other sensory cue(s). Deciphering CMV-associated effects on aphid-tomato interactions would improve our understanding of the transmission of this virus and could inform improved methods of viral disease management.

This study investigated, firstly, the influence of CMV infection on host preference by two CMV vectors, *Myzus persicae* (a generalist aphid) and *Macrosiphum euphorbiae* (a Solanaceae-specialist aphid). Secondly, I investigated whether the effect(s) of CMV on host-vector interactions varied as infection developed in tomato, i.e., at 3, 9 or 21 days post-inoculation (dpi). Thirdly, I initiated an investigation on what plant cues (visual or olfactory) were altered during infection of tomato by CMV. To this end, aphid free-choice assays were performed under light and dark conditions and investigated the effects of different wavelengths on aphid settling preference (Sections 2.8.1 and 2.8.2).

3.2 Results

3.2.1 Symptom development and virus accumulation in tomato plants inoculated with Fny-CMV

Ten-day-old tomato seedlings were inoculated with Fny-CMV or mock-inoculated on the cotyledons, and symptom development was monitored. Plants were asymptomatic at 3 dpi, but CMV-induced symptoms were apparent from 9 dpi, onwards (Fig. 3.1). Symptoms were characterised by a green-yellow mosaic on systemically infected leaves that appeared between 4-6 dpi, decreased leaf size, and stunting of plant growth. Symptoms increased in severity between 6 and 9 dpi, and during this period, the reduced growth of infected plants became apparent compared to the growth of mock-inoculated plants (Fig. 3.1). The most severe developmental symptoms observed from 9 dpi onwards included emerging leaves that were fern-like (Fig. 3.1), described initially as 'wiry' by Lesley & Lesley (1928). Consistent with the observations of Groen et al. (2016), flower buds emerged between 7-10 days earlier in Fny-CMV infected plants as compared to mock-inoculated plants. The presentation of CMV disease symptoms in tomato is consistent with previous findings by Edwardson & Corbett (1962) and Groen et al. (2016). Virus accumulation in non-inoculated upper leaves at 3, 9 and 21 dpi was determined by ELISA (Section 2.3.2). Fny-CMV accumulated to detectable levels by 3 dpi before symptoms had become apparent (Fig. 3.1; Fig. 3.2). Virus accumulation in systemically infected leaves, as measured by ELISA, remained at levels that were not significantly different between stages of infection (Fig. 3.2), despite the increasing severity of the disease symptoms (Fig. 3.1).

3.2.2 The effects of Fny-CMV on aphid-tomato interactions varied over disease development, and pre-symptomatic plants were attractive to both *Myzus persicae* and *Macrosiphum euphorbiae*

The effects of CMV infection on tomato-aphid interactions varied over the development of the infection from the pre-symptomatic stage (3 dpi) to later stages as disease symptoms become increasingly severe (9 dpi to 21 dpi). The preference and settling response of *Myzus persicae* (Fig. 3.3) and *Macrosiphum euphorbiae* (Fig. 3.4) at 3, 9, and 21 dpi was investigated using aphid free-choice assays (Section 2.8.1). Over each time course, I recorded aphid settlement on Fny-CMV infected versus mock-inoculated plants, mock-inoculated versus mock-inoculated plants (control assay), at 1 and 24 hours following aphid release. Each time course, with each aphid species, was carried out on four occasions (i.e., there were four biological replicates).

At 3 dpi, before the onset of visible disease symptoms, plants infected with Fny-CMV did not repel aphids of either species (Fig. 3.3; Fig 3.4). Generally, *Myzus persicae* migrated and settled on Fny-CMV-infected plants compared to mock-inoculated plants at 1 and 24 hours post-release. However, at 1 hour post-release, more *Myzus persicae* aphids significantly chose virus-infected plants over mock-inoculated plants. After 24 hours, aphids could not differentiate between virus-infected plants and mock-inoculated plants. This aphid settling response at 1 and 24 hours post-release was consistently observed in all four experiments. *Macrosiphum euphorbiae* migrated and settled on either mock-inoculated or Fny-CMV-infected plants in equal proportions at 3 dpi for both time intervals (Fig. 3.4). At 9 and 21 dpi, infection by Fny-CMV strongly deterred settling of both aphid species (Fig. 3.3 & Fig. 3.4). The repulsive effect was noticeable for *Macrosiphum euphorbiae* compared to *Myzus persicae*. This result suggests that Fny-CMV infection may cause distinct effects for generalist and specialist aphid vectors depending on the stage of disease.

I further determined if preferential settling by either aphid species achieved a tipping point: a time interval post-infection during which infected tomato plants are infectious, i.e. capable of transmitting the virus to susceptible plants through aphid vectors. Additional free choice assays were performed at 5 dpi. Plotting this data together with data at 3, 9 and 21 dpi (Fig. 3.5) suggested *Myzus persicae* settling tipped at 5 dpi.

Beyond 5 dpi, the number of aphids preferring CMV-infected to mock-inoculated plants began to decline. However, aphids did not wholly avoid infected plants, and between 20-30% consistently migrated to and settled on CMV-infected plants. The tipping point for *Macrosiphum euphorbiae* could not be precisely determined. I repeated this experiment three times with similar results.

3.2.3 CMV may affect plant visual cues influencing aphid interactions with tomato

To test the hypothesis of whether visual or, specifically, colour-associated visual cues were involved in CMV-aphid-tomato interactions, I carried out trapping assays in the dark, light and under different light-transmitting films. Tomato plants at 9 dpi were used to test this hypothesis. The 9 dpi time-point was chosen because both aphid species showed a comparatively strong bias against virus-infected plants compared to 3 dpi and 21 dpi. The plants were also at the correct growth stage (not too young or too old) to work with in trapped free-choice experiments. In each of the experiments, i.e., light, or dark or under monochromatic light, the total number of aphids trapped at the adhesive tape adjacent to CMV-infected plants was compared with those trapped towards mock-inoculated plants.

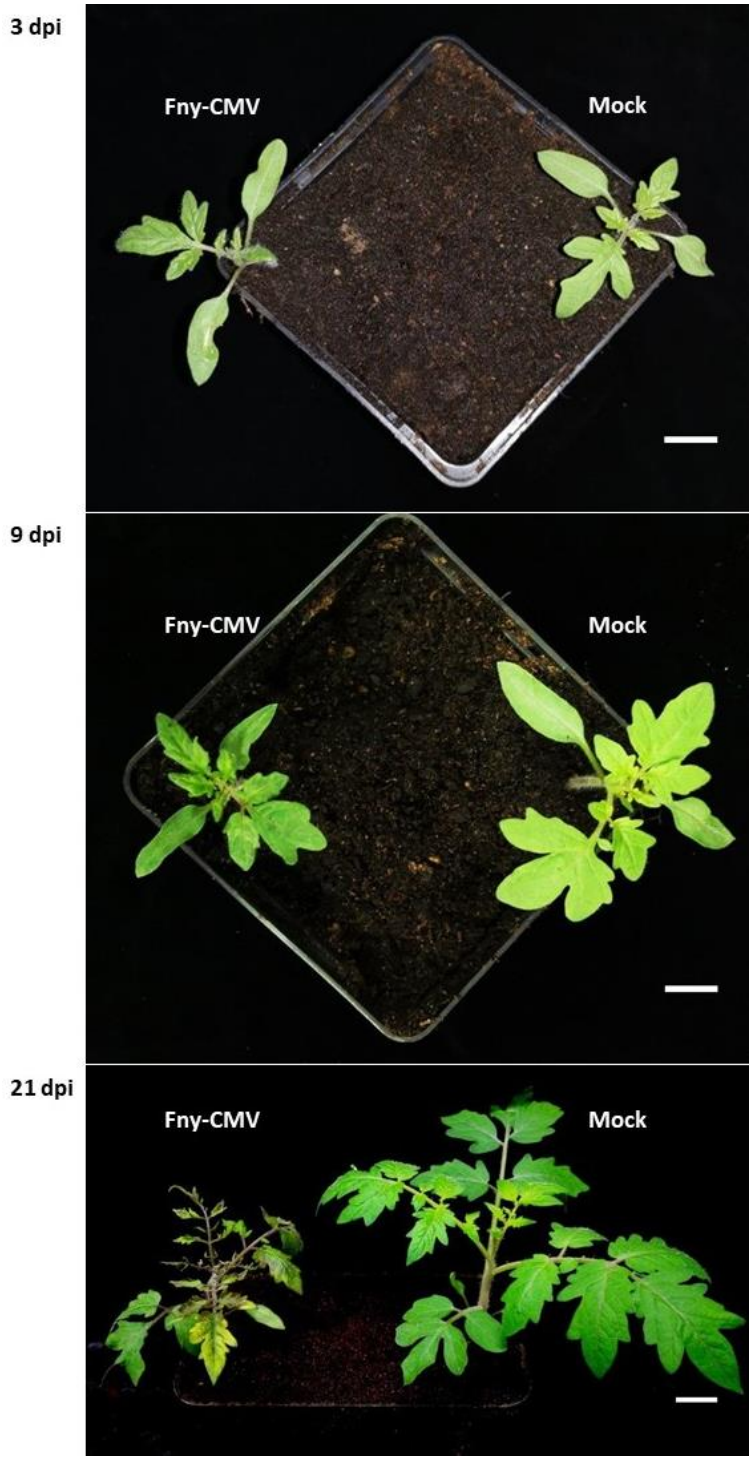


Figure 3.1. Characteristic symptoms of Fny-CMV on tomato at three different time-points post-inoculation. Tomato plants were mechanically inoculated with Fny-CMV virions (500 ng/µl concentration) or mock-inoculated with water (Mock) (Section 2.3.2), and systemic disease symptoms observed over time. The scale bars represent 1 cm.

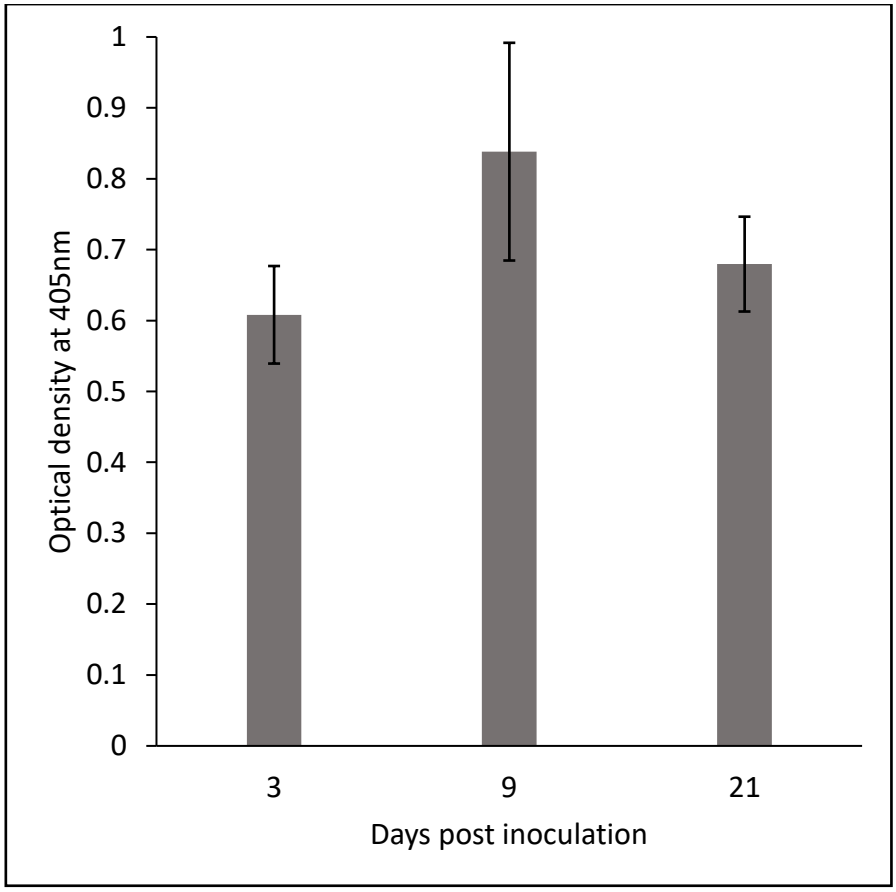


Figure 3.2. Accumulation of CMV in tomato seedlings at 3, 9 and 9 dpi. Newly emerged leaves were harvested, and the same amount of leaf tissue was processed for ELISA (Section 2.4.2). Despite infected plants appearing asymptomatic, the mean viral accumulation at 3 dpi was similar to that at 9 and 21 dpi. It appears Fny-CMV replicates and spreads rapidly in tomato seedlings. It also accumulates stably as the infected plant ages. One-way analysis of variance comparing viral accumulation at 3, 9 and 21 dpi showed no significant difference between the three-time intervals (p= >0.05, df= 2, F-value = 1.25). Statistics were generated from three experiments per time-course. Error bars represent the standard error of the mean (SEM).

Figure 3.3. CMV-infected tomato plants become increasingly repellent to the generalist aphid *Myzus persicae* as infection progresses. In free choice assays *Myzus persicae* preferred to settle on CMV-infected tomato plants at three dpi for a short period. They later migrated away to mock-inoculated plants. In each plot, stacked bars of grey against grey indicates a control group of mock-inoculated plants. The orange versus grey stack represents a comparison pair of Fny-CMV-infected plants and mock-inoculated plants. n is the total number of aphids that made a choice at 1 and 24hr post-release. Treatment pairs with asterisks * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$) or **** ($p<0.0001$) are significantly different when compared using a Binomial test. The error bars represent SEM.

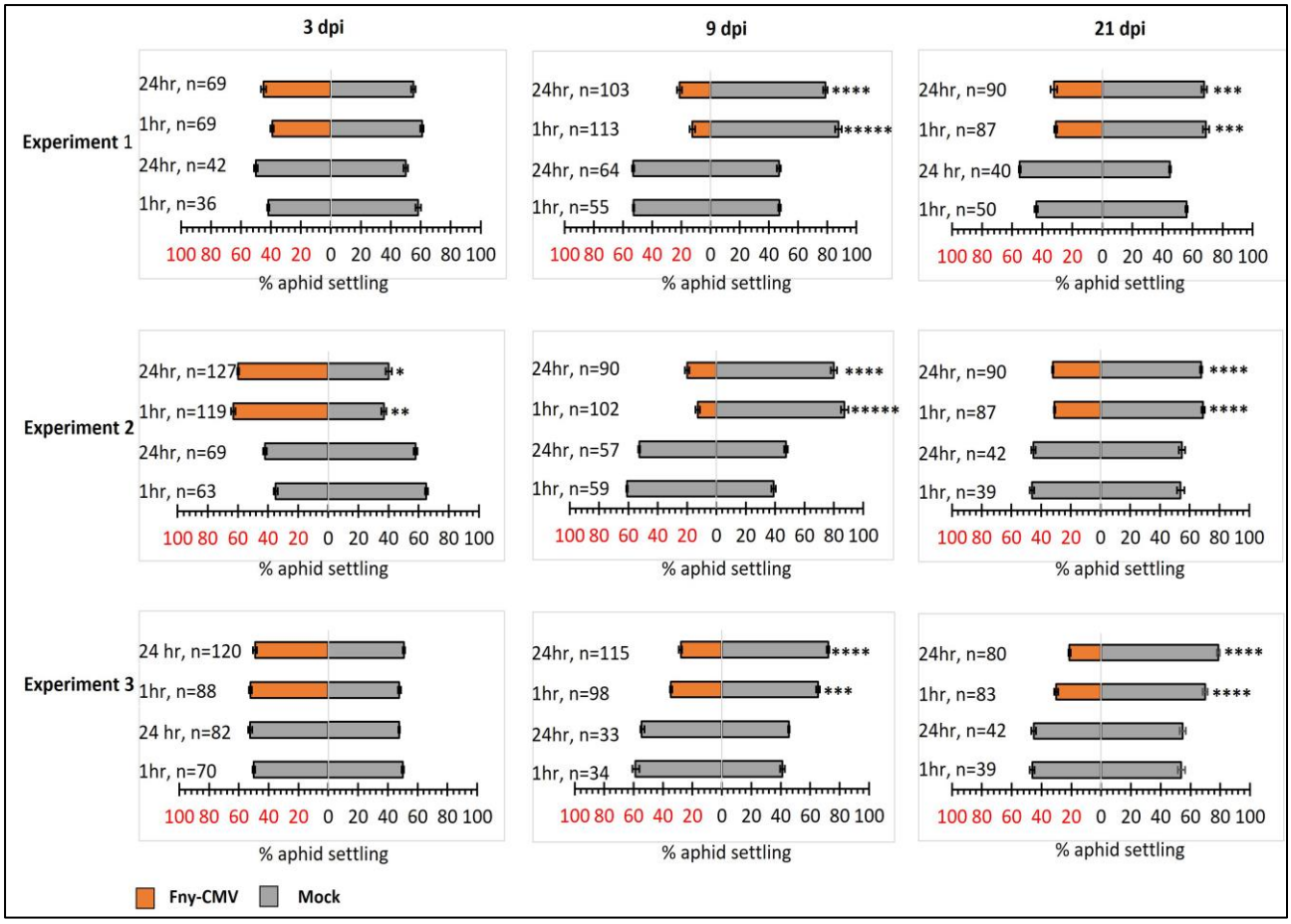


Figure 3.4. CMV-infected tomato plants become increasingly repellent to the specialist aphid *Macrosiphum euphorbiae* as infection progresses. Aphids preference and settlement on CMV-infected and mock-inoculated plants at 3 dpi was neutral after 1 and 24 hr post-release. In each plot, stacked bars of grey against grey indicates a control group of mock-inoculated plants. The orange versus grey stack represents a comparison pair of Fny-CMV-infected plants and mock-inoculated plants. n is the total number of aphids that made a choice at each time point. Treatment pairs with * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$), **** ($p<0.0001$) or ***** ($p<0.00001$) are significantly different when compared using a Binomial test. The error bars represent SEM.

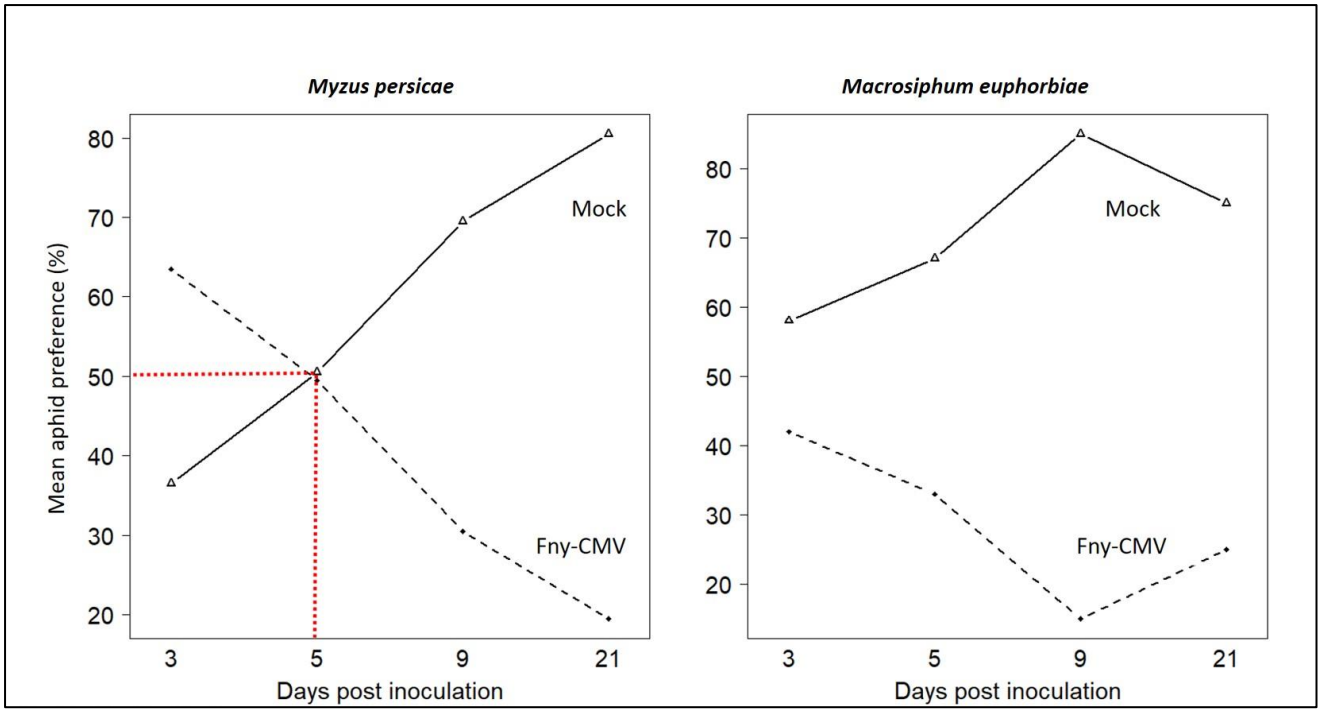


Figure 3.5. The two-way interaction plot reveals a tipping point for *Myzus persicae* at 5 dpi. The proportion of *Myzus persicae* on CMV-infected and mock-inoculated plants equilibrated at 5 dpi, indicated by the red dotted lines. In both plots, the bold black lines represent combined counts of aphids on mock-inoculated tomato plants at 1 and 24 hr post-release, whereas the black dotted lines indicate aphid counts on Fny-CMV-infected plants.

In normal illumination, 72% of *Myzus persicae* and *Macrosiphum euphorbiae* were trapped moving towards mock-inoculated plants compared to CMV-infected plants (Fig. 3.6 & Fig 3.7). This confirmed earlier findings that aphids migrate away from CMV-infected plants (see Fig. 3.3 and Fig. 3.4). Interestingly, in the dark, the response rate, i.e., the proportion of aphids that chose either infected or mock-inoculated plants, plummeted for both species. Response rates dropped to 30 and 35% for *Myzus persicae* and *Macrosiphum euphorbiae*, respectively. For both species of aphids, the few aphids that made choices did not show any bias to either CMV-infected or mock-inoculated plants (Fig. 3.6 & Fig. 3.7). This observation suggested that CMV infection could be affecting aphid visual cues more than olfactory cues.

I further tested whether illumination with specific colours affected preference of aphids to move towards either virus-infected or mock-inoculated tomato plants, using five different light-transmitting cellophane films: clear, blue, green, yellow, or red. These colours were chosen based on prior knowledge that many hemipteran insects, including aphids, are visually active within the violet-blue-green waveband and inactive in the yellow-red waveband (Section 1.7.1). Using a spectrophotometer, light transmittance of the different colours was measured at various wavelengths (Section 2.8.2). The clear transparent film, which allowed passage of all visible wavelengths and was used as a white light control in these experiments, transmitted on average 85% of light over a range of 400 nm to 700 nm (Fig. 3.8). Blue, green, yellow, and red films transmitted light with a maximum transmittance at 460 nm, 520 nm, 580 nm, and 700 nm, respectively.

In the aphid free-choice adhesive trapping experiments, which determine the initial direction of aphid migration, the blue and green films influenced *Myzus persicae* and *Macrosiphum euphorbiae* innate preference in the same way as normal illumination (Fig.3.9 and Fig. 3.10). Aphids of both species migrated towards mock-inoculated plants compared to virus-infected plants. Under yellow and red films, both aphid species behaved as if in the dark: they could not differentiate mock-inoculated tomato plants from Fny-CMV-infected plants. I also recorded the rate of aphid response under the different light exposures. More aphids responded in blue and green light ranges compared to yellow and red. The highest number of aphid responders was recorded under a green filter (56 %) and the lowest under yellow light exposure (34 %). These results taken together suggest that light may be a key stimulus in colour-associated aphid behaviour in CMV-tomato interactions.

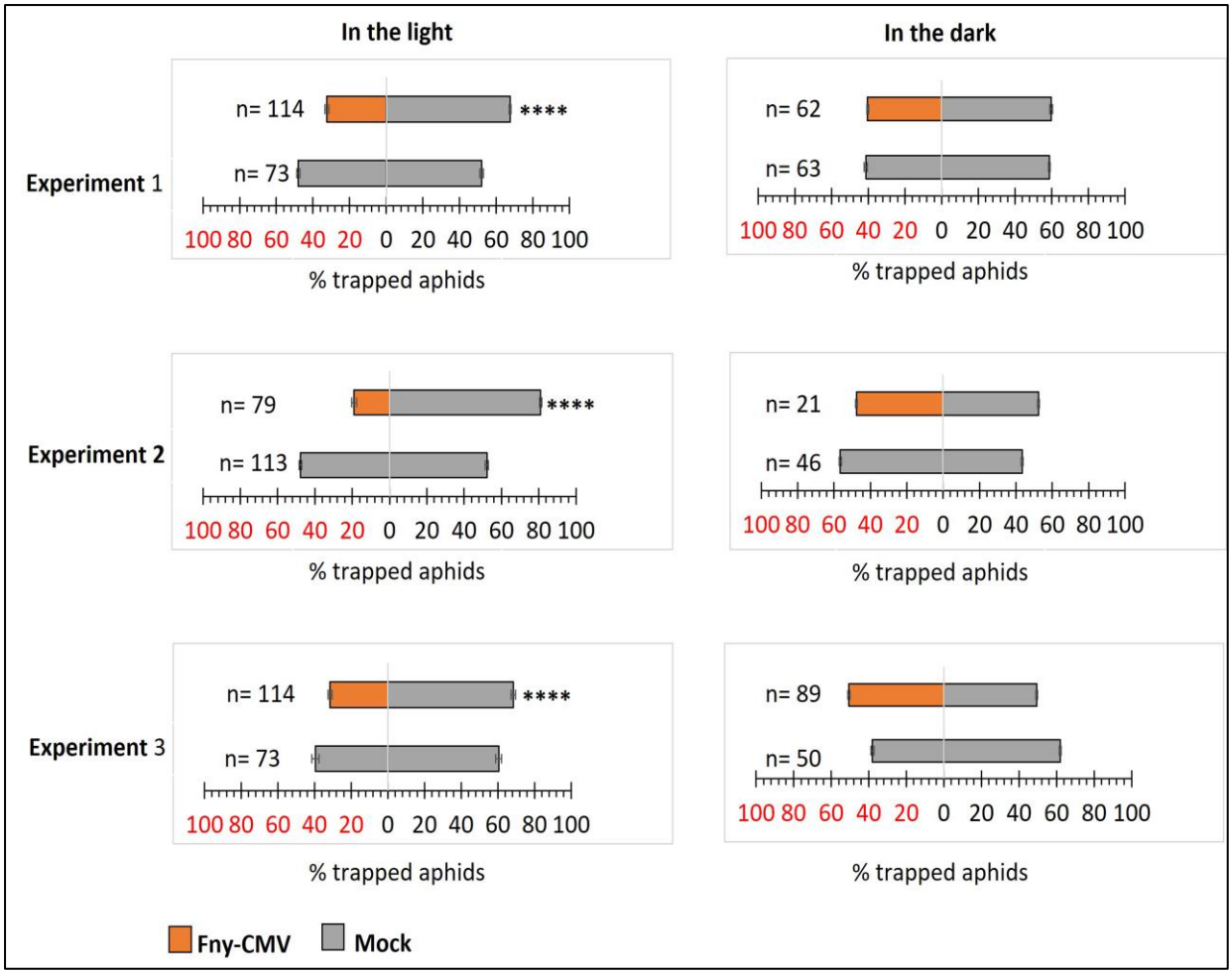


Figure 3.6. Under normal light, more *Myzus persicae* moving towards mock-inoculated plants were trapped compared to aphids moving towards plants infected with Fny-CMV, but there was no bias detected in the dark. Aphids were subjected to free choice trapping assays (Section 2.8.1). Aphid preference of migration direction in the control treatment (mock- versus mock-inoculated plants) remained unchanged in light and dark conditions. n is the total number of aphids that made a choice at each time point. Treatment pairs assigned asterisks****($p<0.0001$) are significantly different (Binomial test). The error bars represent SEM.

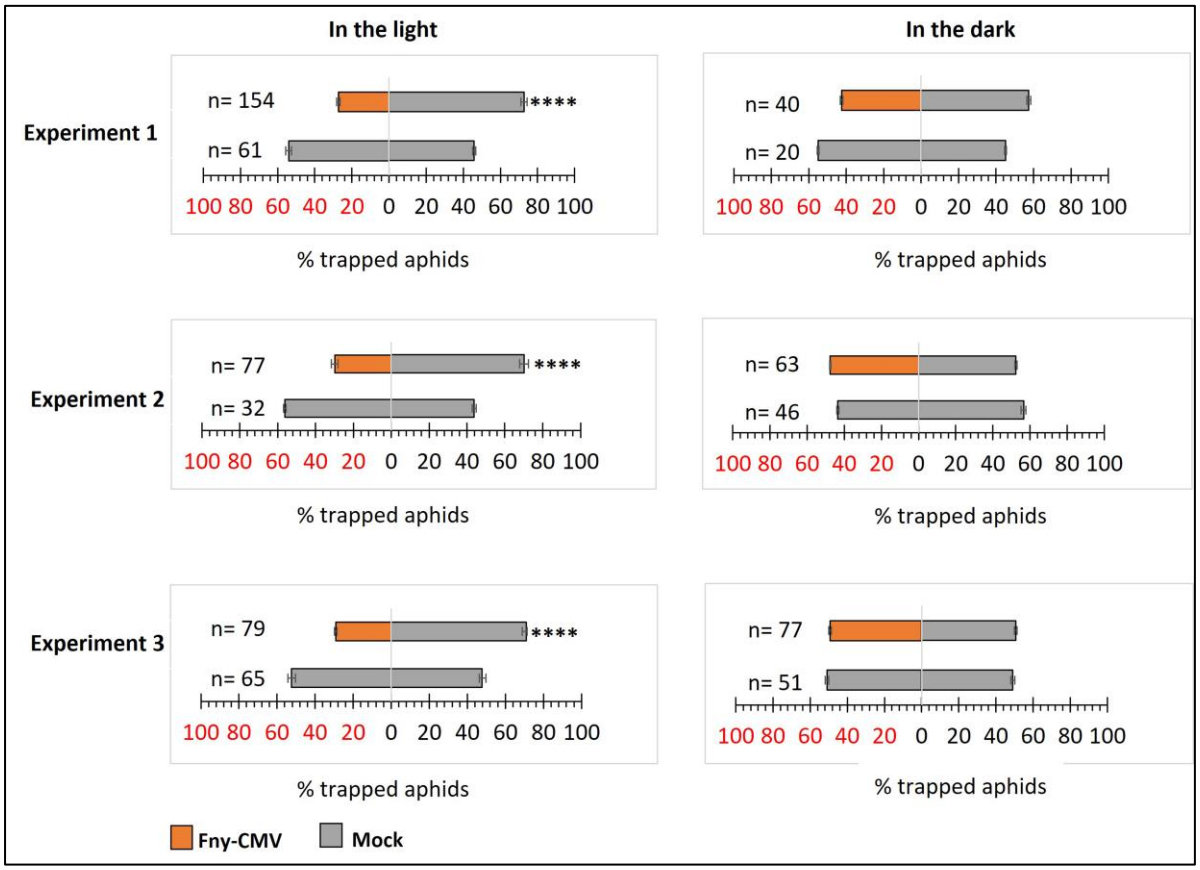


Figure 3.7. The strong innate preference of *Macrosiphum euphorbiae* aphids towards mock-inoculated plants compared to plants infected with Fny-CMV observed in normal illumination does not occur in the dark. In free-choice trapping assays (Section 2.8.1) aphids of this species did not show any bias in migrations towards mock-inoculated plants or Fny-CMV-infected plants in the dark. Aphid preference in the control treatment (mock-inoculated versus mock-inoculated plants) was neutral in either light or dark conditions. n is the total number of aphids that made a choice at each time point. Treatment pairs assigned asterisks ****($p<0.0001$) are significantly different (Binomial test). The error bars represent SEM.

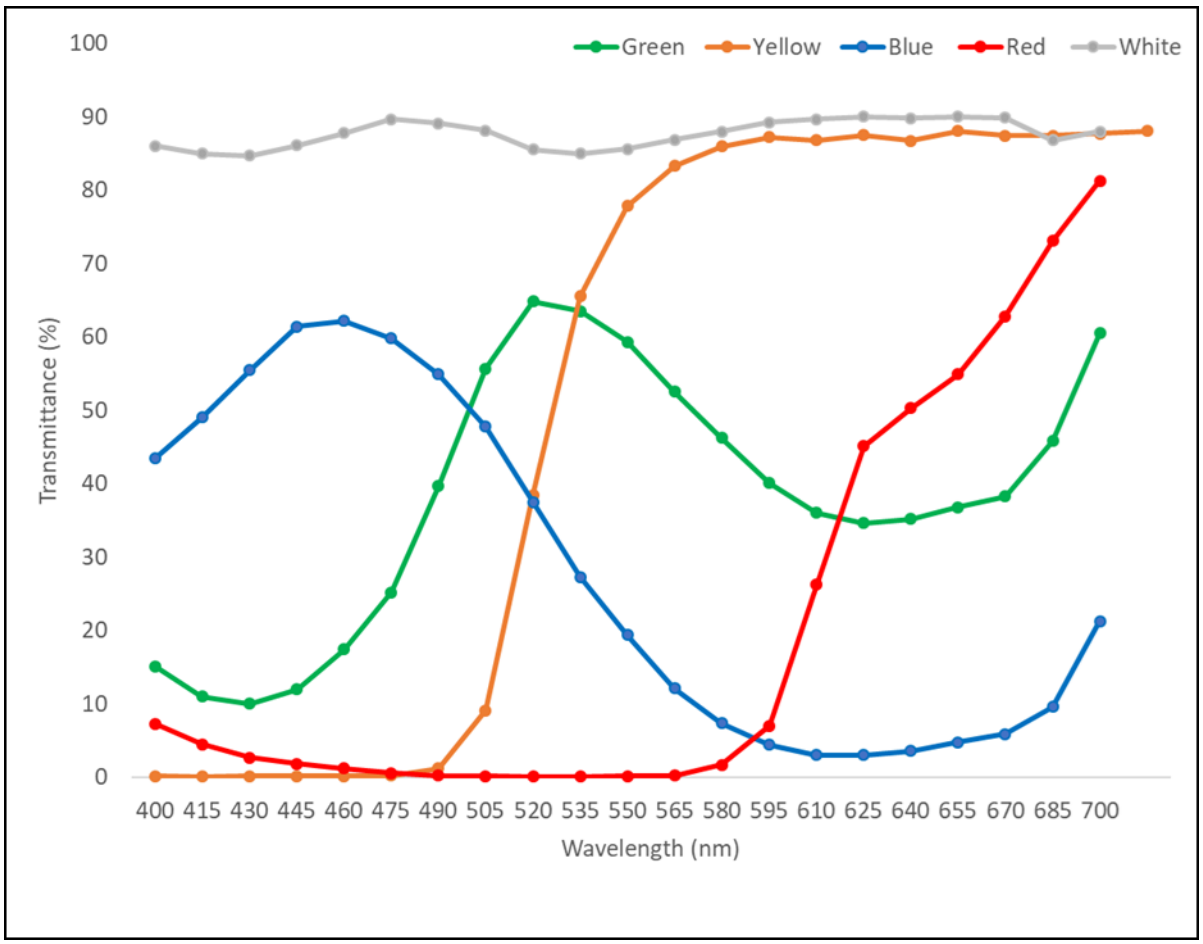


Figure 3.8. Spectral calibration of the different coloured cellophane films. Strips of clear (white control), blue, green, yellow and red transparent films measuring 10 mm wide by 45 mm high were cut and fastened on standard cuvettes. The amount of light transmitted by each film was determined in a spectrophotometer at varying wavelengths, as indicated in the graph. Yellow and red films transmitted more of the received light compared to blue and green-coloured films. The clear film consistently transferred 85% of light received between 400 nm-700 nm.

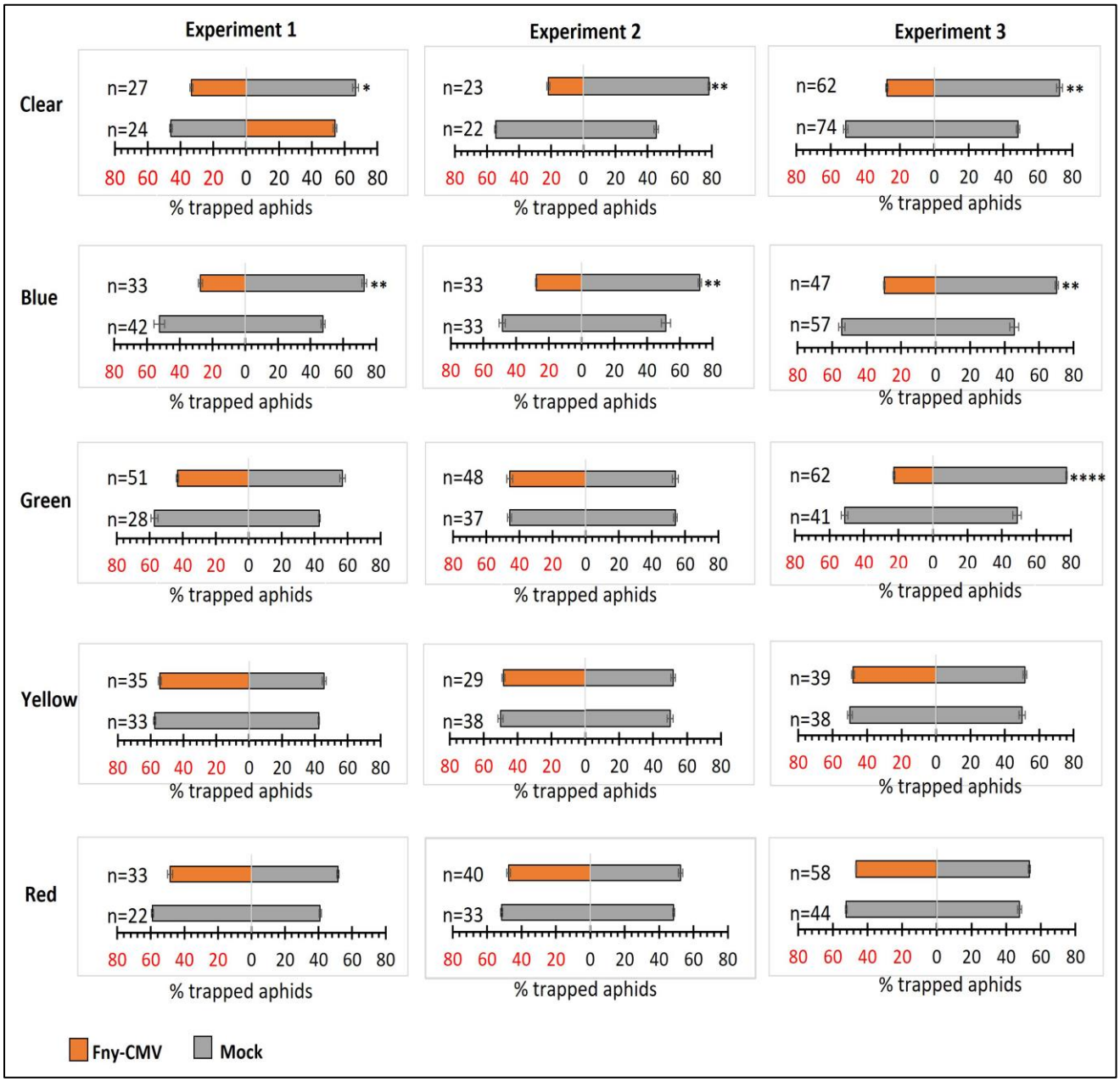


Figure 3.9. Tomato plants infected with Fny-CMV were consistently repellent to *Myzus persicae* aphids under white light and blue light. Aphid trapping experiments were carried out with pairs of mock-inoculated plants or plants infected with Fny-CMV covered with the indicated light filters (Section 2.8.1). Treatment pairs with asterisks *($p<0.05$), **($p<0.01$) or ****($p<0.0001$) indicate migration choices that are significantly different (Binomial test). n represents the total number of trapped aphids per treatment pair. The error bars represent SEM.

Figure 3.10. Under white, blue and green light, *Macrosiphum euphorbiae* aphids markedly preferred mock-inoculated tomato plants compared to virus-infected plants. Migration of aphids towards mock-inoculated plants over plants infected with Fny-CMV was greater in green light compared to blue light and similar to the pattern seen under white light (clear filter) in trapping assays (Section 2.8.1). Treatment pairs with asterisks *($p<0.05$), **($p<0.01$), ***($p<0.001$) or ****($p<0.0001$) are significantly different (Binomial test). n represents the total number of trapped aphids per treatment pair. The error bars represent SEM.

3.3 Discussion

3.3.1 CMV alters aphid preference and settling response in tomato

In the light, aphid preference and settling response to virus-infected plants and non-infected tomato plants is plastic in nature. The attractiveness of plants infected with Fny-CMV to *Myzus persicae* and *Macrosiphum euphorbiae* aphids diminished as infection progressed from 3 to 21 dpi. More aphids, especially *Myzus persicae,* were initially attracted to virus-infected plants compared to mock-inoculated plants. However, by 9 dpi and beyond, a more aphids, irrespective of species, migrated to and settled on mock-inoculated plants rather than infected plants. These results suggest a strong association between the effect(s) of the virus on host-vector interactions and stage of infection. These findings were consistent with insect behaviours have been observed in cucumber (Mauck et al., 2010a), tobacco (X. Shi et al., 2016a), watermelon (Shrestha et al., 2019) and Arabidopsis (Murphy et al. unpublished data) under normal light.

On *Cucurbita pepo*, *Myzus persicae* performance significantly declined on CMV-infected plants as infection progressed from 7-14 dpi (Mauck et al., 2010a). In both field and greenhouse experiments, Mauck and colleagues showed that more aphids preferentially migrated and settled on mock-inoculated and untouched plants compared to CMV-infected plants. The aphid preference rate for mock-inoculated plants was more substantial at 14 dpi compared to 7 dpi. *Myzus persicae* showed a similar pattern in CMV-infected tobacco plants (Shi et al., 2016). Using aphid free-choice assays, these workers showed that CMV-infected tobacco plants enhanced aphid preference and reproduction during the initial stages of infection (3-9 dpi). Beyond 9 dpi, aphids performed better on mock-inoculated plants compare to their diseased counterparts. In watermelon, squash vein yellowing virus (SqVYV), a geminivirus that is semi-persistently transmitted by whiteflies (*Bemisia tabaci*), altered whitefly alighting behaviour and fitness as infection progressed (Shrestha et al., 2017; 2019). Earlier than 9 dpi, the proportion of whiteflies that migrated and settled on either virus-infected or mock-inoculated watermelon plants were equal. Beyond 9 dpi, whiteflies markedly preferred mock-inoculated compared to virus-infected plants. Using insect cages to monitor whitefly reproduction, Shrestha and colleagues (2019) also showed that female whiteflies produced more eggs on mock-inoculated plants compared to geminivirus-infected plants at 10-12 dpi. Shrestha et al. (2019) suggested that the incremental development of yellowing symptoms in SqVYV-infected watermelon negatively altered whitefly alighting, settling and oviposition properties in a way that could promote SqVYV transmission. Interestingly, there was no significant difference in Fny-CMV accumulation between time courses (3, 9, 21 dpi) in tomato.

The innate preferences of *Myzus persicae* and *Macrosiphum euphorbiae* to migrate towards or settle on either Fny-CMV-infected plants or mock-inoculated plants under normal illumination are not exhibited when assays are carried out in the dark. Aphids of neither species made any distinction between virus-infected plants and mock-inoculated tomato plants. Some viruses target plant-derived visual cues to influence significant ecological interactions between plants and their insect vectors. Ajayi & Dewar (1983) found that plant colour changes in barley and oat plants caused by barley yellow dwarf virus infection influenced migration and settling properties of two cereal aphids, *Sitobion avenae* and *Metopolophium dirhodum*. In flight chamber experiments, two pea aphid species were more visually attracted to yellow-looking virus-infected leaves than to dark green health leaves. In tic beans, *Acyrthosiphon pisum* was more attracted to bean yellow mosaic-infected plants, that display yellow symptoms, than to healthy plants (Hodge & Powell, 2008). In this study, reduced rate of response and lack of bias by *Myzus persicae* and *Macrosiphum euphorbiae* in the dark suggests that CMV-associated visual changes such as leaf colour or other factors such as decreased plant size or modified morphology may play roles in aphid-tomato interactions. However, it remains to be determined if other cues such as taste and VOCs are also involved.

3.3.2 Blue and green light might influence aphid settling preference on tomato plants infected with CMV

Under blue and green light, aphids of both species preferentially migrated towards mock-inoculated plants compared to Fny-CMV-infected tomato plants, as they did under white light. The bias for mock-inoculated plants was stronger under green light for *Macrosiphum euphorbiae* than for *Myzus persicae*. When yellow and red light was shone on plants, aphids of neither species showed any preference for mock-inoculated or infected plants. Though these wavelength-dependent aphid preference properties were reported, to my knowledge, for the first time in aphid free-choice assays involving viral infection, several previous no-choice bioassays found that specific light wavelength can modify take-off, attraction, settling and feeding properties of thrips, whiteflies and aphids [see Fennell et al. (2019) and references therein].

Recently, Fennell et al. (2020) showed that apterous *Myzus persicae* aphids were sensitive to colour changes and prone to avoidance behaviour as their colour space is biased towards short wavelengths light such as UV and blue. The probability of *Myzus persicae* avoidance increased under blue or green light illumination (Fennell et al., 2020). Green light enhanced *Myzus persicae* feeding (Thomas Felix Döring & Chittka, 2007). In the same study, it was shown that under blue light aphids fed less than under white light, suggesting that blue light may inhibit feeding behaviour. *Aphis fabae* under blue or

green illumination are more attracted to green objects than blue (Hardie, 1989). Blue light may be unfamiliar to many insects, including aphids, because plant surfaces absorb most blue light and transmit or reflect more green light (Rabideau et al., 1946). The current study suggests possibly that the capacity of Fny-CMV-infected plants to absorb blue light is very low, making virus-infected plants look less attractive to aphids. It is also possible that Fny-CMV infection enhances the accumulation of anthocyanin (physiological indicators of plant phenolic status). Anthocyanin-rich plant surfaces reflect more blue light (Gitelson et al., 2009). Therefore, exposure of virus-infected plants to blue light may reveal their defensive status to aphids, and thus increase deterrence.

Hardie (1989) showed that *Aphis fabae* was unresponsive in light in the range 600 nm to 700 nm (yellow-red). Similarly, yellow and red light did not alter *Myzus persicae* and *Macrosiphum euphorbiae* innate attraction for either Fny-CMV-infected tomato plants or mock-inoculated plants. Most aphid species lack a red light receptor (Döring & Chittka, 2007). Results in this chapter suggests that *Myzus persicae* and *Macrosiphum euphorbiae* can see in blue or green light but not in yellow or red, which may appear dark to aphids. A recent study showed that red light stimulates a mutualistic relationship between *Bemisia tabaci* and begomoviruses by inhibiting JA-mediated host defence (Zhao et al., 2020). Zhao and colleagues showed that red light stabilises the interaction between begomovirus-encoded βC1 and MYC2 (see Section 1.6.1 for details) and PIF (PHYTOCHROME-INTERACTING FACTOR) transcription factors, thus inhibiting terpene biosynthesis. Terpenes are key JA-regulated VOCs and may affect aphid-plant interactions and virus transmission (see Section 1.6.1). Taken together the data in this chapter suggest that light plays a role in CMV-induced effects on aphid-tomato interactions. More research is required to investigate the connection between light, CMV viral proteins and host factors at a molecular level.

Chapter 4. Changes in VOC emission induced by Fny-CMV infection in tomato influence aphid behaviour

4.1 Introduction

In Chapter 3, it was shown that aphid settlement on tomato plants was affected by Fny-CMV in a time- and light-dependent manner. *Myzus persicae* and *Macrosiphum euphorbiae* were deterred under normal light from migrating to and settling on Fny-CMV-infected plants compared to mock-inoculated plants. This effect did not occur under darkness, and it was inferred that colour cues are important in the relationship between aphids and CMV-infected tomato plants. However, the effect of volatile cues could not be ruled out, which led to a fresh hypothesis that dark conditions might alter the blend of VOCs emitted by tomato plants. In this study, I investigated whether virus-induced changes in VOC emission were significant in aphid behaviour. The effect of light and darkness on VOC emission by virus-infected and mock-inoculated tomato plants was also tested.

4.2 Results

4.2.1 In the absence of other cues, aphids preferred plant odours emitted by plants infected with Fny-CMV

Y-tube olfactometry (Section 2.9) was used to examine whether volatile cues were critical in determining CMV-induced changes in aphid-tomato interactions. In this assay design, the aphids cannot be influenced by visual or tactile cues from the plants. Olfactometry showed that both *Myzus persicae* and *Macrosiphum euphorbiae* preferred odours of Fny-CMV-infected plants compared to those of mock-inoculated plants (Fig. 4.1 and Fig. 4.2). In three experiments performed for each aphid species, increased preference for tomato plants infected with Fny-CMV was more consistent for *Macrosiphum euphorbiae* than for *Myzus persicae*. These results showed that *Myzus persicae* and *Macrosiphum euphorbiae* have an innate preference for odours emitted from CMV-infected over those emitted by mock-inoculated tomato plants.

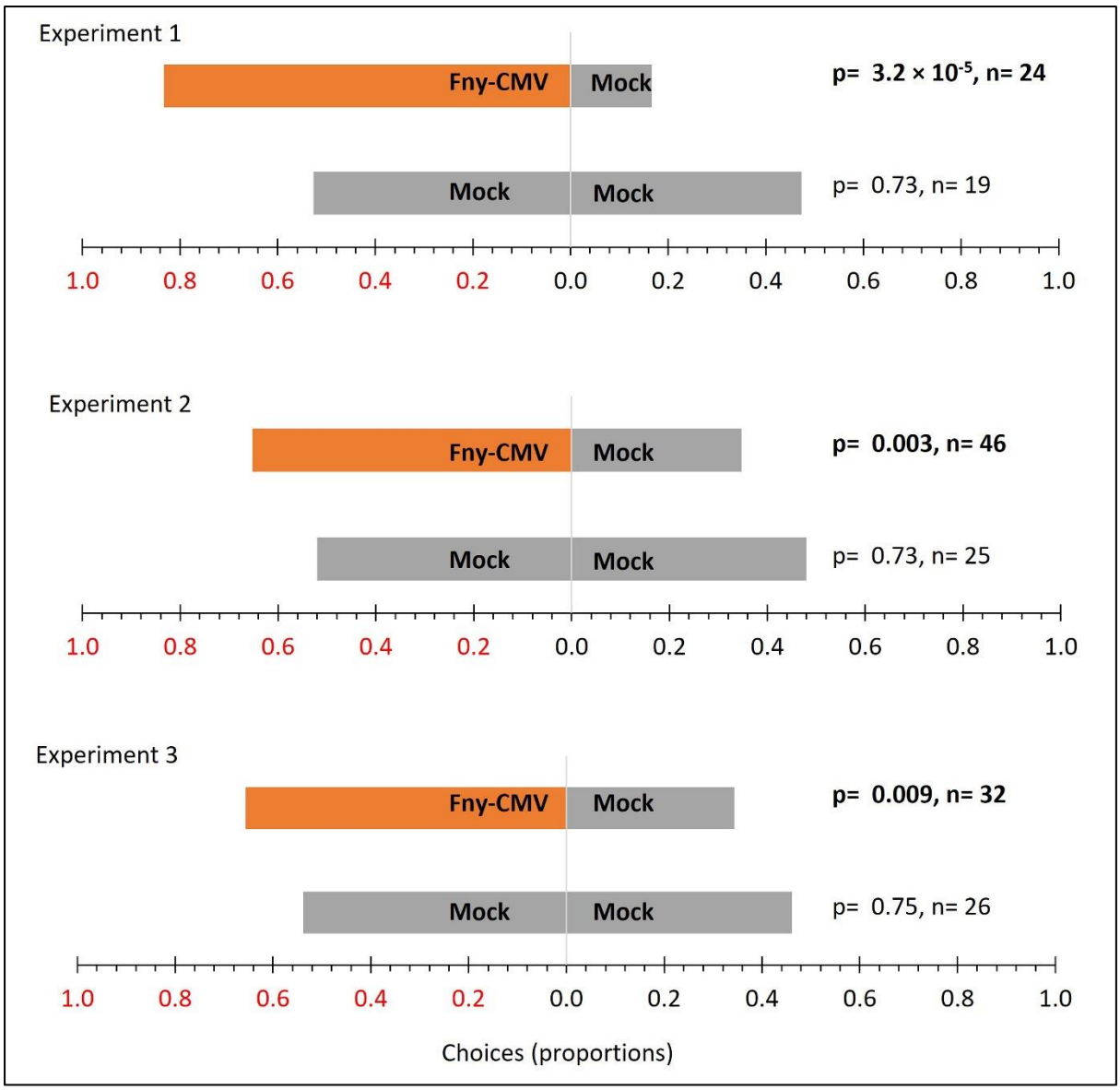


Figure 4.1. *Myzus persicae* aphids preferentially migrated towards odours of tomato plants infected with Fny-CMV compared to mock-inoculated plants. Y-tube olfactometry was used to determine which odour blend of virus-infected and mock-inoculated plants was more attractive to aphids. The bars show proportions of aphids that made choices in the treatment group (Fny-CMV versus mock) and controls (mock versus mock). The orange and grey bars represent Fny-CMV-infected plants and mock-inoculated plants, respectively. P-values (Binomial test) indicating significant difference are in bold. The total number of aphids that chose between odours pumped from chambers containing virus-infected plants and mock-inoculated plants is denoted by n. In the control group, i.e., mock versus mock, a high proportion of aphids did not make any choices at all. This experiment was repeated three times**.** All experiments were performed under normal light conditions**.**

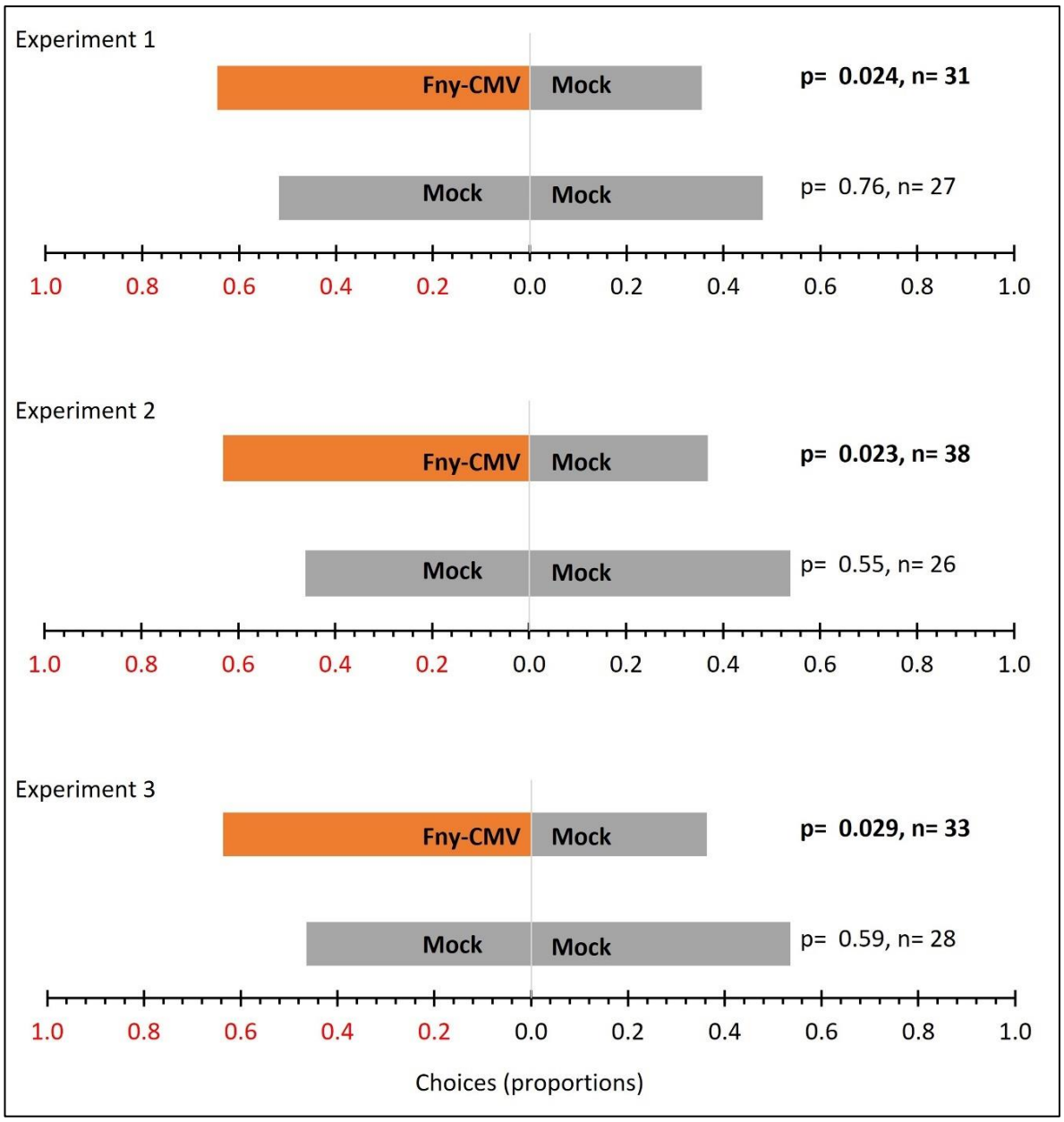


Figure 4.2. *Macrosiphum euphorbiae* aphids were consistently attracted to the odours of Fny-CMV-infected tomato plants compared to mock-inoculated plants. Y-tube olfactory was used to determent whether aphids differentiated odours of virus-infected plants and mock-inoculated plants. The bars represent proportions of aphids that made choices. P-values in bold, which were generated from a binomial test, are significantly different. The total number of aphids that chose between odours pumped from chambers containing virus-infected plants and mock-inoculated plants is indicated by n. This experiment was repeated three times under normal light conditions**.**

4.2.2 Fny-CMV infection enhanced emission of VOCs by tomato plants in the dark

Tomato plant headspace VOCs were collected from Fny-CMV-infected and mock-inoculated plants at 9 dpi (14 days post-sowing) and analysed by GC-MS (Section 2.11). The most abundant VOCs were identified by comparison of the MS data with known metabolites in the NIST database and with authentic standards for α-pinene, carene, *p*-cymene, (-)-*trans*-caryophyllene and nonanal (Figure 4.3).

Tomato plants infected with Fny-CMV emitted a significantly greater quantity of VOCs compared to mock-inoculated plants ($p= 1.5 \times 10^{-9}$, d=1, F-value= 44.8; Fig. 4.4A) in both illuminated and dark conditions. Tomato plants infected with Fny-CMV emitted more VOCs in the dark than under normal illumination (ANOVA with *post hoc* Tukey HSD test, p = 0.045). In contrast, mock-inoculated plants emitted similar levels of total VOCs in the light and the dark (Fig. 4.4A). VOCs were disaggregated by chemical category, and it was found that the emission rate of different volatile categories by Fny-CMV infected plants varied significantly between light and dark conditions (ANOVA test: $p= 2.81 \times 10^{-15}$, df =11, F value = 15.2) (Fig. 4.4B).

The most abundant VOCs in blends emitted by both virus-infected plants and mock-inoculated plants were: green leaf volatiles [GLVs: C_6 molecules emitted by upon pathogen infection by most green plants and are used insects as volatile cues during host selection (Scala et al., 2013)], including isopropyl acetate, 2-ethyl-1-hexanol and nonanal; benzenoids (benzaldehyde and naphthalene), and the aromatic terpenes (α-terpine, α-pinene and p-cymene) (Fig. 4.6). Both infected and mock-inoculated plants produced more GLVs, and to a lesser extent benzenoid and monoterpenoid emission was increased. In darkness no *p*-cymene was detected in blends emitted by tomato plants.

Although virus-infected tomato plants emitted more VOCs in light and dark conditions, findings from free-choice trapping assays in the light and the dark suggest that olfactory cues do not influence aphid preference and settling behaviour (Section 3.2.3). Trapping assays in the light showed that fewer aphids migrated towards virus-infected plants. In trapping assays in the dark (Chapter 3: Figs. 3.6 & 3.7), aphids did not migrate to virus-infected plants in preference to mock-inoculated plants, but in Y-tube olfactometry bioassays, the odours of virus-infected plants were shown to be more attractive to aphids (Fig. 4.1 & Fig.4.2). These olfactory findings sharply contrast aphid preference response observed in the trapping assays.

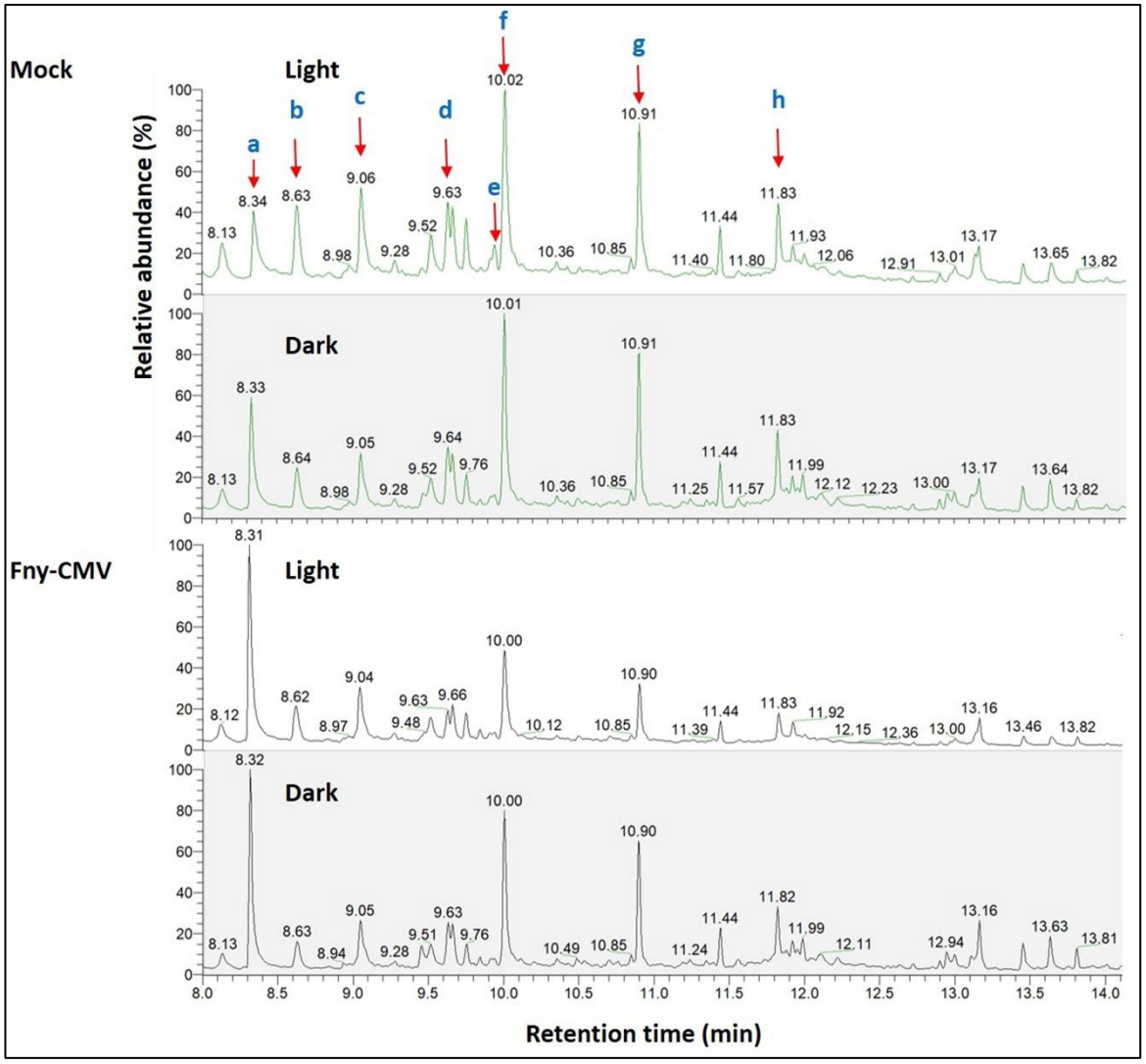


Figure 4.3. Chromatographs of volatile organic compounds (VOCs) emitted by Fny-CMV-infected plants (black) and mock-inoculated plants (green) in light (white plot area) and dark (grey plot area) conditions. Chromatographs were obtained by gas chromatography-mass spectrometry of samples of VOCs extracted by dynamic headspace trapping from tomato plants that had been infected with Fny-CMV and mock-inoculated (see Section 2.10 & Section 2.11 for detailed methods). The y-axis represents the percentage peak area of each VOC normalised with the peak area of Isopropyl acetate (retention time 8.33). Numerals above each peak show GC-MS retention time. Alphabets in the first panel represent identified peaks for most abundant volatile compounds per treatment and light exposure: a= isopropyl acetate, b= α-pinene, c= benzaldehyde, d= α-terpine, e= *p*-cymene, f= 2-ethyl-1-hexanol, g= nonanal, and h= naphthalene.

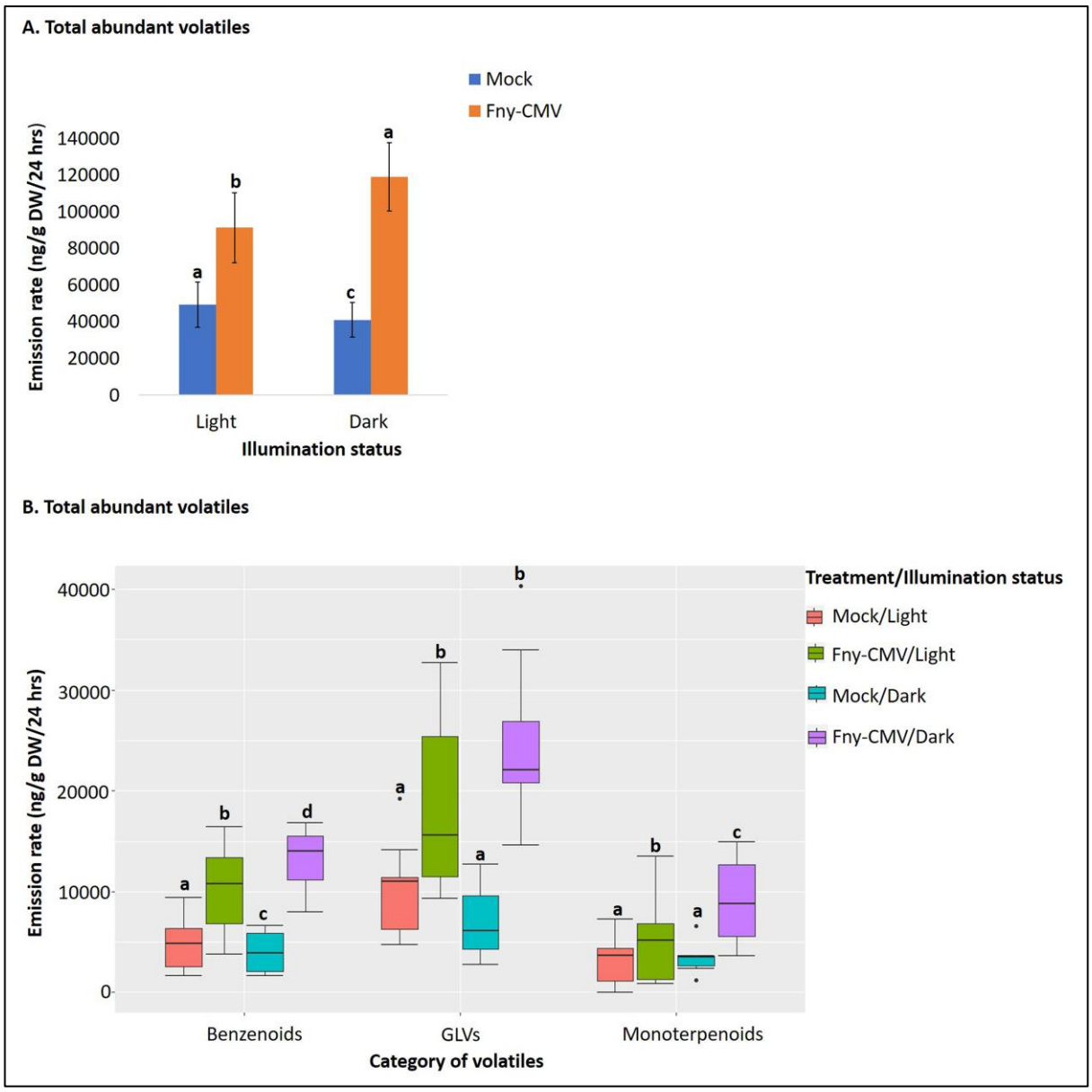


Figure 4.4. Infection of tomato by Fny-CMV increased the quantity of VOCs emitted. VOCs from virus-infected plants and mock-inoculated plants were captured by dynamic headspace trapping and quantified by GC-MS. Panel **A** shows the emission of three groups of VOCs that were relatively abundant in emitted blends from tomato (identified in Fig. 4.4), in the light and dark in nanograms per gram dry weight (DW). Virus-infected plants emitted more VOCs in the dark compare to light. Though marginal, the reverse was true for mock-inoculated plants: they released more VOCs in normal illumination. Panel **B** show boxplots of three different categories of VOCs emitted by virus-infected plants and mock-inoculated plants. Pink boxes indicate the emission rate of mock-inoculated plants in the light; green boxes: virus-infected plants in the light; blue and purple boxes: dark-treated mock-inoculated plants and virus-infected, respectively. In both panels, the letters above error bars indicate the level of statistical significance determined using Tukey's post hoc HSD test with ANOVA at $\alpha = 0.05$. Mean values for emission within each chemical group (benzenoids, GLVs and monoterpenoids) with the same lower case letter are not significantly different.

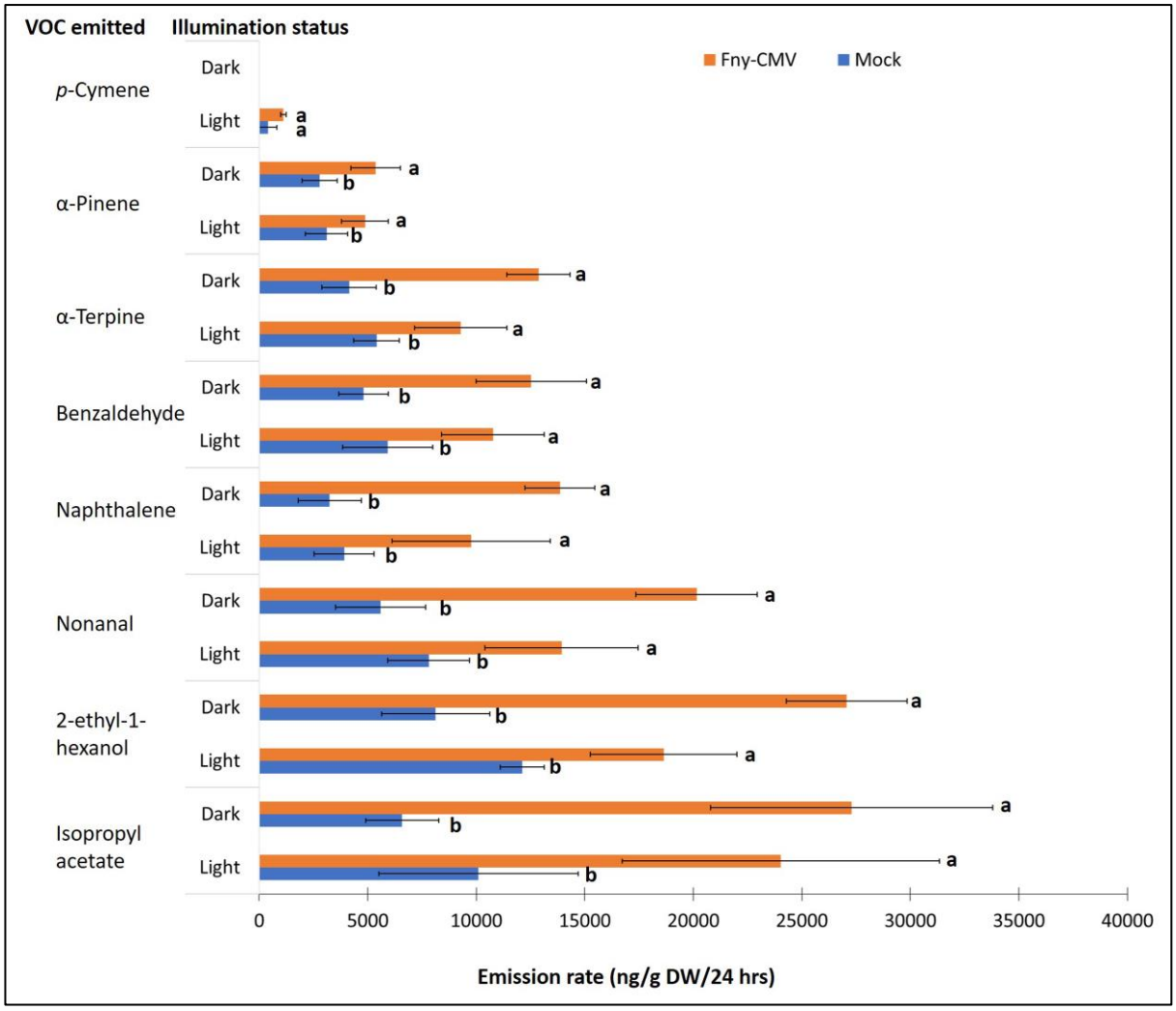


Figure 4.5. Fny-CMV infection increased the emission rate of several ionised VOCs by tomato plants in the dark. Samples of VOCs were extracted by dynamic headspace trapping from virus-infected plants and mock-inoculated tomato plants. Collected VOCs were further separated and ionised by electron impact in a Thermo Scientific TG-SQC capillary column (Section 2.11). Fny-CMV-infected plants emitted larger quantities of isopropyl acetate, 2-ethyl-1-hexanol, nonanal, naphthalene and benzaldehyde. Other VOCs released by virus-infected plants include α-terpine, α-pinene and p-cymene. The error bars indicate SEM, and the letters above represent Tukey's post hoc test with ANOVA. In each VOC emitted, bars with different letters indicate mean values for emission that are significantly different.

4.3 Discussion

I found that aphids, in the absence of visual and contact cues, were influenced by olfactory cues and preferred VOCs emitted by tomato plants infected with Fny-CMV. When the olfactometry assays were carried out in the light, *Myzus persicae* and *Macrosiphum euphorbiae* aphids preferentially migrated and settled in the olfactometer arm presenting odours emitted by Fny-CMV-infected plants. GC-MS analysis confirmed that tomato plants infected with Fny-CMV, although smaller than mock-inoculated plants (approximately 50%: Fig. 3.1), emitted more VOCs in both light and darkness. A few studies indicate that plants often release volatiles in distinct diurnal or nocturnal patterns (De Moraes et al., 2001; Lei, 2017; Lerdau & Gray, 2003; Martin et al., 2003). That VOC emission by Fny-CMV-infected tomato plants is increased in the dark is to my knowledge a novel observation.

The responses of aphids to plant odour reported in this chapter are consistent with the data reported by Mauck et al. (2010a), who showed that VOCs emitted by Fny-CMV-infected squash plants were attractive to *Myzus persicae* and *Aphis gossypii*. Similarly, it was reported that CMV infection in Arabidopsis induced odour-dependent attraction to *Myzus persicae* aphids (Wu et al., 2017b). Odour-dependent changes in behaviour have been reported for insects that are not CMV vectors. Fny-CMV-infected tomato plant leaf odours attracted bumblebees (Groen et al., 2016b), and whiteflies (*Bemisia tabaci*) preferred odours produced CMV-infected *Capsicum annuum* L. over those produced by healthy plants (Saad et al., 2019).

The olfactometry results reported in this chapter appear to contradict free choice trapping assay findings described in Section 3.2.3. In trapping tests in the light, aphids preferred to migrate towards mock-inoculated plants compared to Fny-CMV-infected tomato plants (Figures 3.6 & 3.7). In olfactory assays, aphids chose odours by virus-infected plants (Figures 4.1 & 4.2). This inconsistency in aphid preference may imply that aphids employ precedence in a complex environment involving multiple cues. In the case of trapping tests in the light, visual cues overshadowed olfactory cues, increasing the chances of vectoring CMV to healthy tomato plants. The ranking of signals by aphids in CMV-tomato interactions agrees with the insect vector sequential cues hypothesis by Silva and Clarke (2020). Using mathematical models, Silva and Clarke postulated that insects could prioritise specific cues from a complex mixture of signals while identifying and selecting suitable host plants. Findings in this chapter and Chapter 3 suggest that *Myzus persicae* and *Macrosiphum euphorbiae* aphids rank cues in CMV-tomato interactions.

Alternatively, the contrast between aphid responses in trapping assays and olfactory bioassays may be technical, i.e., due to variation in experimental set-up. In trapping assays, test plants for pair-wise comparison were planted 9 cm apart in the same pot, increasing the chances of volatile mixing. In olfactory bioassays, mixing of VOCs is minimised because individual test plants were planted in separate pots. Also, by design, the Y-tube limits the mixing of plant volatiles.

Taken together, positive odour-associated aphid response revealed by olfactometry (this chapter) and deterrence in trapping bioassays, as shown in Section 3.2.3, suggests that both visual and olfactory cues are essential in CMV-tomato interactions. However, visual cues play a critical role in influencing aphid behaviour in tomato. The significance of visual cues compared to volatile cues is supported by no preference of aphids in the trapping assays performed in the dark despite evidence from GC-MS analysis that darkness enhances volatile emission by Fny-CMV-infected tomato plants.

Chapter 5. The cucumber mosaic virus 2b protein is a determinant of aphid deterrence in tomato

5.1 Introduction

As described in Section 1.3, the CMV genome comprises three RNA segments, RNAs 1, 2 and 3, that encode five proteins. There is growing evidence that specific CMV RNA segments and/or the proteins they encode directly or indirectly influence aphid-host interactions in various plant species (Rhee et al., 2020; Tungadi et al., 2020; Westwood et al., 2013, 2014). Tungadi et al. (2020) recently demonstrated that both the 1a protein encoded by RNA1 as wells as the 2b protein encoded by RNA 2 influence CMV-induced aphid resistance in tobacco. In Arabidopsis, the 1a, 2a and 2b proteins all influence aphid-host interactions (Westwood et al., 2013). Chapter 3 of this study showed that Fny-CMV alters aphid preference and settling behaviour in tomato. At 9 dpi, Fny-CMV infection discouraged aphids from migrating to and settling on virus-infected tomato plants (Section 3.2.3), but it is unclear if the repulsion of aphids from tomato plants infected with CMV is associated with one or more viral proteins. Therefore, I sought to identify Fny-CMV RNA segments and viral gene products involved in CMV-induced aphid deterrence in tomato. I used the approach of constituting inter-strain pseudorecombinant viruses (see Section 1.8; Tungadi et al., 2020) to identify the responsible Fny-CMV gene product(s). Initially, I investigated whether there were virus strain-specific differences in how CMV influences aphid preference and settling properties in tomato. I chose to use the LS-CMV strain to do this because: it does not affect aphid-host interactions in Arabidopsis (Westwood et al., 2013); in tobacco, the 1a protein of LS-CMV does not induce resistance to aphids, unlike the orthologous 1a protein of Fny-CMV (Tungadi et al., 2020); LS-CMV causes mild or symptomless infections in tomato, and tomato supports the replication of a recombinant CMV in which the Fny-CMV 2b coding sequence has been replaced with that of LS-CMV (Cillo et al., 2009a). These factors make LS-CMV a useful experimental tool for understanding the aphid resistance induced in tomato by Fny-CMV.

5.2 Results

5.2.1 The LS strain of CMV did not change the settling preference of aphids on tomato plants.

I compared the settling behaviour of *Myzus persicae* and *Macrosiphum euphorbiae* on mock-inoculated tomato plants and plants infected with LS-CMV using aphid free-choice bioassays (Section 2.8.1). LS-CMV infections were authenticated using ELISA (Section 2.3.3). Seven-day-old wingless aphids were allowed to make a choice to migrate either to virus-infected or to mock-inoculated tomato

plants. The proportion of aphids settling on either virus or mock-inoculated plants was recorded at 1 and 24 hours post-release.

Neither the aphids of *Myzus persicae* nor those of *Macrosiphum euphorbiae* showed any bias in settling on either tomato plants infected with LS-CMV or on mock-inoculated plants (Fig. 5.1 & Fig. 5.2). This was confirmed multiple times in subsequent experiments reported in this chapter. It appears that in tomato, LS-CMV infection does not affect the attractiveness of plants to either the generalist aphid *Myzus persicae* or to the specialist, *Macrosiphum euphorbiae*. On the contrary, tomato plants infected with Fny-CMV were repellent to both species of aphids. I confirmed using ELISA that LS-CMV accumulates lower than Fny-CMV, albeit comparable (Fig. 5.4), showing that the difference in the effects of the two strains on aphid-host interactions are not due to differences in titre. These experiments demonstrated that the effects of CMV infection on aphid settling are strain-specific in tomato, which allowed me to utilise the pseudorecombinant virus approach to identify which CMV viral protein or RNA(s) are responsible for deterring aphids.

I made six pseudorecombinant viruses by mixing *in vitro*-synthesized RNA segments of Fny-CMV (RNAs indicated by 'F') with those of LS-CMV (RNAs denoted by 'L') (see Section 2.3). At 9 dpi, four out of six pseudorecombinant viruses caused leaf mosaic and growth stunting symptoms in infected tomato plants (Fig 5.3). These four pseudorecombinant viruses, i.e., $L_1L_2F_3$, $F_1L_2F_3$, $F_1F_2L_3$, and $L_1F_2F_3$, caused symptoms similar in some respects to those induced by Fny-CMV (leaf distortion) (Fig. 5.3). LS-CMV did not induce strong disease symptoms (Fig. 5.3). These pseudorecombinants caused whole plant stunting and reduced the leaf size in infected tomato plants. Infection with $F_1L_2F_3$, $F_1F_2L_3$, and $L_1F_2F_3$ caused severe mosaic, which was not observed in tomato plants infected with $L_1L_2F_3$. Virus accumulation in newly emerged leaves systemically infected with $L_1L_2F_3$, $F_1L_2F_3$ and $F_1F_2L_3$, was comparable to that of the wild-type parental viruses, Fny-CMV and LS-CMV (Fig.5.4). Notably, the pseudorecombinant virus $L_1F_2F_3$ accumulated to much lower levels compared to $L_1L_2F_3$, $F_1L_2F_3$ and $F_1F_2L_3$ despite causing plant stunting as severe as that induced by Fny-CMV.

The pseudorecombinant virus $F_1L_2L_3$ induced weaker symptoms than Fny-CMV and most of the other pseudorecombinant viruses (except for $L_1F_2L_3$) and induced yellowing of the leaves, although its accumulation was detectable by ELISA. $L_1F_2L_3$ did not cause noticeable symptoms and accumulated poorly in infected tomato plants. ELISA, which was the standard assay for confirming infection, did not detect $L_1F_2L_3$ infection. When RT-PCR was used infection by $L_1F_2L_3$ was confirmed although the

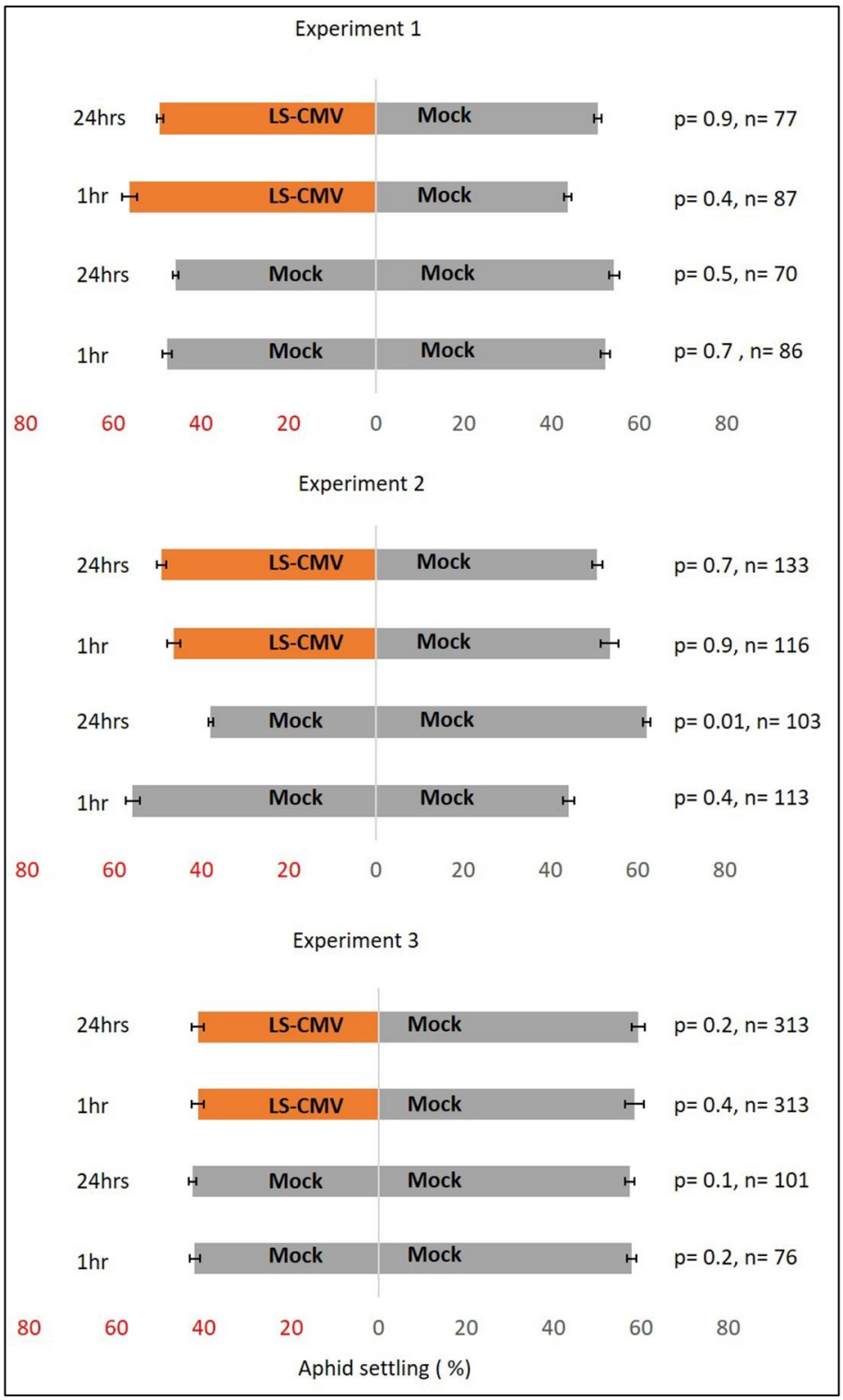


Figure 5.1. LS-CMV did not affect *Myzus persicae* settling preference in tomato. A settling assay described in Section 2.8.1 was used to evaluate aphid settling preference. Plot bars labelled LS-CMV and Mock represent tomato plants infected with LS-CMV and plants inoculated with sterile water as mock treatment, respectively. Aphid choices were recorded after 1 hour and at 24 hours post-release in the three independent experiments denoted as experiment 1, experiment 2, and experiment 3 in the plot. The error bars represent SEM. The p-values against each control/treatment comparison indicate a binomial test for statistical significance at $\alpha = 0.05$, and n represents the total number of aphids settled per treatment pair.

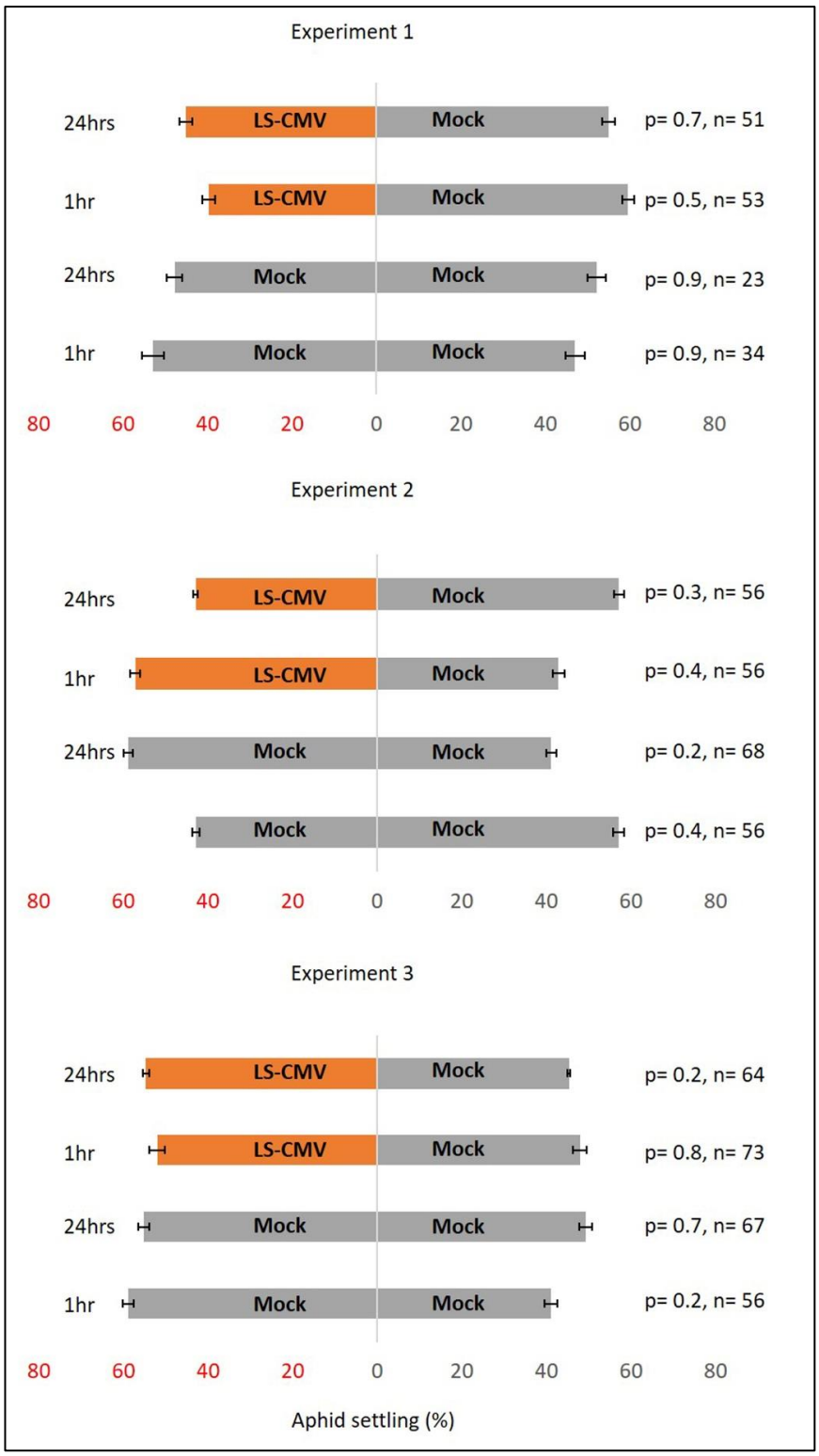


Figure 5.2. *Macrosiphum euphorbiae* settling behaviour was not influenced by LS-CMV infection. A settling assay was used to determine aphid settling preference. In three independent experiments (indicated as experiment 1-3), the proportion of aphids that settled on LS-CMV-infected tomato plants and mock-inoculated plants did not differ at both time intervals (1hr and 24 hours post aphid release). The error bars represent SEM. The p-values against each control/treatment comparison indicate a binomial test for statistical significance at $\alpha = 0.05$.

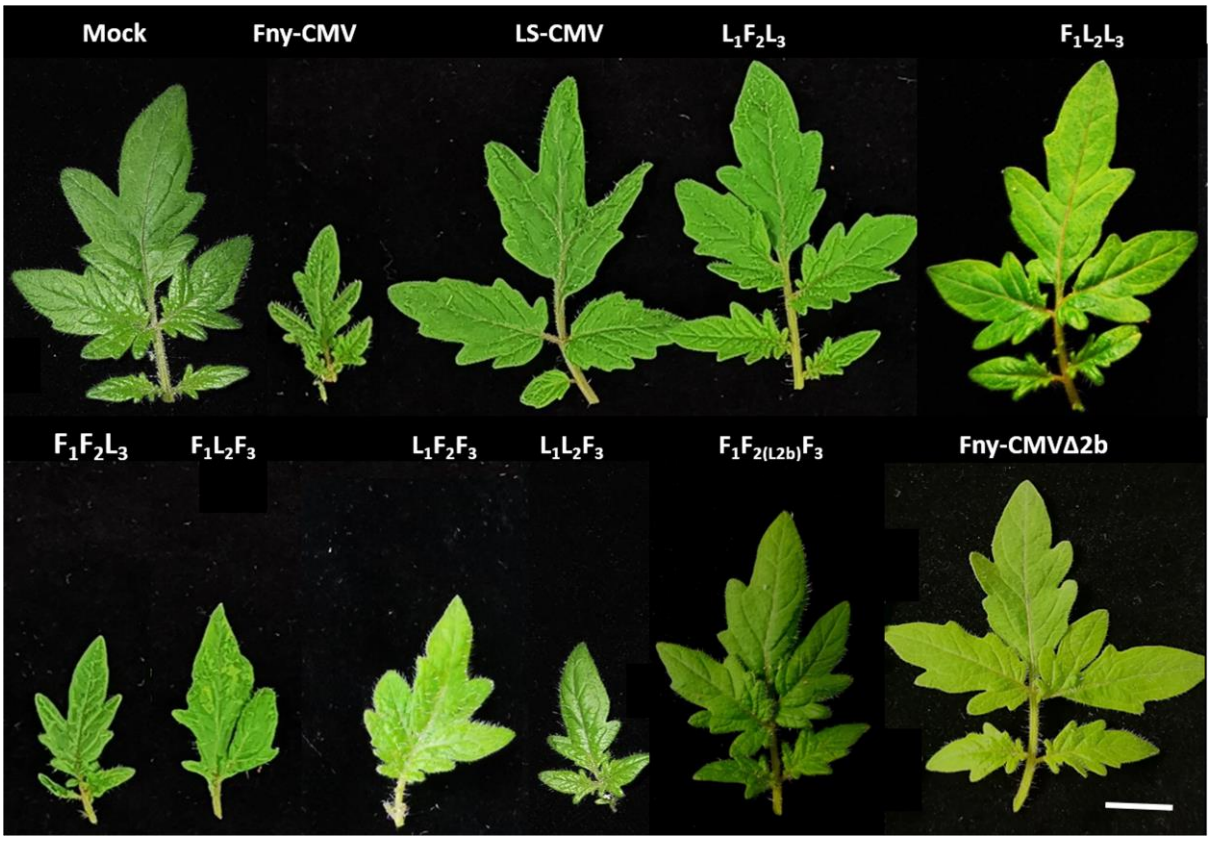


Figure 5.3. Symptoms induced by different CMV pseudorecombinants on newly emerged leaves of tomato plants at 9 dpi. Viral pseudorecombinant viruses were generated using combinations of CMV genomic RNAs 1, 2 and 3 (subscripts) of Fny-CMV and LS-CMV. Tomato plants were mechanically infected with purified virions of each pseudorecombinant virus, mutant virus and wild-type viruses at a concentration of 500 ng/µl or mock-inoculated with water (Mock) (Section 2.3.2). Tomato plants infected with $F_1L_2F_3$, $F_1F_2L_3$, $L_1F_2F_3$, and $L_1L_2F_3$ expressed severe mosaic and stunting symptoms similar to tomato plants infected by Fny-CMV. $L_1F_2L_3$ and $F_1L_2L_3$ pseudorecombinants caused similar symptoms as LS-CMV. Virus-infected plants showed mild to no mosaic symptoms in tomato and no stunting symptoms. The scale bar represents 1 cm.

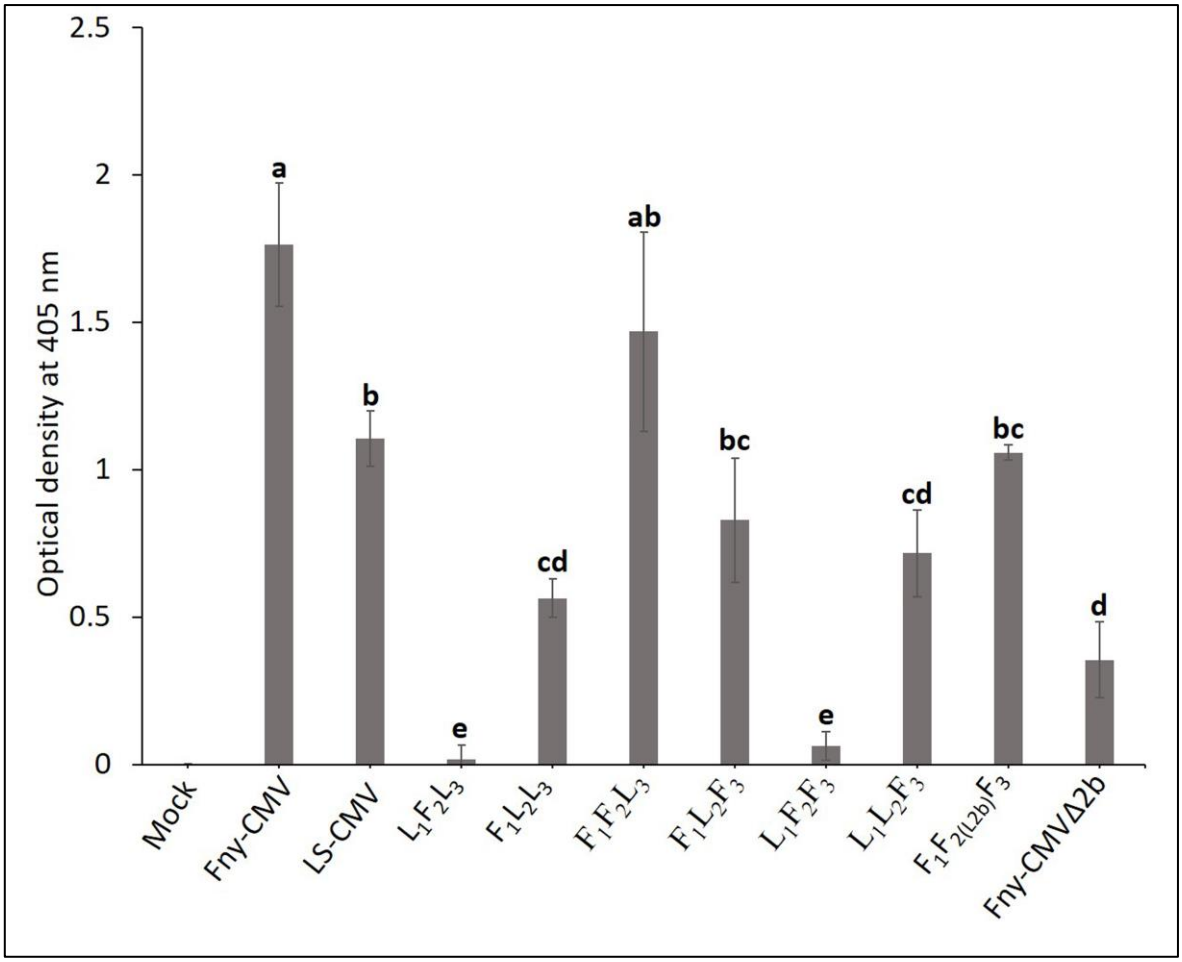


Figure 5.4. Virus accumulation of CMV pseudorecombinants, Fny-CMV and LS-CMV in tomato plants. ELISA was used to measure virus accumulation in infected tomato plants at 9 dpi. Mock indicates a mock-inoculated plant. Different letters (a-e) are assigned to statistically different results (ANOVA with post hoc Tukey's HSD tests). Error bars represent SEM.

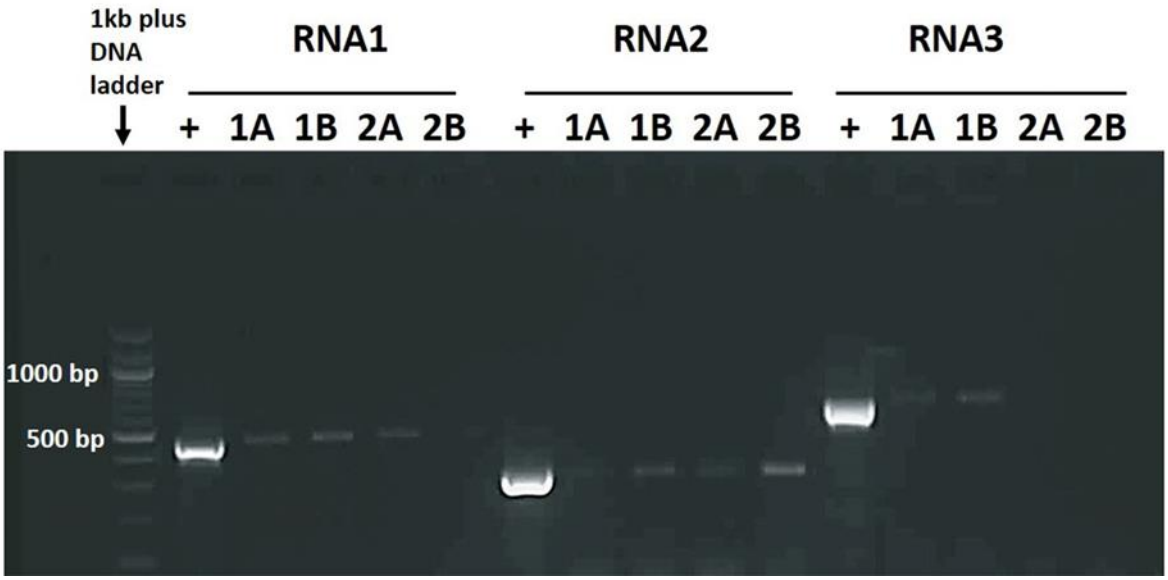


Figure 5.5. RT-PCR of $L_1F_2L_3$-infected tomato plants at 9 dpi using primers specific for CMV RNA1, RNA2 and RNA3. I performed RT-PCR with total RNA extracted from intermediate (A) and newly emerged (B) leaves of two randomly selected $L_1F_2L_3$ -infected plants (indicated by 1 and 2). Expected amplicons for RNA1 (465 bp), RNA2 (311 bp) and RNA3 (643 bp) were obtained for two samples analysed. The + indicates an Fny-CMV positive control. The weak or absent signals for RNA 3 bands in sample 2A and 2B suggests why ELISA, which detects the CMV coat protein, did not detect $L_1F_2L_3$ since CMV RNA 3 encodes the coat protein.

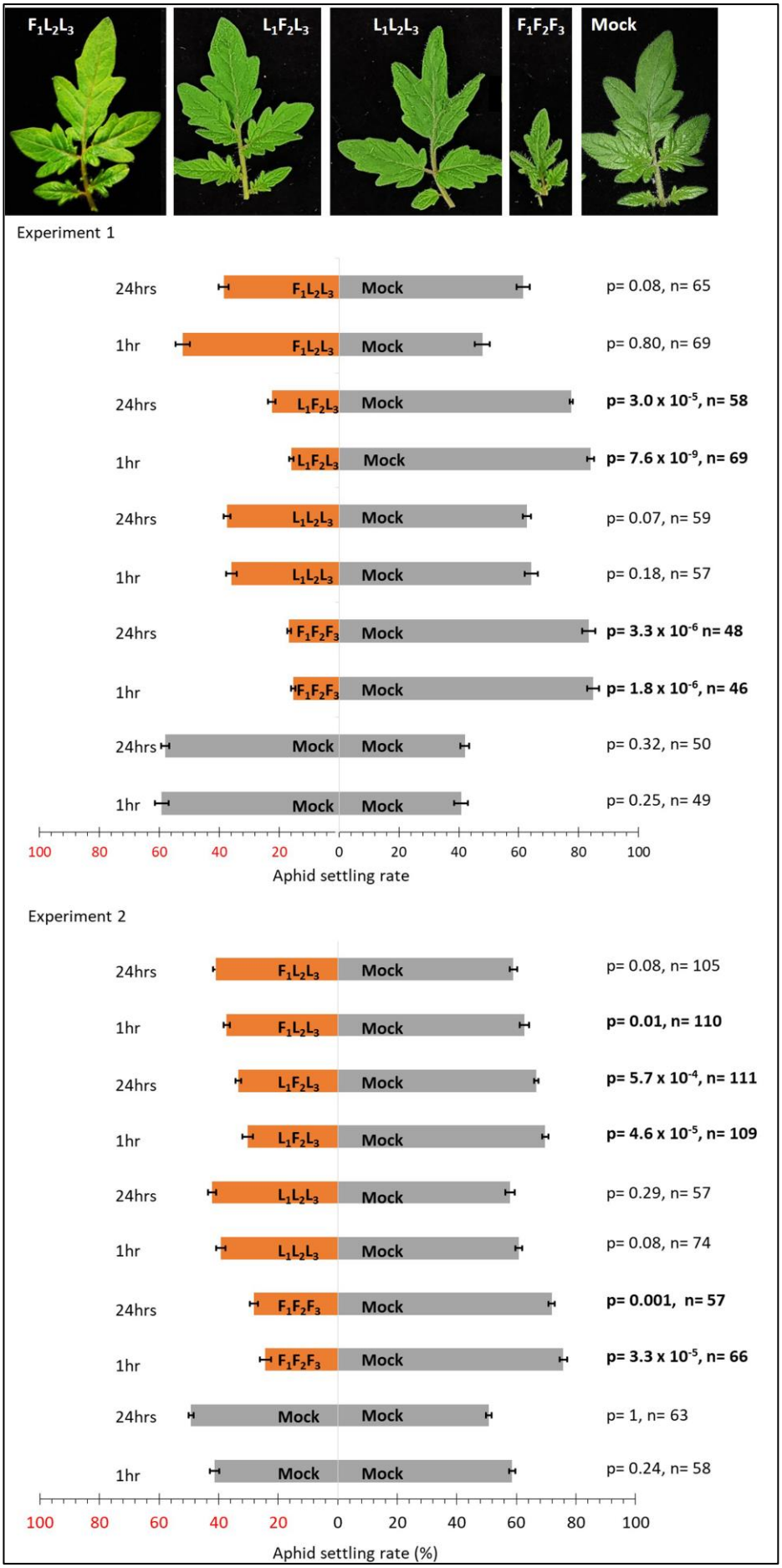
accumulation of viral RNA was lower than in some other infections, which may explain the weakness of the symptoms induced by this pseudorecombinant virus in tomato, and why the accumulation of this virus was below the limit of detection for ELISA (Fig. 5.5).

5.2.2 The Fny-CMV RNA2 appears to be a major determinant of virus-induced aphid deterrence in tomato plants

Choice tests were carried out with six pseudorecombinant viruses (Fig. 5.6, Fig. 5.7, Fig. 5.8, & Fig. 5.9). Two pseudorecombinant viruses, $L_1F_2L_3$ and $F_1F_2L_3$, consistently affected the relationship between aphids and tomato plants (see results summarised in Table 5.1). When aphids were allowed to choose between tomato plants infected with $L_1F_2L_3$ or $F_1F_2L_3$ and mock-inoculated plants, more aphids settled on mock-inoculated plants than on plants infected with either of these pseudorecombinant viruses and this was seen with both *Myzus persicae* and *Macrosiphum euphorbiae* (Fig. 5.6, Fig. 5.7, Figs. 5.8 & Fig. 5.9). Across experiments, the effects of $F_1F_2L_3$ on aphid settling behaviour was seen consistently in all experiments with both *Macrosiphum euphorbiae* and *Myzus persicae* (Table 5.1). However, plants infected with $L_1F_2L_3$ were less susceptible to aphid settlement by *Myzus persicae* in four out of four experiments, but in one out of the four experiments with *Macrosiphum euphorbiae*, infected plants and mock-inoculated plants were similarly susceptible to aphid settlement (Table 5.1). $L_1F_2L_3$ accumulates to much lower levels than the other pseudorecombinant viruses (Fig.5.4), which may explain the less consistent results with *Macrosiphum euphorbiae,* if it is hypothesized that this specialist aphid might be less susceptible than the generalist *Myzus persicae* to a virus-induced anti-aphid mechanism.

The results with the pseudorecombinant viruses $L_1F_2L_3$ and $F_1F_2L_3$ suggested that RNA2, or one of the two proteins encoded by this viral RNA (the 2a and 2b proteins), was responsible for the induction of some form of repellence or resistance to aphid settlement on tomato plants. The results obtained with the pseudorecombinant viruses $F_1L_2F_3$ and $L_1L_2F_3$, which did not induce deterrence, support this idea (Table 5.1). However, the idea appears to be contradicted by the results with $L_1F_2F_3$, which did not induce deterrence to aphid settlement (Fig. 5.8 & Fig. 5.9) (Table 5.1). The neutral aphid response observed with $L_1F_2F_3$, which accumulates poorly in tomato, suggests that viral titre load may also contribute to aphid deterrence.

The pseudorecombinant virus $F_1L_2L_3$ had a transient effect on *Myzus persicae* settling behaviour. In three out of four independent experiments, *Myzus persicae* aphids initially (at 1 hour post-release) settled in significantly higher numbers on tomato plants over mock-inoculated plants (Fig. 5.6). However, this initial-preference was not sustained, and by 24 hours post-release, the aphids settled in


F1L2L3
L1F2L3
L1L2L3
F1F2F3
Mock
Experiment 1
24hrs F1L2L3 Mock p= 0.08, n= 65
1hr F1L2L3 Mock p= 0.80, n= 69
24hrs L1F2L3 Mock p= 3.0 x 10-5, n= 58
1hr L1F2L3 Mock p= 7.6 x 10-9, n= 69
24hrs L1L2L3 Mock p= 0.07, n= 59
1hr L1L2L3 Mock p= 0.18, n= 57
24hrs F1F2F3 Mock p= 3.3 x 10-6 n= 48
1hr F1F2F3 Mock p= 1.8 x 10-6, n= 46
24hrs Mock Mock p= 0.32, n= 50
1hr Mock Mock p= 0.25, n= 49
100 80 60 40 20 0 20 40 60 80 100
Aphid settling rate
Experiment 2
24hrs F1L2L3 Mock p= 0.08, n= 105
1hr F1L2L3 Mock p= 0.01, n= 110
24hrs L1F2L3 Mock p= 5.7 x 10-4, n= 111
1hr L1F2L3 Mock p= 4.6 x 10-5, n= 109
24hrs L1L2L3 Mock p= 0.29, n= 57
1hr L1L2L3 Mock p= 0.08, n= 74
24hrs F1F2F3 Mock p= 0.001, n= 57
1hr F1F2F3 Mock p= 3.3 x 10-5, n= 66
24hrs Mock Mock p= 1, n= 63
1hr Mock Mock p= 0.24, n= 58
100 80 60 40 20 0 20 40 60 80 100
Aphid settling rate (%)

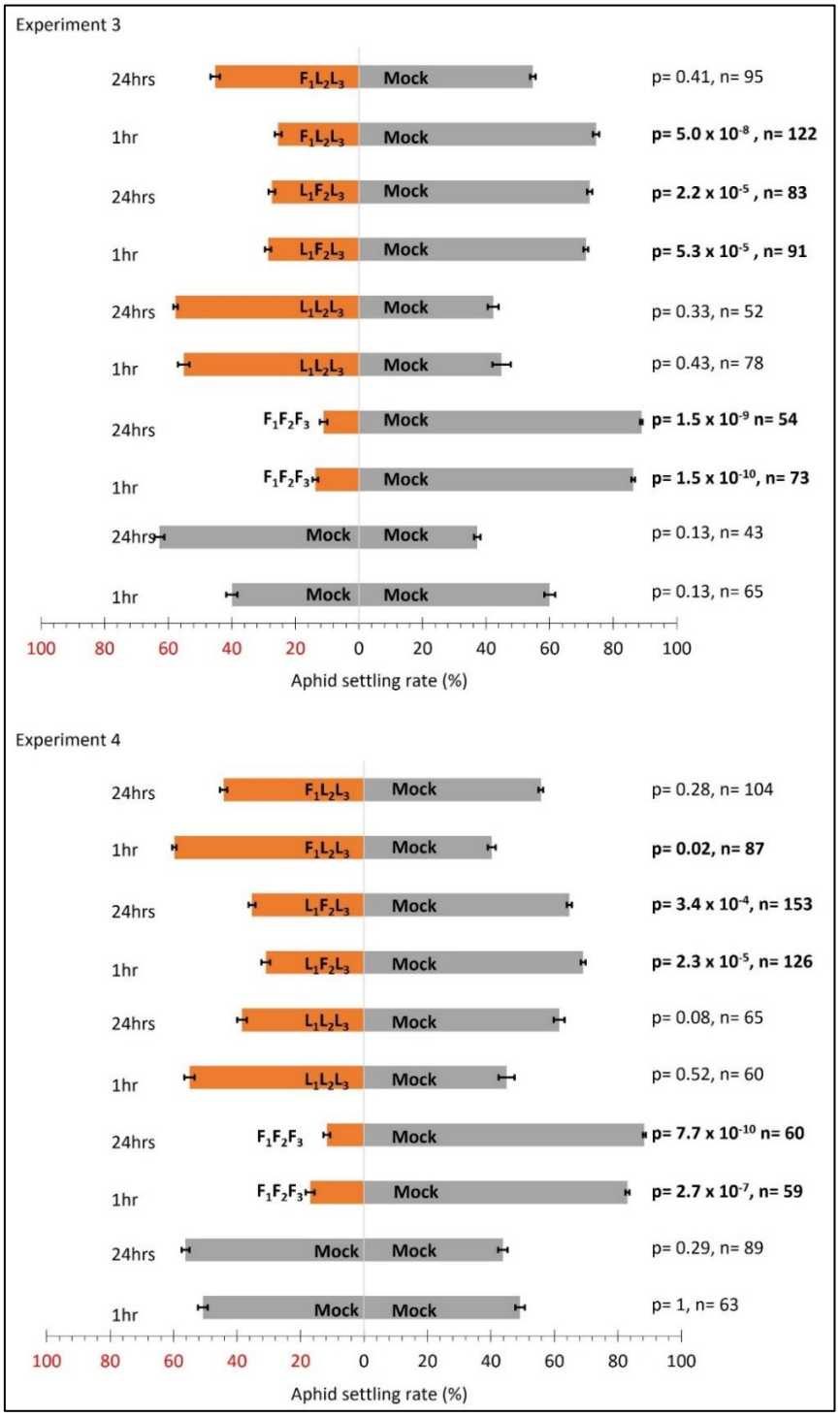
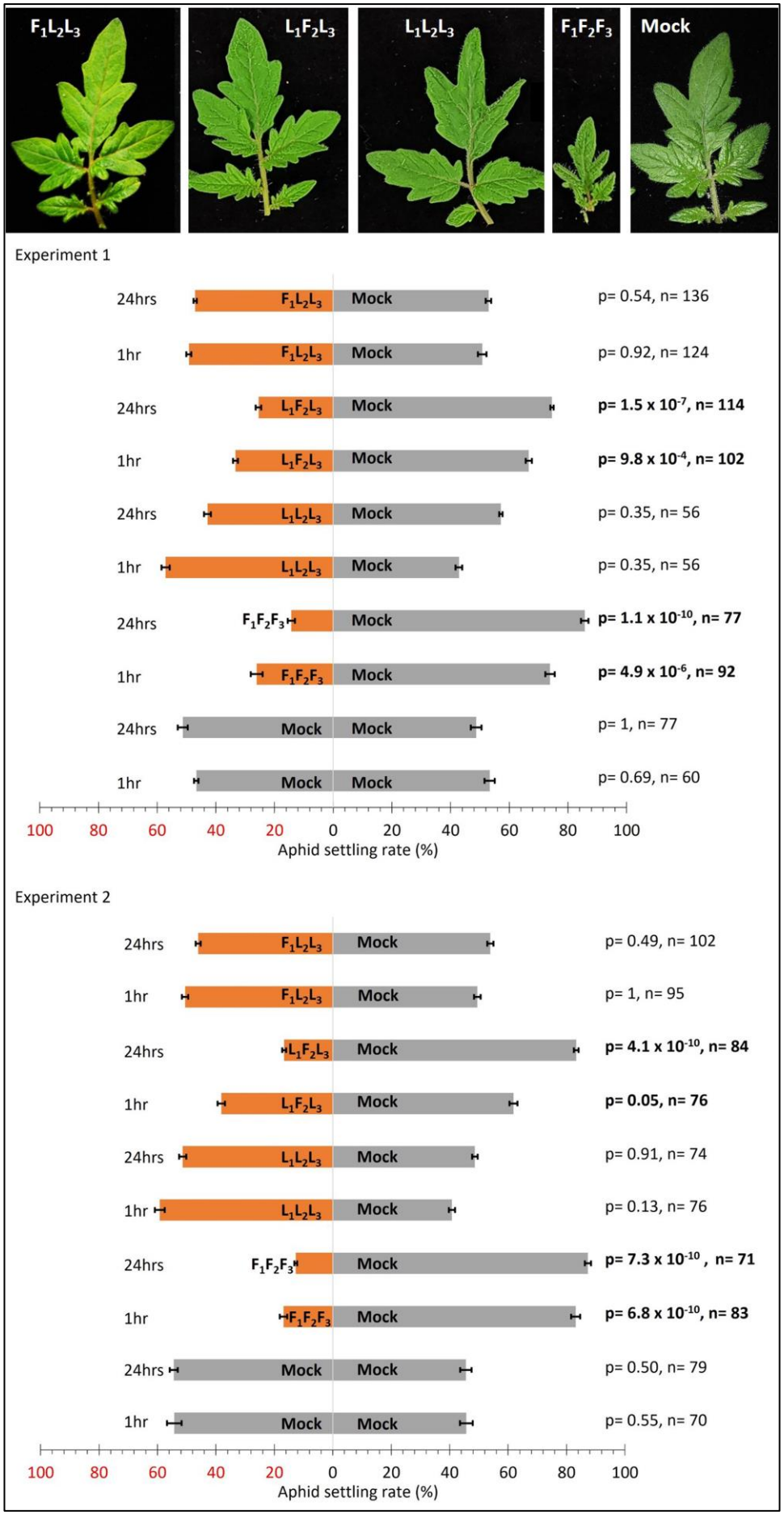


Figure 5.6. *Myzus persicae* settling on tomato plants infected with pseudorecombinant viruses, $F_1L_2L_3$ and $L_1F_2L_3$. A settling assay was used to determine aphid settling preference(Section 2.8.1). Plot bars labelled Mock indicate plants inoculated with water, and $L_1L_2L_3$ and $F_1F_2F_3$ represent plants infected with reconstituted LS-CMV and Fny-CMV viruses. The two wild-type viruses were used as controls against which the changes in aphid responses in plants infected with pseudorecombinant virus, $F_1L_2L_3$ and $L_1F_2L_3$, were compared. In each treatment comparison, aphid choices were recorded at 1 and 24 hours post-release in four independent experiments (1-4).Error bars represent SEM. The p-values indicate outcomes of a binomial test of statistical significance at $\alpha = 0.05$, and n represents the total number of aphids that made choices in each control/treatment comparison pair at each timepoint. Stacked bars with p-values in bold are statistically significant.


$F_1L_2L_3$
$L_1F_2L_3$
$L_1L_2L_3$
$F_1F_2F_3$
Mock
Experiment 1
24hrs $F_1L_2L_3$ Mock p= 0.54, n= 136
1hr $F_1L_2L_3$ Mock p= 0.92, n= 124
24hrs $L_1F_2L_3$ Mock p= 1.5 x 10-7, n= 114
1hr $L_1F_2L_3$ Mock p= 9.8 x 10-4, n= 102
24hrs $L_1L_2L_3$ Mock p= 0.35, n= 56
1hr $L_1L_2L_3$ Mock p= 0.35, n= 56
24hrs $F_1F_2F_3$ Mock p= 1.1 x 10-10, n= 77
1hr $F_1F_2F_3$ Mock p= 4.9 x 10-6, n= 92
24hrs Mock Mock p= 1, n= 77
1hr Mock Mock p= 0.69, n= 60
100 80 60 40 20 0 20 40 60 80 100
Aphid settling rate (%)
Experiment 2
24hrs $F_1L_2L_3$ Mock p= 0.49, n= 102
1hr $F_1L_2L_3$ Mock p= 1, n= 95
24hrs $L_1F_2L_3$ Mock p= 4.1 x 10-10, n= 84
1hr $L_1F_2L_3$ Mock p= 0.05, n= 76
24hrs $L_1L_2L_3$ Mock p= 0.91, n= 74
1hr $L_1L_2L_3$ Mock p= 0.13, n= 76
24hrs $F_1F_2F_3$ Mock p= 7.3 x 10-10 , n= 71
1hr $F_1F_2F_3$ Mock p= 6.8 x 10-10, n= 83
24hrs Mock Mock p= 0.50, n= 79
1hr Mock Mock p= 0.55, n= 70
100 80 60 40 20 0 20 40 60 80 100
Aphid settling rate (%)

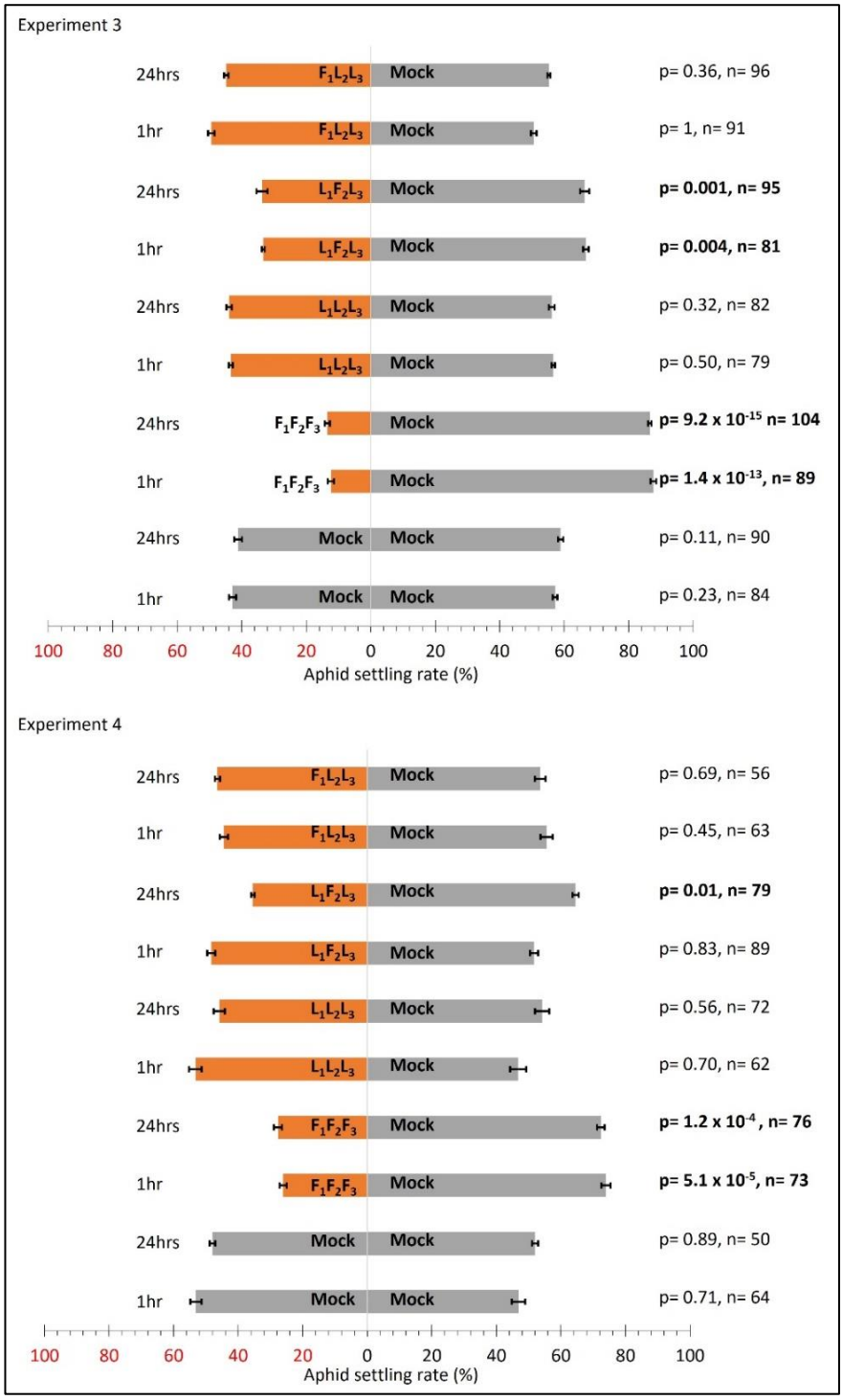
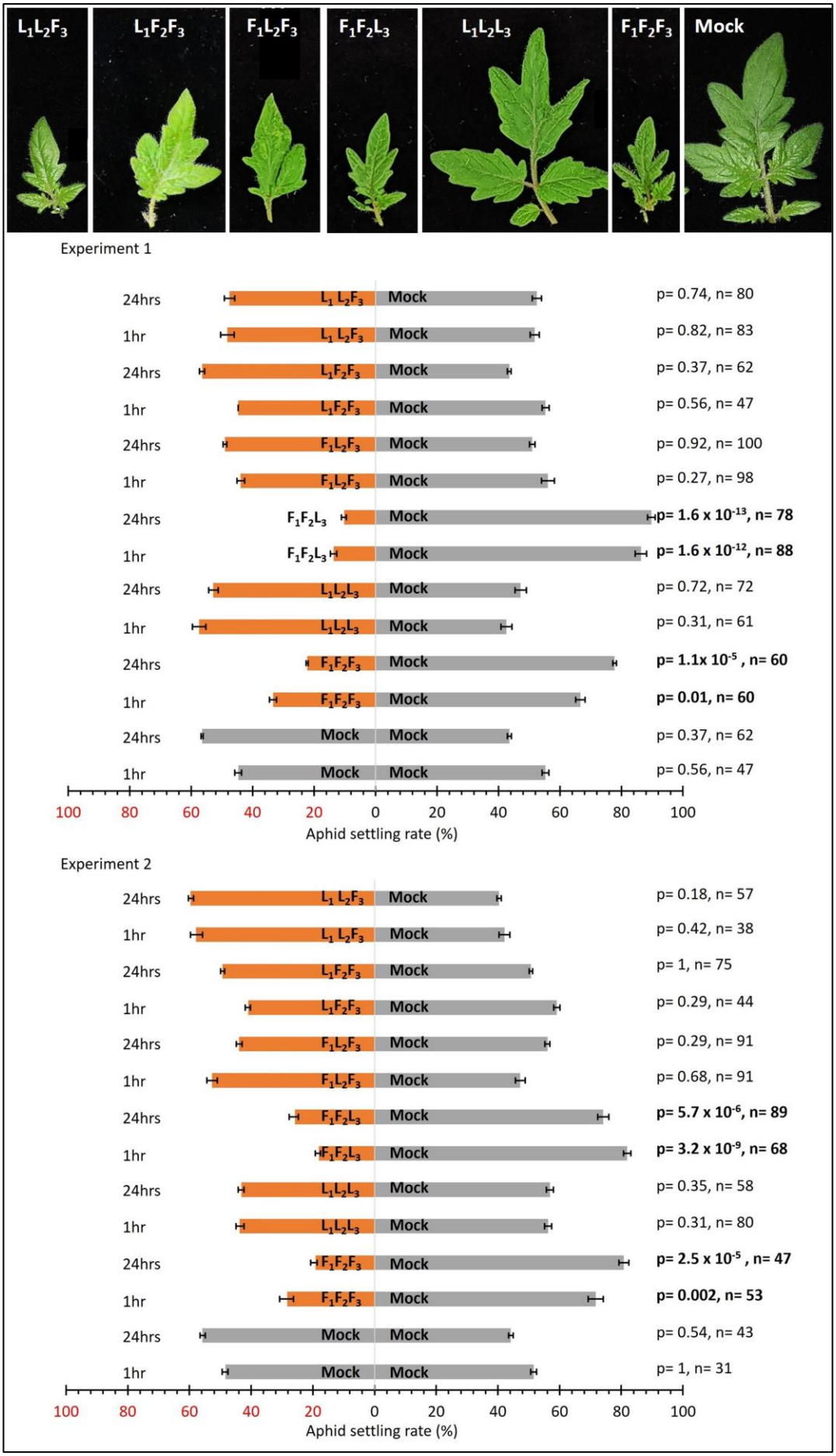


Figure 5.7. Effects of $F_1L_2L_3$ and $L_1F_2L_3$ pseudorecombinant viruses on settling properties of *Macrosiphum euphorbiae*. A settling assay was used to determine aphid settling preference. Plot bars labelled Mock indicate tomato plants inoculated with water. $L_1L_2L_3$ and $F_1F_2F_3$ represent plants infected with reconstituted wild-type LS-CMV and Fny-CMV viruses. The wild-type viruses were used as virus controls against which the changes in *Macrosiphum euphorbiae* responses in plants infected with pseudorecombinant virus, $F_1L_2L_3$ and $L_1F_2L_3$, were compared. In each treatment comparison, aphid choices were recorded at 1 and 24 hours post-release in four independent experiments (1-4). Error bars represent SEM. The p-values indicate outcomes of a binomial test of statistical significance at α =0.05, and n represents the total number of aphids that made choices in each control/treatment comparison pair at each timepoint. Stacked bars with p-values in bold are statistically significant.


$L_1L_2F_3$
$L_1F_2F_3$
$F_1L_2F_3$
$F_1F_2L_3$
$L_1L_2L_3$
$F_1F_2F_3$
Mock
Experiment 1
24hrs $L_1L_2F_3$ Mock p= 0.74, n= 80
1hr $L_1L_2F_3$ Mock p= 0.82, n= 83
24hrs $L_1F_2F_3$ Mock p= 0.37, n= 62
1hr $L_1F_2F_3$ Mock p= 0.56, n= 47
24hrs $F_1L_2F_3$ Mock p= 0.92, n= 100
1hr $F_1L_2F_3$ Mock p= 0.27, n= 98
24hrs $F_1F_2L_3$ Mock p= 1.6 x 10^{-13}, n= 78
1hr $F_1F_2L_3$ Mock p= 1.6 x 10^{-12}, n= 88
24hrs $L_1L_2L_3$ Mock p= 0.72, n= 72
1hr $L_1L_2L_3$ Mock p= 0.31, n= 61
24hrs $F_1F_2F_3$ Mock p= 1.1x 10^{-5} , n= 60
1hr $F_1F_2F_3$ Mock p= 0.01, n= 60
24hrs Mock Mock p= 0.37, n= 62
1hr Mock Mock p= 0.56, n= 47
100 80 60 40 20 0 20 40 60 80 100
Aphid settling rate (%)
Experiment 2
24hrs $L_1L_2F_3$ Mock p= 0.18, n= 57
1hr $L_1L_2F_3$ Mock p= 0.42, n= 38
24hrs $L_1F_2F_3$ Mock p= 1, n= 75
1hr $L_1F_2F_3$ Mock p= 0.29, n= 44
24hrs $F_1L_2F_3$ Mock p= 0.29, n= 91
1hr $F_1L_2F_3$ Mock p= 0.68, n= 91
24hrs $F_1F_2L_3$ Mock p= 5.7 x 10^{-6}, n= 89
1hr $F_1F_2L_3$ Mock p= 3.2 x 10^{-9}, n= 68
24hrs $L_1L_2L_3$ Mock p= 0.35, n= 58
1hr $L_1L_2L_3$ Mock p= 0.31, n= 80
24hrs $F_1F_2F_3$ Mock p= 2.5 x 10^{-5} , n= 47
1hr $F_1F_2F_3$ Mock p= 0.002, n= 53
24hrs Mock Mock p= 0.54, n= 43
1hr Mock Mock p= 1, n= 31
100 80 60 40 20 0 20 40 60 80 100
Aphid settling rate (%)

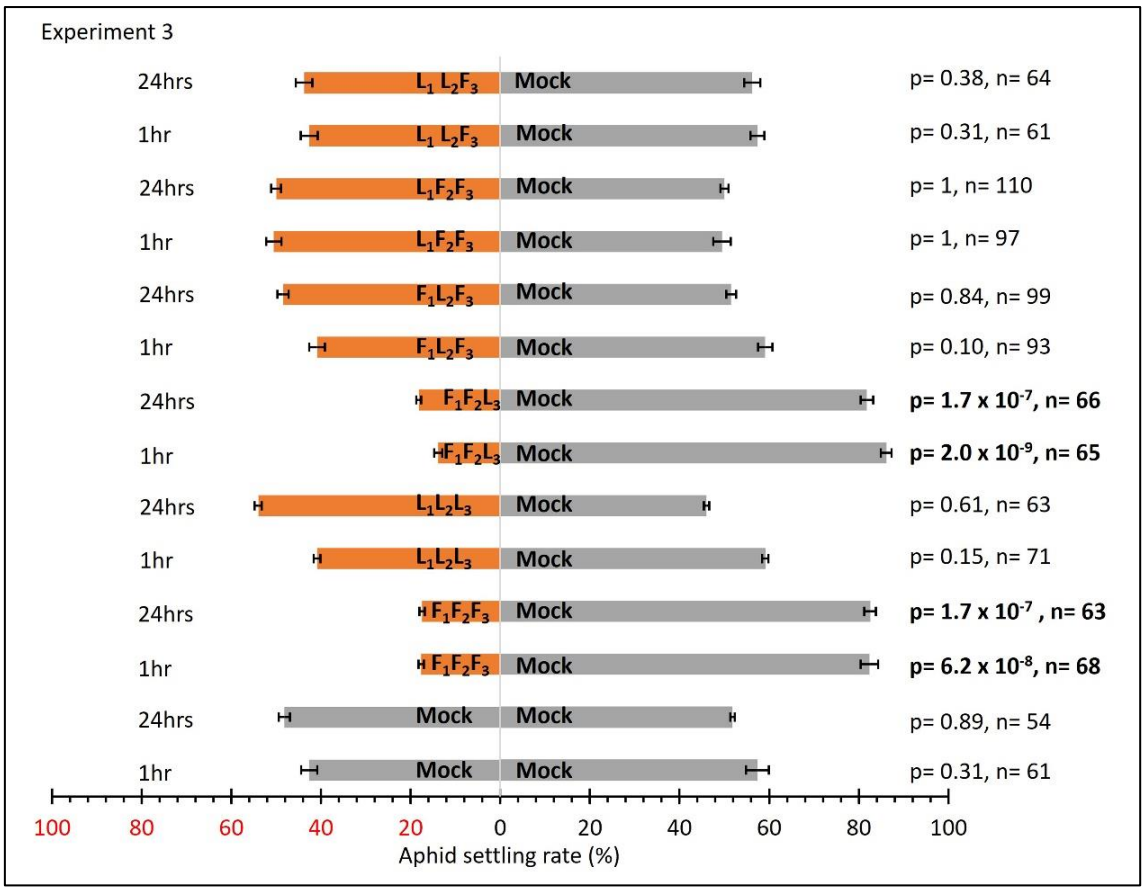
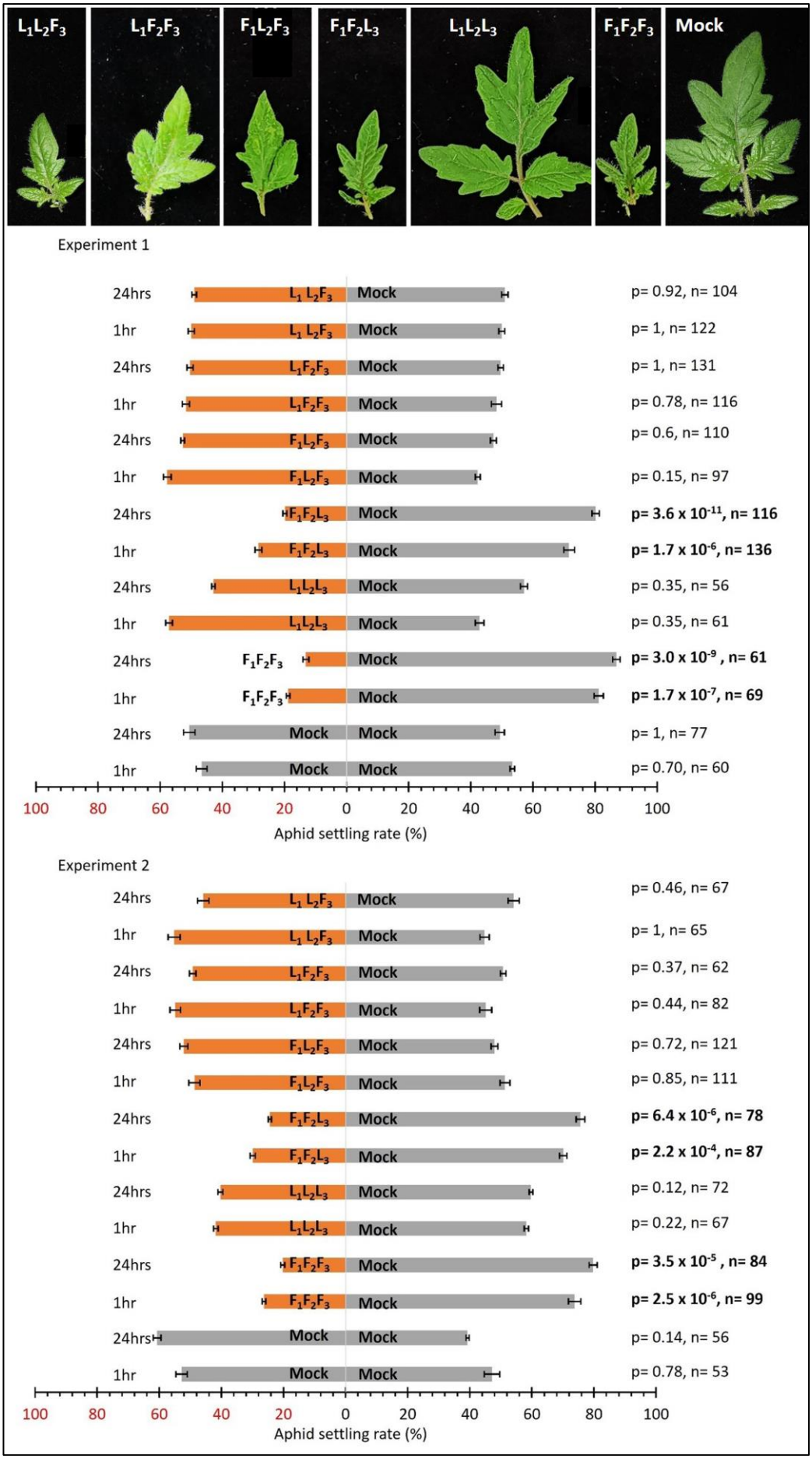


Figure 5.8. *Myzus persicae* preferred not to settle on tomato plants infected with $F_1F_2L_3$. Aphid settling preference was examined in a settling bioassay (Section 2.8.1). Plot bars labelled Mock indicate plants inoculated with water, and $L_1L_2L_3$ and $F_1F_2F_3$ labels represent plants infected with reconstituted wild-type LS-CMV and Fny-CMV viruses, which were used as controls against which changes in aphid responses induced by different pseudorecombinant viruses could be compared. $L_1L_2F_3$, $L_1F_2F_3$ $F_1L_2F_3$ and $F_1F_2L_3$ indicate pseudorecombinant viruses used to investigate aphid settling responses at 1 and 24 hours post-release in three independent experiments (1-3). Error bars represent SEM. The p-values indicate outcomes of a binomial test of statistical significance at α= 0.05, and n represents the total number of aphids that made choices per treatment pair. The p-values in bold indicate differences in aphid settlement that are statistically significant.


$L_1L_2F_3$
$L_1F_2F_3$
$F_1L_2F_3$
$F_1F_2L_3$
$L_1L_2L_3$
$F_1F_2F_3$
Mock
Experiment 1
24hrs $L_1L_2F_3$ Mock p= 0.92, n= 104
1hr $L_1L_2F_3$ Mock p= 1, n= 122
24hrs $L_1F_2F_3$ Mock p= 1, n= 131
1hr $L_1F_2F_3$ Mock p= 0.78, n= 116
24hrs $F_1L_2F_3$ Mock p= 0.6, n= 110
1hr $F_1L_2F_3$ Mock p= 0.15, n= 97
24hrs $F_1F_2L_3$ Mock p= 3.6 x 10-11, n= 116
1hr $F_1F_2L_3$ Mock p= 1.7 x 10-6, n= 136
24hrs $L_1L_2L_3$ Mock p= 0.35, n= 56
1hr $L_1L_2L_3$ Mock p= 0.35, n= 61
24hrs $F_1F_2F_3$ Mock p= 3.0 x 10-9 , n= 61
1hr $F_1F_2F_3$ Mock p= 1.7 x 10-7, n= 69
24hrs Mock Mock p= 1, n= 77
1hr Mock Mock p= 0.70, n= 60
100 80 60 40 20 0 20 40 60 80 100
Aphid settling rate (%)
Experiment 2
24hrs $L_1L_2F_3$ Mock p= 0.46, n= 67
1hr $L_1L_2F_3$ Mock p= 1, n= 65
24hrs $L_1F_2F_3$ Mock p= 0.37, n= 62
1hr $L_1F_2F_3$ Mock p= 0.44, n= 82
24hrs $F_1L_2F_3$ Mock p= 0.72, n= 121
1hr $F_1L_2F_3$ Mock p= 0.85, n= 111
24hrs $F_1F_2L_3$ Mock p= 6.4 x 10-6, n= 78
1hr $F_1F_2L_3$ Mock p= 2.2 x 10-4, n= 87
24hrs $L_1L_2L_3$ Mock p= 0.12, n= 72
1hr $L_1L_2L_3$ Mock p= 0.22, n= 67
24hrs $F_1F_2F_3$ Mock p= 3.5 x 10-5 , n= 84
1hr $F_1F_2F_3$ Mock p= 2.5 x 10-6, n= 99
24hrs Mock Mock p= 0.14, n= 56
1hr Mock Mock p= 0.78, n= 53
100 80 60 40 20 0 20 40 60 80 100
Aphid settling rate (%)

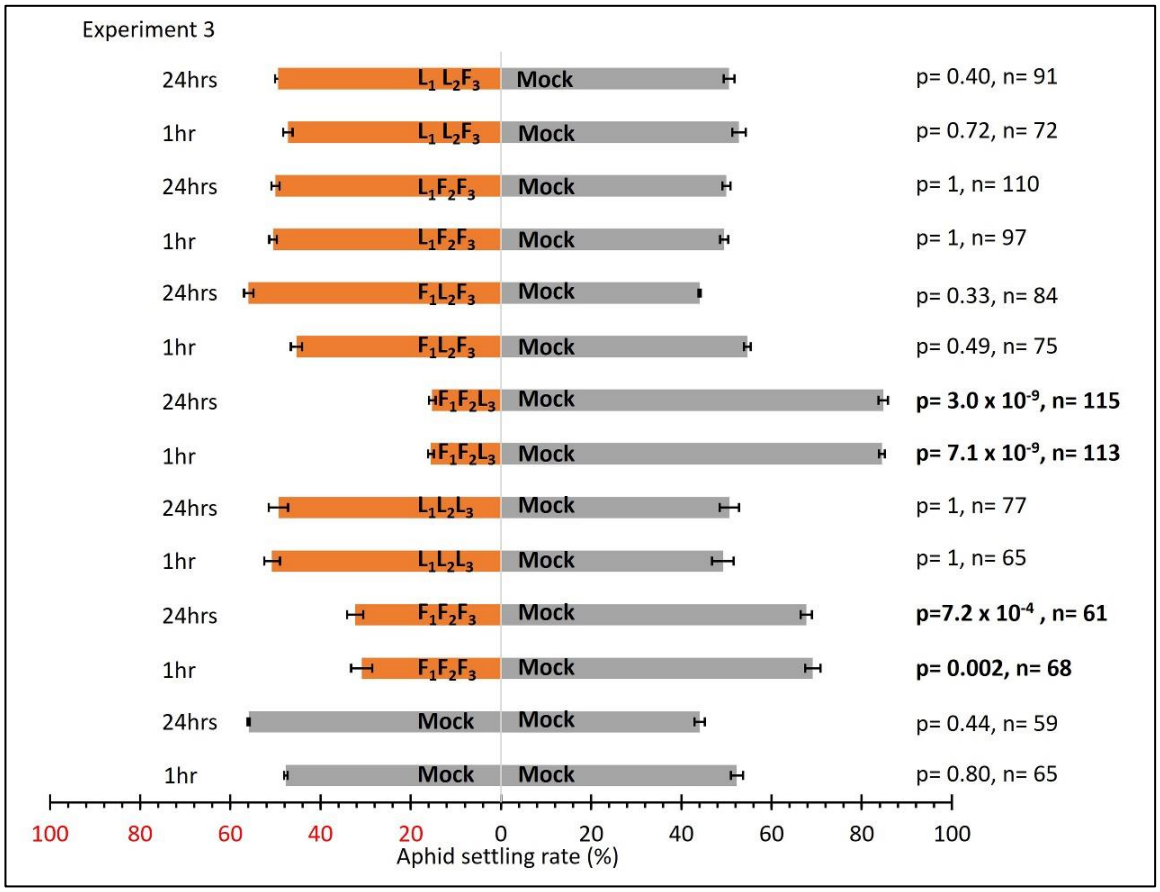


Figure 5.9. Fewer aphids (*Macrosiphum euphorbiae*) preferred to settle on tomato plants infected with $F_1F_2L_3$. Aphid settling preference was examined in a settling bioassay. Plot bars labelled Mock indicates plants inoculated with water. $L_1L_2L_3$ and $F_1F_2F_3$ represent plants infected with reconstituted LS-CMV and Fny-CMV viruses used as viral controls against which changes in aphid responses induced by different pseudorecombinant viruses were measured. $L_1L_2F_3$, $L_1F_2F_3$ $F_1L_2F_3$ and $F_1F_2L_3$ show pseudorecombinant viruses used to investigate aphid settling responses at 1hour and 24 hours post-release at four independent experiments (1-3). Error bars represent SEM. The p-values indicate outcomes of a binomial test of statistical significance at $\alpha = 0.05$. n represents the total number of aphids that made choices per treatment pair. The p-values in bold indicate aphid responses that are statistically significant.

Table 5.1. Summary of the effects of wild-type LS-CMV and Fny-CMV and pseudorecombinant viruses on aphid settlement on tomato based on data in Figures 5.6 -5.9

Virus	Experiments/total experiments in which aphid settlement was inhibited on virus-infected plants			
	Myzus persicae		*Macrosiphum euphorbiae*	
	1h post-release	24h post-release	1h post-release	24h post-release
Fny-CMV ($F_1F_2F_3$)	7/7	7/7	7/7	7/7
LS-CMV ($L_1L_2L_3$)	0/7	0/7	0/7	0/7
$L_1F_2L_3$	4/4	4/4	3/4	3/4
$F_1L_2L_3$	3/4*	0/4	0/4	0/4
$F_1F_2L_3$	3/3	3/3	3/3	3/3
$F_1L_2F_3$	0/3	0/3	0/3	0/3
$L_1F_2F_3$	0/3	0/3	0/3	0/3
$L_1L_2F_3$	0/3	0/3	0/3	0/3

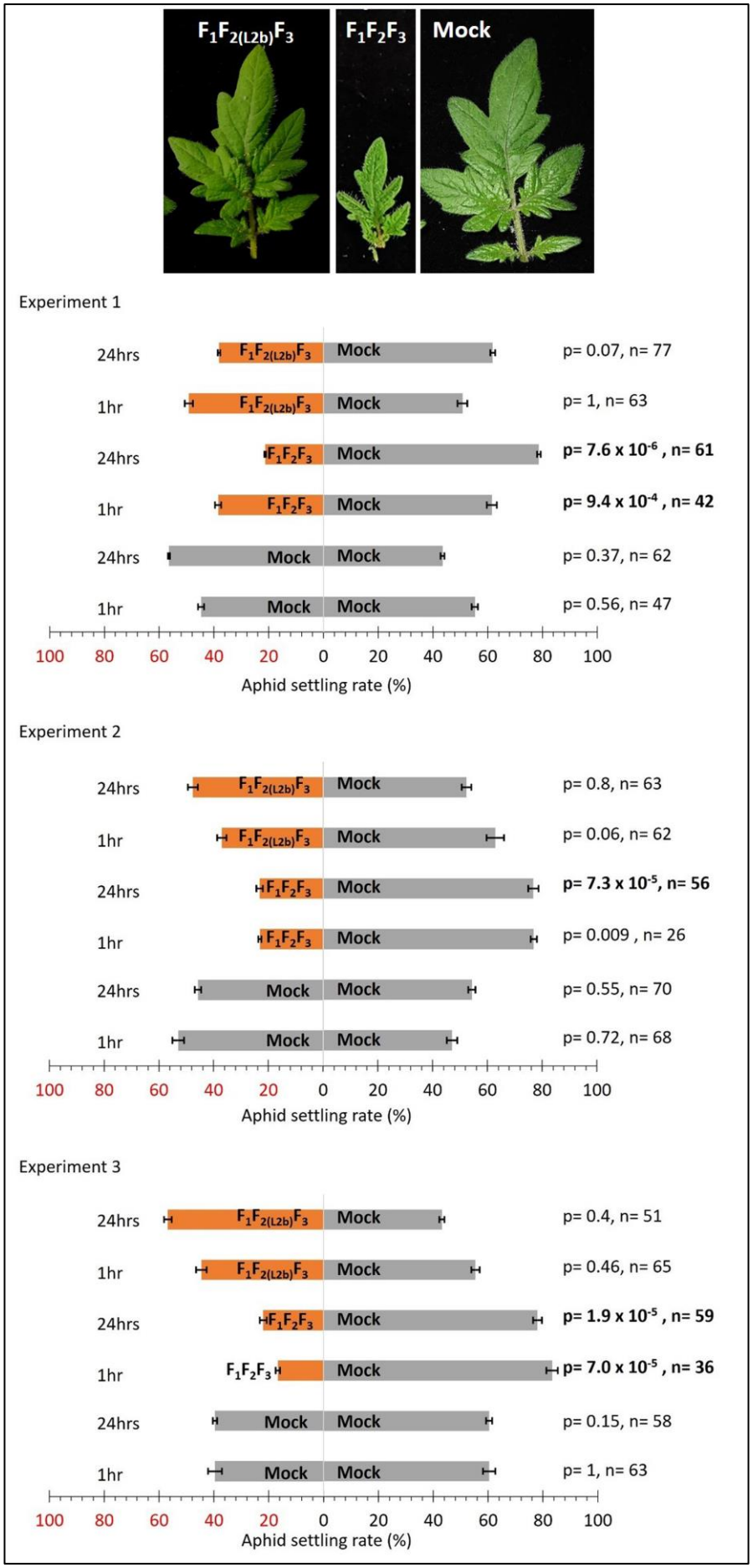
* In the case of pseudorecombinant virus $F_1L_2L_3$, *Myzus persicae* settlement on infected plants was higher than on mock-inoculated plants at 1 h post-release in three out of four experiments.

similar numbers on mock-inoculated plants and on plants infected with $F_1L_2L_3$ (Fig. 5.6). In contrast, the pseudorecombinant virus $F_1L_2L_3$ had no significant effect on the settling behaviour of aphids of *Macrosiphum euphorbiae* when allowed to choose between plants infected with this virus and mock-inoculated tomato plants (Fig. 5.7).

5.2.3 The 2b protein appears to be a major determinant of the deterrence to aphid settlement on tomato plants infected with Fny-CMV

Since the results described in Section 5.2.2 pointed to the likelihood that one or both of the proteins encoded by CMV RNA2 determines aphid-tomato interactions, I mixed a recombinant RNA 2 [$F_{2(L2b)}$] (Pita & Roossinck, 2013), which encodes the Fny-CMV 2a protein and the LS-CMV 2b protein, with wild type Fny-CMV RNAs 1 and 3 to constitute the recombinant CMV, $F_1F_{2(L2b)}F_3$. Aphid settling preferences on tomato plants infected with the CMV recombinant were investigated using free choice assays (Table 5.2; Fig. 5.10 & Fig. 5.11). In five independent experiments, *Myzus persicae* aphids did not show any significant differences in settling on tomato plants infected with $F_1F_{2(L2b)}F_3$ versus mock-inoculated plants at either 1 or 24 hours post-release (Fig. 5.10). In assays using *Macrosiphum euphorbiae*, these aphids did not initially show any bias for settling on plants that had been mock-inoculated or infected with $F_1F_{2(L2b)}F_3$ (Fig. 5.11). However, by 24 hours post-release, in four out of five experiments, most aphids of *Macrosiphum euphorbiae* had migrated away from plants infected with $F_1F_{2(L2b)}F_3$ and had settled on mock-inoculated plants (Table 5.2; Fig. 5.11).

To further explore the effects of the Fny-CMV 2b protein on aphid behaviour in tomato, I used the *2b* gene deletion mutant virus, Fny-CMVΔ2b (Soards et al., 2002), which cannot express the 2b protein, to test the settling preference of *Myzus persicae*. I recorded the proportion of aphids settling in three different treatment combinations: Fny-CMV versus Fny-CMVΔ2b; mock versus Fny-CMVΔ2b, and mock versus mock (Fig. 5.12). In the Fny-CMV versus Fny-CMVΔ2b experiment, aphids markedly preferred to settle on Fny-CMVΔ2b-infected tomato plants than Fny-CMV-infected plants after one hour and 24 hours post-release. No preferential settling was observed in mock-inoculated versus Fny-CMVΔ2b-infected and mock-inoculated versus mock-inoculated treatment comparisons at both time points. The experiment was repeated thrice with similar results. Taken together, results from the recombinant virus experiment and Fny-CMVΔ2b mutant virus indicate that the 2b protein is a major determinant of the interaction between aphids and tomato plants infected with Fny-CMV.


$F_1F_{2(L2b)}F_3$
$F_1F_2F_3$
Mock
Experiment 1
24hrs $F_1F_{2(L2b)}F_3$ Mock p= 0.07, n= 77
1hr $F_1F_{2(L2b)}F_3$ Mock p= 1, n= 63
24hrs $F_1F_2F_3$ Mock p= 7.6 x 10-6 , n= 61
1hr $F_1F_2F_3$ Mock p= 9.4 x 10-4 , n= 42
24hrs Mock Mock p= 0.37, n= 62
1hr Mock Mock p= 0.56, n= 47
100 80 60 40 20 0 20 40 60 80 100
Aphid settling rate (%)
Experiment 2
24hrs $F_1F_{2(L2b)}F_3$ Mock p= 0.8, n= 63
1hr $F_1F_{2(L2b)}F_3$ Mock p= 0.06, n= 62
24hrs $F_1F_2F_3$ Mock p= 7.3 x 10-5, n= 56
1hr $F_1F_2F_3$ Mock p= 0.009 , n= 26
24hrs Mock Mock p= 0.55, n= 70
1hr Mock Mock p= 0.72, n= 68
100 80 60 40 20 0 20 40 60 80 100
Aphid settling rate (%)
Experiment 3
24hrs $F_1F_{2(L2b)}F_3$ Mock p= 0.4, n= 51
1hr $F_1F_{2(L2b)}F_3$ Mock p= 0.46, n= 65
24hrs $F_1F_2F_3$ Mock p= 1.9 x 10-5 , n= 59
1hr $F_1F_2F_3$ Mock p= 7.0 x 10-5 , n= 36
24hrs Mock Mock p= 0.15, n= 58
1hr Mock Mock p= 1, n= 63
100 80 60 40 20 0 20 40 60 80 100
Aphid settling rate (%)

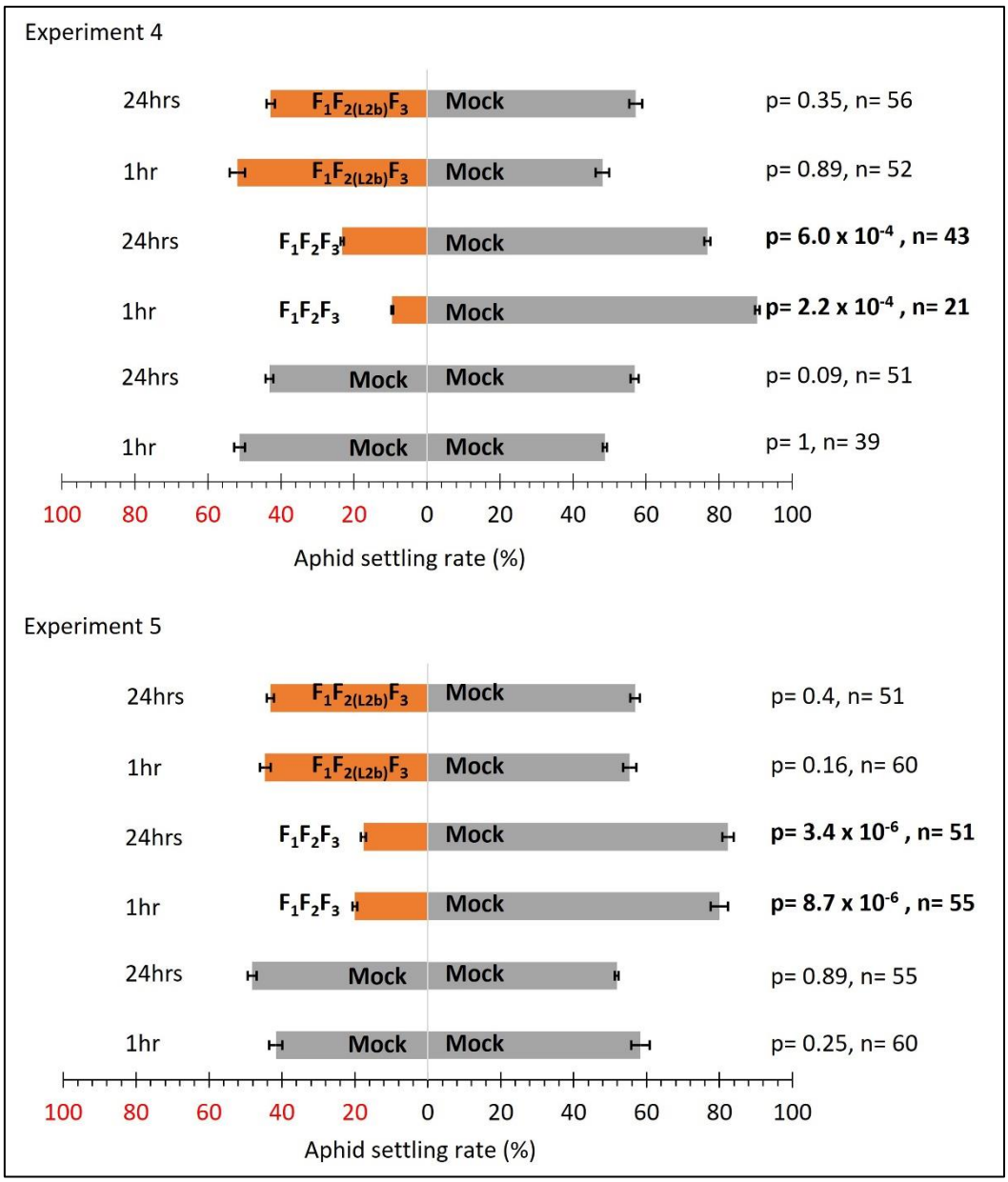
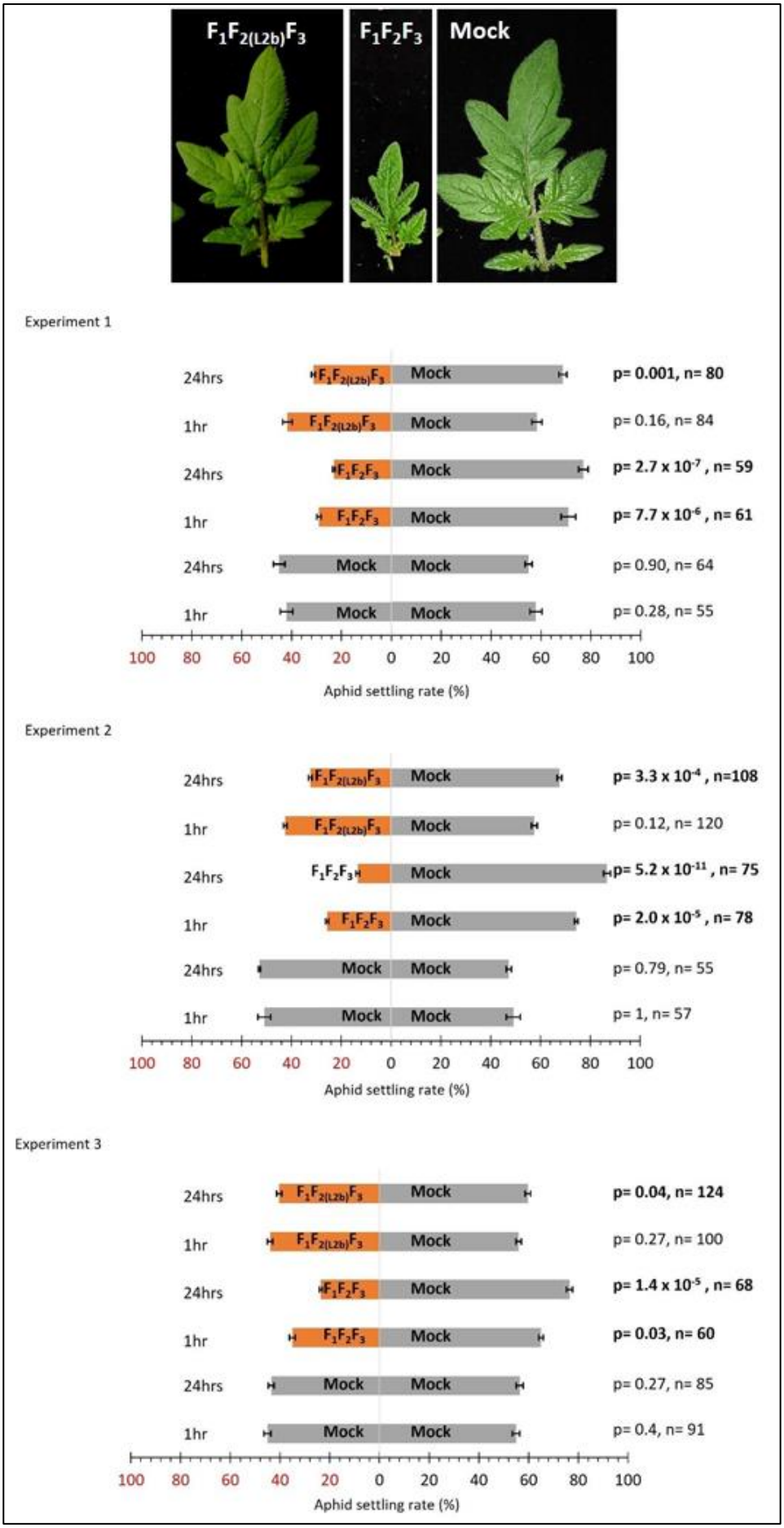


Figure 5.10. A recombinant CMV virus possessing the LS-CMV 2b coding sequence does not induce repellence in tomato plants against *Myzus persicae*. A settling assay was used to measure aphid settling preference. Plot bars labelled Mock indicate tomato plants inoculated with water. $F_1F_2F_3$ and $F_1F_{2(L2b)}F_3$ labels represent plants infected with the Fny-CMV virus and recombinant CMV. Aphid settling responses were recorded in five independent experiments at two-time intervals, 1 h and 24 h. Aphid settling rate on the x-axis was computed as a proportion of aphids that chose a particular plant in a given control or treatment pair of plants. n is the total number of aphids that made choices. The error bars indicate SEM. The p-values indicate a binomial test at α= 0.05, and values in bold indicate significantly different settling rates.


F1F2(L2b)F3
F1F2F3
Mock
Experiment 1
24hrs
F1F2(L2b)F3
Mock
p= 0.001, n= 80
1hr
F1F2(L2b)F3
Mock
p= 0.16, n= 84
24hrs
F1F2F3
Mock
p= 2.7 x 10-7 , n= 59
1hr
F1F2F3
Mock
p= 7.7 x 10-6 , n= 61
24hrs
Mock
Mock
p= 0.90, n= 64
1hr
Mock
Mock
p= 0.28, n= 55
100 80 60 40 20 0 20 40 60 80 100
Aphid settling rate (%)
Experiment 2
24hrs
F1F2(L2b)F3
Mock
p= 3.3 x 10-4 , n=108
1hr
F1F2(L2b)F3
Mock
p= 0.12, n= 120
24hrs
F1F2F3
Mock
p= 5.2 x 10-11 , n= 75
1hr
F1F2F3
Mock
p= 2.0 x 10-5 , n= 78
24hrs
Mock
Mock
p= 0.79, n= 55
1hr
Mock
Mock
p= 1, n= 57
100 80 60 40 20 0 20 40 60 80 100
Aphid settling rate (%)
Experiment 3
24hrs
F1F2(L2b)F3
Mock
p= 0.04, n= 124
1hr
F1F2(L2b)F3
Mock
p= 0.27, n= 100
24hrs
F1F2F3
Mock
p= 1.4 x 10-5 , n= 68
1hr
F1F2F3
Mock
p= 0.03, n= 60
24hrs
Mock
Mock
p= 0.27, n= 85
1hr
Mock
Mock
p= 0.4, n= 91
100 80 60 40 20 0 20 40 60 80 100
Aphid settling rate (%)

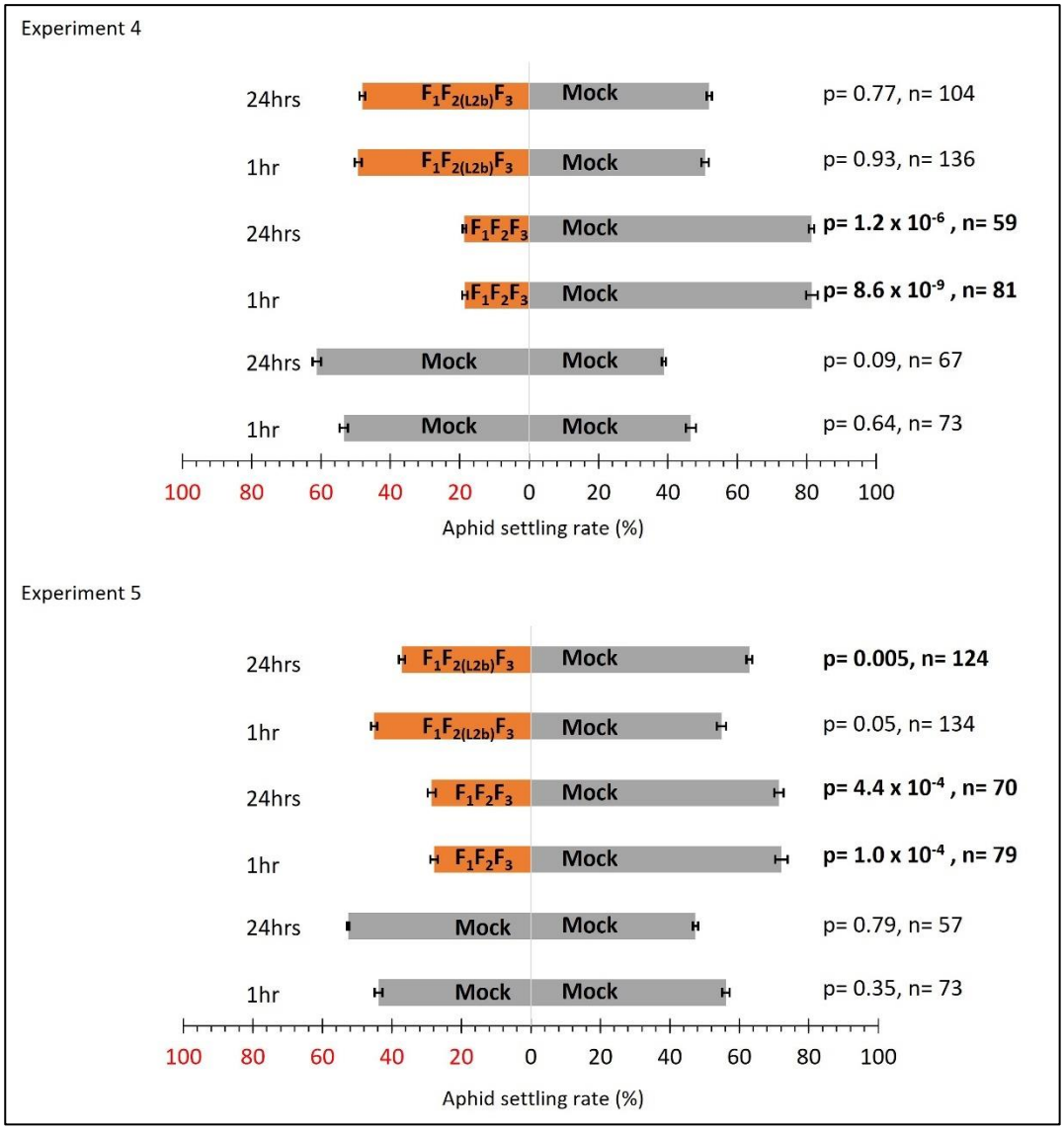


Figure 5.11. Investigation of the role of the 2b protein in the induction of repellence to *Macrosiphum euphorbiae* settlement. A settling bioassay was used to determine aphid settling preference behaviour. Plot bars labelled Mock indicate tomato plants inoculated with water. $F_1F_2F_3$ and $F_1F_{2(L2b)}F_3$ labels represent plants infected with Fny-CMV virus and the recombinant version of Fny-CMV. Aphid settling responses were recorded in five independent experiments at two-time intervals, 1 and 24 hours post-release. Aphid settling rate on the x-axis was computed as a proportion of aphids that chose a particular plant in a given control or treatment pair of plants. n is the total number of aphids that made choices. The error bars indicate SEM. The p-values indicate a binomial test at α= 0.05, and values in bold indicate significantly different settling rates. More aphids showed a bias towards mock-inoculated plants compared to plants infected with a recombinant virus. By and large, the strength of repellent effects of $F_1F_{2(L2b)}F_3$ against *Macrosiphum euphorbiae* is much lower compared to that of $F_1F_2F_3$.

Table 5.2. Summary of the effects of wild-type Fny-CMV compared with those of the recombinant virus $F_1F_{2(L2b)}F_3$ on settlement by a generalist (*Myzus persicae*) and a specialist (*Macrosiphum euphorbiae*) aphid on tomato based on data in Figures 5.10 and 5.11

Virus	Experiments/total experiments in which aphid settlement was inhibited			
	Myzus persicae		*Macrosiphum euphorbiae*	
	1h post-release	24h post-release	1h post-release	24h post-release
Fny-CMV ($F_1F_2F_3$)	5/5	5/5	5/5	5/5
$F_1F_{2(L2b)}F_3$	0/5	0/5	0/5	4/5

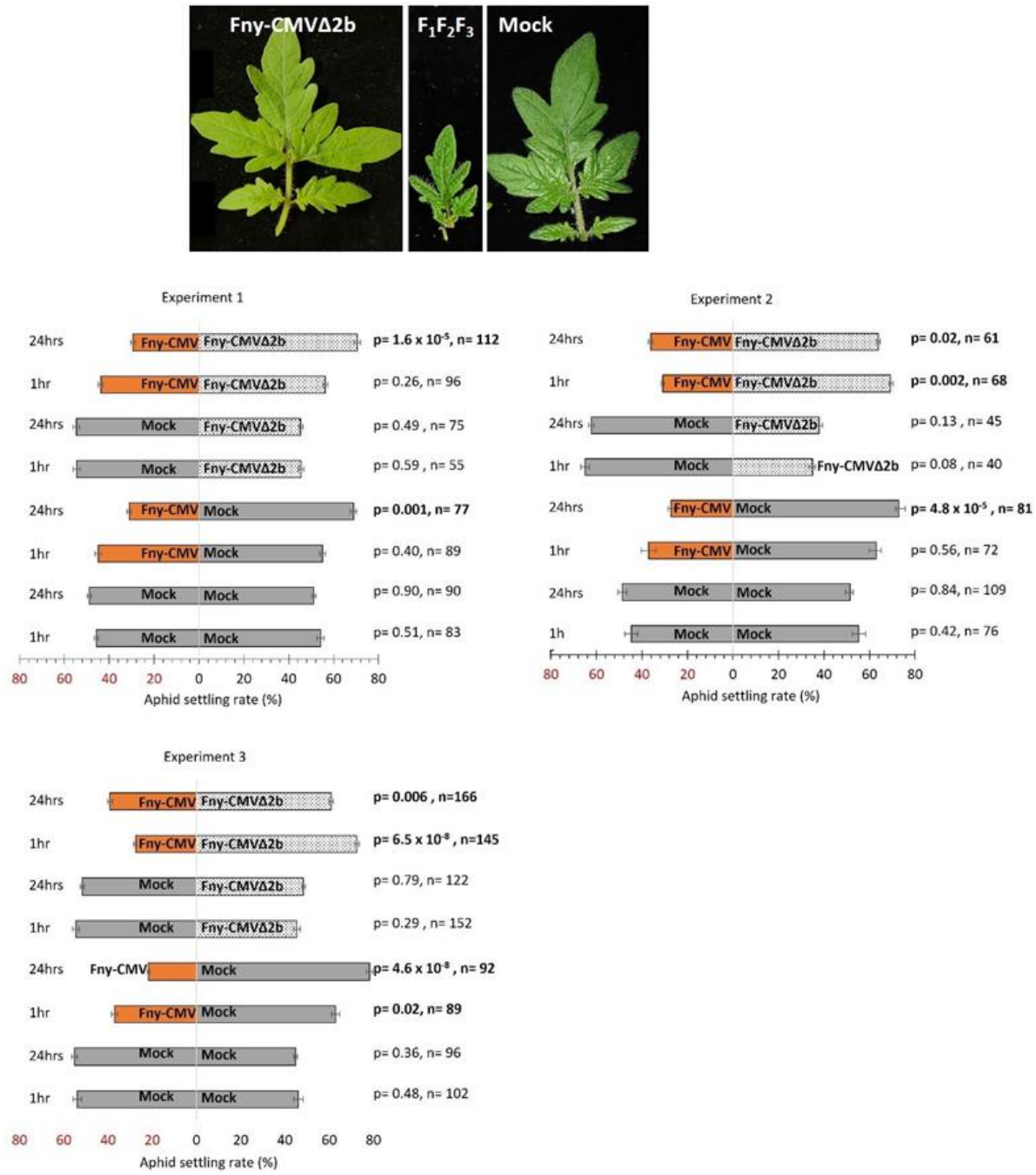


Figure 5.12. ***Myzus persicae*** **preferred to settle on tomato plants infected with Fny-CMVΔ2b-infected than plants infected with Fny-CMV**. In all three settling assays performed, aphids consistently chose to settle on Fny-CMVΔ2b-infected plants compared to Fny-CMV-infected plants for a long period. Probability (*p*) values show outcomes of binomial tests, and n represents the total number of settled aphids per treatment pair. The p-values in bold indicate significantly different settling rates. The error bars represent SEM.

5.3 Discussion

In Arabidopsis and tobacco, as well as in common bean, infection with CMV influences aphid-plant interactions in ways that may affect virus transmission by aphids, sometimes by inhibiting aphid settlement on infected plants (Rhee et al., 2020; Tungadi et al., 2020; Wamonje et al., 2020; Watt et al., 2020; Westwood et al., 2013). I have shown that this is also true for Fny-CMV in tomato (see Chapters 3 and 4 of this thesis). It was found that the Subgroup II CMV strain LS had no effect on interactions of tomato plants with aphids, but that aphid settlement on plants infected with the Subgroup IA strain Fny-CMV was significantly inhibited. These strain differences were exploited to identify which viral gene product was the major determinant of tomato plant repellency to aphids. The main finding of the work described in this chapter was that the CMV 2b protein has a major role in controlling the repellence to settlement by the aphids *Myzus persicae* (a polyphagous generalist) and *Macrosiphum euphorbiae* (a specialist on Solanaceous hosts) on tomato plants infected with Fny-CMV. I further demonstrated that viral titre might play an important role in the relationship between CMV, aphids and tomato plants.

Tomato plants infected with $L_1F_2L_3$ and $F_1F_2L_3$, were repellent to aphids of both species. This suggested that one or both of the RNA2-encoded proteins, 2a or 2b, were involved in modifying the interaction between tomato and members of the two aphid species. To resolve whether one of both proteins were influencing aphid-tomato interactions, I carried out experiments with the RNA2 recombinant virus, $F_1F_{2(L2b)}F_3$, in which the Fny-CMV 2b protein sequence has been replaced by the orthologous sequence from LS-CMV, and with the deletion mutant CMVΔ2b, which cannot express the 2b protein. Both these sets of experiments suggest that the 2b protein of Fny-CMV strain, but not the 2a protein, influences aphid-tomato plant interactions. However, the results indicate that the Fny-CMV 2b protein exerts the greatest effects on the interaction of infected plants with the generalist aphid, *Myzus persicae*, since with the recombinant virus $F_1F_{2(L2b)}F_3$, in which the Fny-CMV 2b protein coding sequence has been replaced by that of LS-CMV, the ability of the virus to induce repellence is not apparent at either time point (1 and 24 hours post-release), and appears to have been abolished. However, the result of experiments with *Macrosiphum euphorbiae* and $F_1F_{2(L2b)}F_3$ suggest the possibility that the Fny-CMV 2b protein may not be the sole determinant of the repellence induced by Fny-CMV, which can only be confirmed in future when the behaviour of *Macrosiphum euphorbiae* on CMVΔ2b-infected plants is tested. Whilst this Solanaceous specialist aphid settles on mock-inoculated plants and plants infected by $F_1F_{2(L2b)}F_3$ with apparently equal facility at 1 hour post-release, by 24 hours post-placement, aphids of *Macrosiphum euphorbiae* show a significant preference (in four out of five experiments) to settle on mock-inoculated plants, rather than on plants infected with the recombinant virus. Thus, the repellence against aphid settlement induced by Fny-CMV may possibly involve more than one

mechanism (causing differential effects on generalist versus specialist aphids) and other CMV gene product(s) in addition to the 2b protein must play a role for the full effect to be manifested. Works in Arabidopsis and tobacco by previous members of the Carr group supports the hypothesis that multiple CMV gene products may contribute to tomato repellency against aphids. Westwood and colleagues (2013) showed the interaction between the 2b, 1a and 2a proteins discourage prolonged feeding of *Myzus persicae* on Arabidopsis plants infected with CMV, a behaviour that promotes aphid preference for mock-inoculated plants compared to virus-infected plants. Recently, experiments using site-directed mutagenesis to produce CMV recombinant viruses with specific amino acid residue exchanges between the 2a protein sequences of LS-CMV and Fny-CMV confirmed that a specific amino acid residue (valine) in the Fny-CMV 2a protein is a crucial inducer of antixenosis against aphids in CMV-infected Arabidopsis plants (Rhee et al., 2020). In tobacco, the CMV 2b protein inhibits 1a-induced resistance to *Myzus persicae* performance on CMV-infected plants (Tungadi et al., 2020). It is plausible that the 1a protein may be involved in CMV-aphid-tomato interactions. This hypothesis is partially supported by volatility in *Myzus persicae* preference in choice tests of $F_1L_2L_3$-infected plants versus mock-inoculated plants. More aphids had an initial preference for mock-inoculated plants over $F_1L_2L_3$-infected plants in two independent experiments, but this effect diminished after 24 hours post-release. Aphids markedly settled on $F_1L_2L_3$-infected plants in one experiment compared to mock-inoculated plants at 1 hour post-release. These variations in *Myzus persicae* settling preference may imply that the Fny-CMV 1a protein may be required during the initial induction of aphid resistance in tomato. Interestingly, plants infected with the $F_1L_2L_3$ did not alter *Macrosiphum euphorbiae* settling preference which may imply that, in tomato, the 1a protein contributes to repellence of specific aphid species, in this case, *Myzus persicae*. But, alternatively, it may suggest that in tomato *Myzus persicae* has a higher tolerance to CMV-induced repellent effect than *Macrosiphum euphorbiae*.

This study also showed that pseudorecombinant viruses that accumulated poorly in tomato (except $L_1F_2L_3$:Fig. 5.4) did not inhibit aphid settlement, suggesting a possible link between viral titre (and by extension viral gene dosage) and aphid settling behaviour. Aphids preferred to settle on tomato plants infected with $L_1F_2F_3$ and Fny-CMVΔ2b (Tab. 5.1 & Fig. 5.12). The titres of these viruses were significantly lower in infected tomato plants than the wild-type viruses (Fig. 5.4). This result agrees with Shi and colleagues (2016), who reported a relationship between viral titre and effects on *Myzus persicae* settling and reproductive behaviour on tobacco. Aphids settled in significantly higher numbers and reproduced better on mock-inoculated plants than on CMV-infected tobacco plants from 10-15 days post-inoculation when viral titre was high (Shi et al., 2016). It appears that CMV confers a reverse effect on aphid settling preference in tomato: a high viral tire increases host resistance against aphid settlement.

Chapter 6. Salicylic acid influences CMV-induced changes in aphid settling preference on tomato plants

6.1 Introduction

Aphid settling on tomato is affected when host plants are infected by CMV strain Fny (Chapter 3), and I have shown that the CMV 2b protein is a crucial determinant of CMV-induced changes in aphid-tomato interactions (Chapter 5). The defensive signal compound SA accumulates in plants infected with Fny-CMV (Lewsey et al., 2010; Xu et al., 2008; Zhou et al., 2014), and it is conceivable that it might influence aphid-tomato interactions. Shi et al. (2016) reported that in tobacco (cv. Samsun) high titres of CMV enhanced SA accumulation, which decreased *Myzus persicae* growth rate and survival. *NahG*-transgenic tobacco plants do not accumulate normal levels of SA because it is converted to catechol due to the constitutive expression of SA hydroxylase, which is encoded by the *NahG* transgene (Delaney et al., 1994). In this chapter, it was hypothesised that SA accumulation in tomato plants infected with Fny-CMV might influence the settling preference of two different aphid species, thus facilitating CMV transmission.

Using choice tests, the preferences of *Myzus persicae* and *Macrosiphum euphorbiae* for non-transgenic and *NahG*-transgenic tomato plants infected with Fny-CMV were examined. In different treatment combinations, the settling preference of seven-day-old aphids was recorded at 1 and 24 hours post-release. Contrary to previous choice tests (Chapter 3 and Chapter 5), tomato plants were used at 7 dpi instead of 9 dpi because beyond 7 dpi, *NahG*-transgenic tomato plants infected with Fny-CMV suddenly wilted (Fig. 6.2). For each aphid species, experiments with different treatment combinations were repeated 3 to 4 times.

6.2 Results

6.2.1 *NahG*-transgenic tomato plants infected with Fny-CMV showed an early dramatic disease phenotype

Ten days post sowing, *NahG*-transgenic and non-transgenic tomato plants (background: cv. Moneymaker) were inoculated with Milli-Q water (mock-inoculated) or with purified virions of Fny-CMV. Daily, plants were observed for development of systemic disease symptoms, which became visible between 4 dpi and 6 dpi. At 7 dpi, mock-inoculated *NahG*-transgenic and non-transgenic tomato plants showed no visible difference in leaf shape, size and plant vigour (Fig. 6.1). Leaves of *NahG*-

transgenic plants infected with Fny-CMV curled downwards compared to Fny-CMV-infected non-transgenic tomato plant leaves, which faced upright. Plants of both lines exhibited green-yellow symptoms, though mosaic symptoms on *NahG*-transgenic plants were less apparent than on non-transgenic plants. Fny-CMV infection caused more severe stunting in *NahG*-transgenic plants compared to non-transgenic tomato plants. By 9 dpi, older leaves of virus-infected *NahG*-transgenic plants wilted and compared to non-transgenic plants, had a greater tendency to abscise (Fig. 6.2) (see Chapter 3). Similar exacerbation of symptoms was previously reported for virus-infected *NahG*-transgenic tomato plants (López-Gresa et al., 2016) and potato plants (Baebler et al., 2011).

6.2.2 The disruption of SA accumulation alone does not influence *Myzus persicae* settling on tomato, but SA is necessary for CMV-induced deterrence against *Myzus persicae* settlement

To examine the effect of SA accumulation on *Myzus persicae* settlement on CMV-infected plants, in free-choice settling assays aphids were allowed to choose between *NahG*-transgenic tomato plants and non-transgenic tomato plants following their infection with Fny-CMV or mock inoculation. By 24 hours post-release more aphids had settled on virus-infected *NahG*-transgenic tomato plants than on virus-infected non-transgenic plants (Fig. 6.3). At 1 hour post-release, aphids did not show any bias in settling preference to either type of plant except in one experiment. Aphids did not show a preference for either mock-inoculated *NahG*-transgenic plants or for mock-inoculated non-transgenic tomato plants. However, in one of the four experiments, aphids preferentially settled on *NahG*-transgenic mock-inoculated plants at 1 hour post-release (see Fig. 6.3, experiment 2), though this attraction towards *NahG*-transgenic mock-inoculated plants was not sustained and not detectable at 24 h post-release. There was no difference in aphid settling preference in control experiments when aphids were presented with choices between two mock-inoculated *NahG*-transgenic tomato plants (Fig.6.4). The neutrality in aphid settling preference recorded in the two different combinations of mock-inoculated plants suggests that the disruption of SA accumulation alone does not influence *Myzus persicae* settling on tomato, but that SA is necessary for CMV-induced deterrence against settling by *Myzus persicae*.

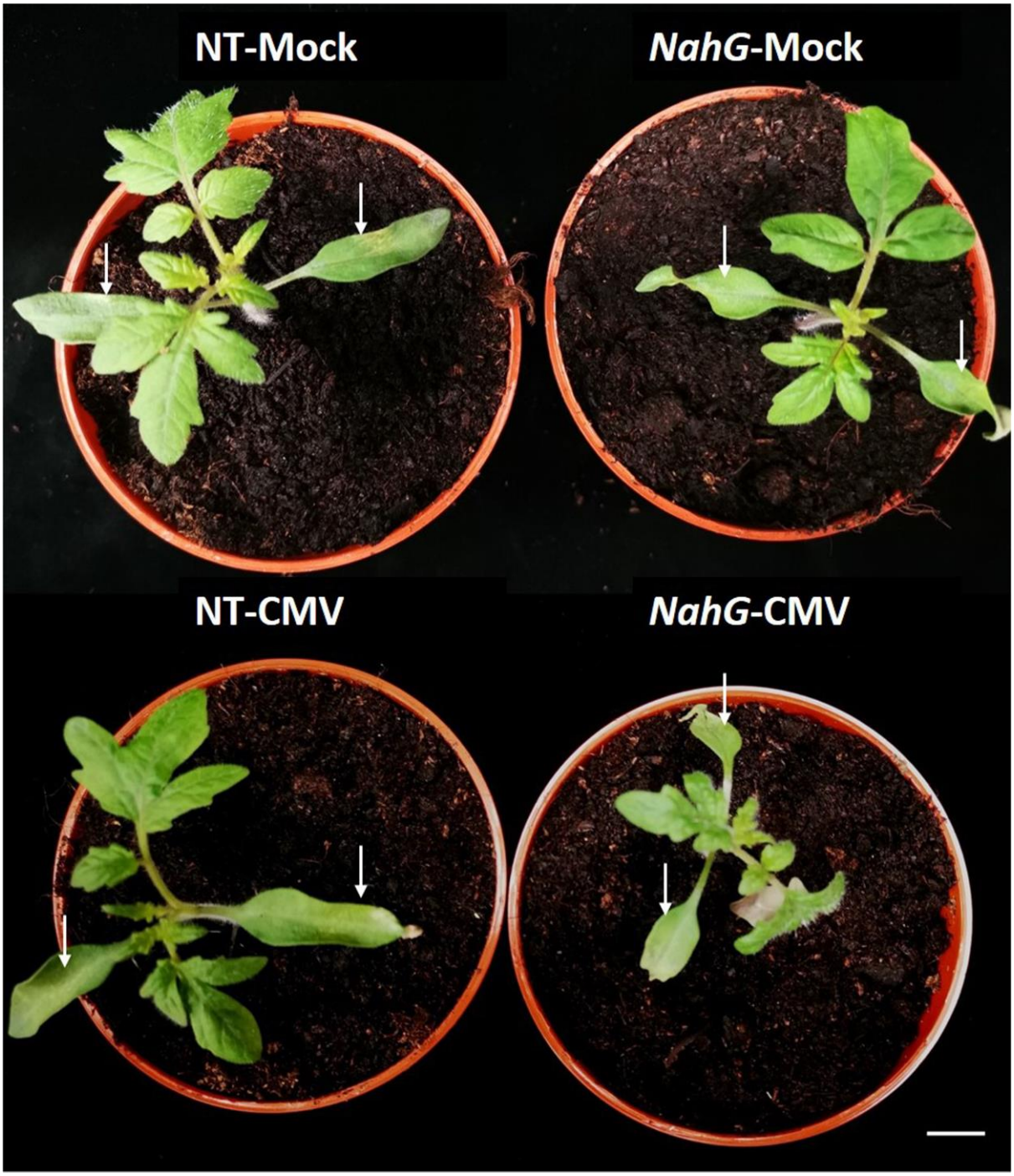


Figure 6.1. Phenotypic characteristics of virus-infected or mock-inoculated non-transgenic and *NahG*-transgenic tomato plants. The plant labelled as NT-Mock represent a non-transgenic plant inoculated with milli-Q water. *NahG*-Mock is a transgenic plant inoculated with milli-Q water. NT-CMV and *NahG*-CMV indicate a non-transgenic plant and transgenic plant infected with Fny-CMV. Plants were inoculated when 10 days old and photographed at 9 days after inoculation or mock inoculation on the indicated lower leaves (indicated by arrows). The scale bar is equivalent to 1 cm.

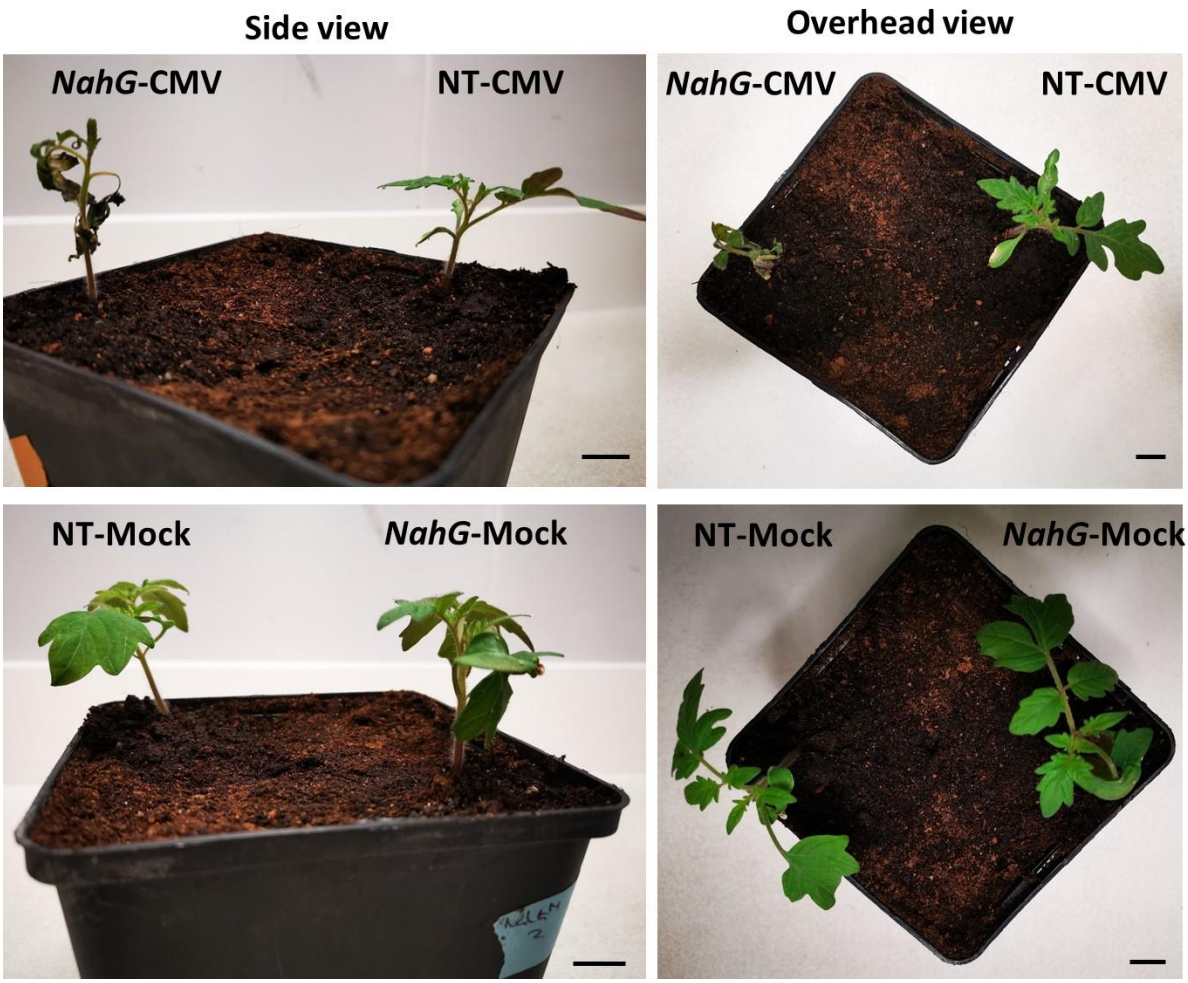
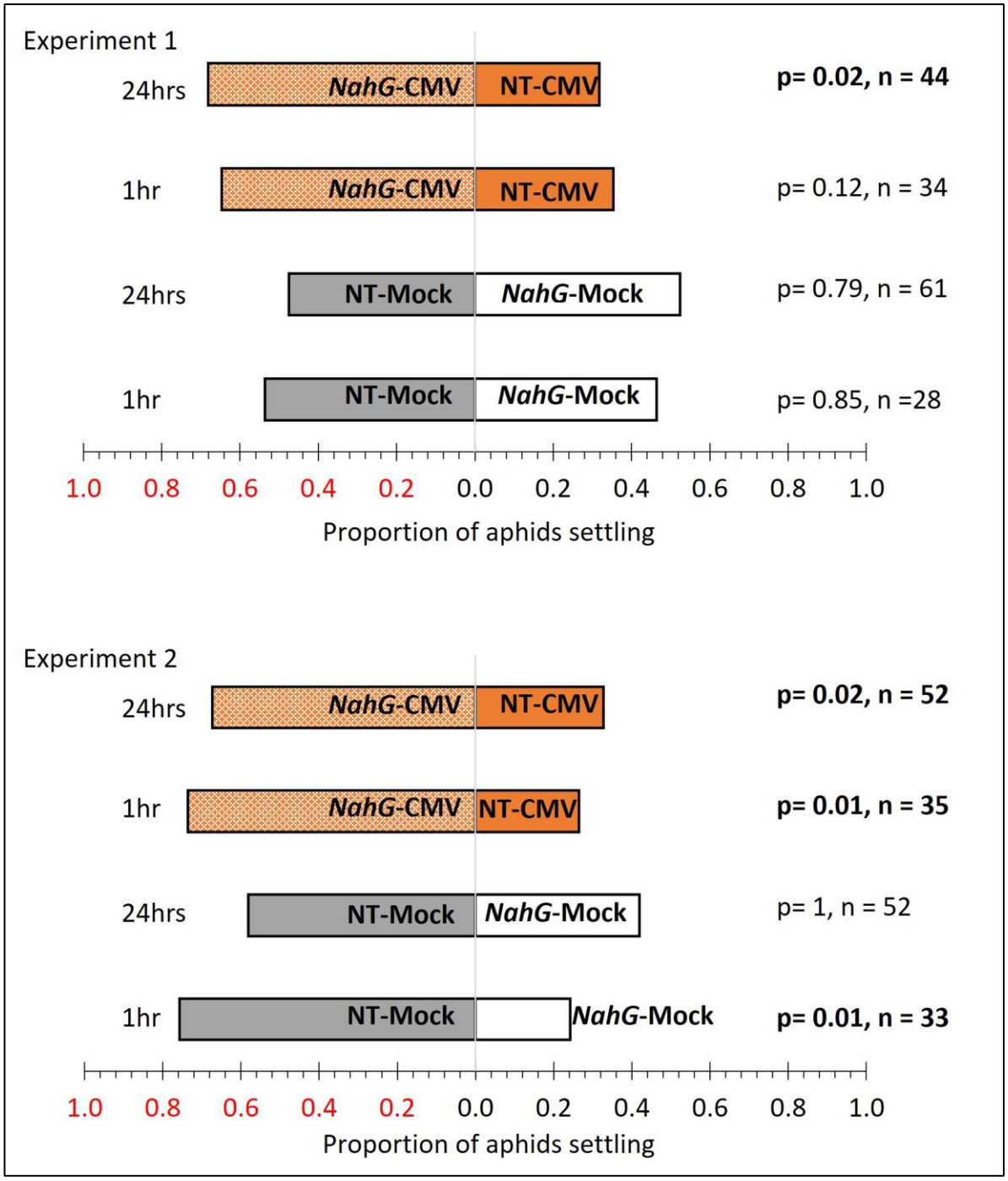


Figure 6.2. *NahG*-transgenic plants are hypersusceptible to CMV-induced pathogenesis. *NahG*-transgenic plants infected with Fny-CMV showed drastic wilting, starting with older leaves. Newly emerged leaves had necrotic lesions and dried off starting from leaf tips. See the legend of Fig 6.1 for additional details. The scale bars represent 1cm.


Experiment 1
24hrs
NahG-CMV
NT-CMV
p= 0.02, n = 44
1hr
NahG-CMV
NT-CMV
p= 0.12, n = 34
24hrs
NT-Mock
NahG-Mock
p= 0.79, n = 61
1hr
NT-Mock
NahG-Mock
p= 0.85, n =28
1.0 0.8 0.6 0.4 0.2 0.0 0.2 0.4 0.6 0.8 1.0
Proportion of aphids settling
Experiment 2
24hrs
NahG-CMV
NT-CMV
p= 0.02, n = 52
1hr
NahG-CMV
NT-CMV
p= 0.01, n = 35
24hrs
NT-Mock
NahG-Mock
p= 1, n = 52
1hr
NT-Mock
NahG-Mock
p= 0.01, n = 33
1.0 0.8 0.6 0.4 0.2 0.0 0.2 0.4 0.6 0.8 1.0
Proportion of aphids settling

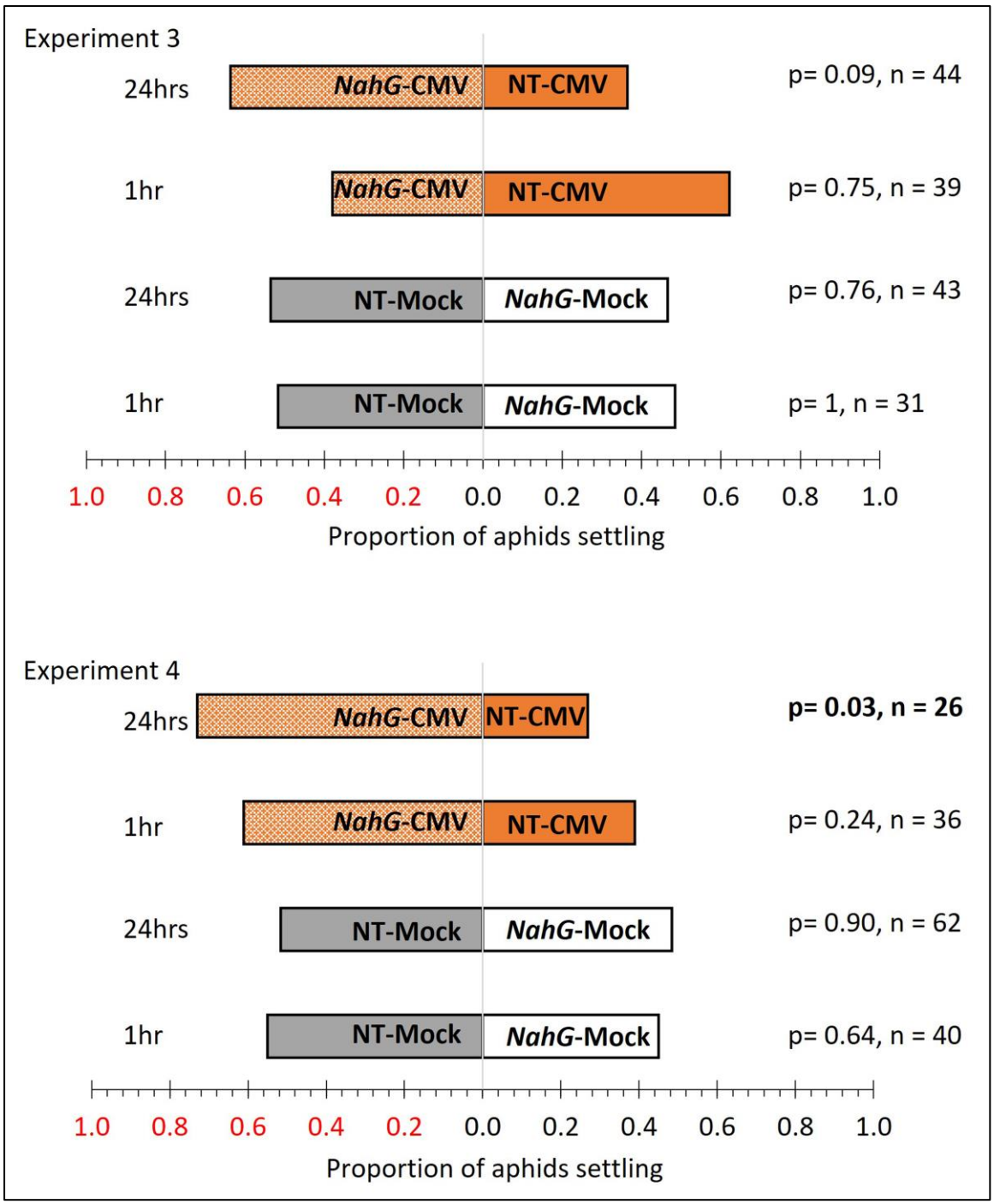
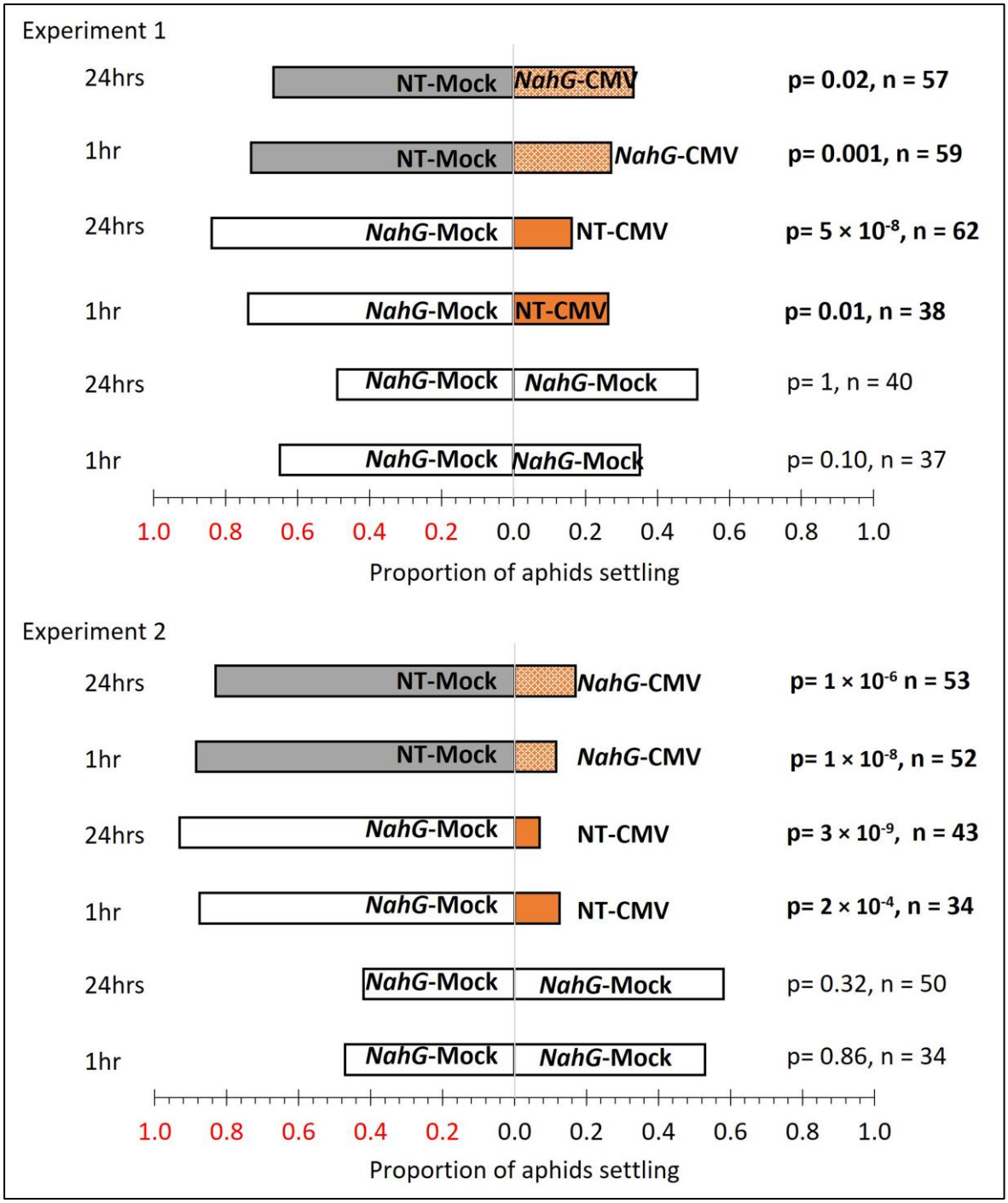


Figure 6.3. *Myzus persicae* preferentially settled on *NahG*-transgenic plants infected with Fny-CMV compared to non-transgenic tomato plants infected with the same virus. Plot bars labelled *NahG*-CMV and NT-CMV, respectively, indicate *NahG*-transgenic and non-transgenic tomato plants infected with Fny-CMV. NT-Mock and *NahG*-Mock represent non-transgenic and *NahG*-transgenic tomato plants inoculated with milli-Q water as control plants. The number of aphids settling per plant in each treatment pair was recorded in each experiment after 1 hour and 24 hours post-release. Four independent experiments denoted in the plot as experiment 1, experiment 2, experiment 3, and experiment 4 were performed. The p-values against each control/treatment pair of plants indicate a binomial test for statistical significance at $\alpha = 0.05$, and n represents the total number of aphids that settled per treatment pair. The p-values in bold indicate treatments that significantly altered aphid settling preference.


Experiment 1
24hrs
NT-Mock
NahG-CMV
p= 0.02, n = 57
1hr
NT-Mock
NahG-CMV
p= 0.001, n = 59
24hrs
NahG-Mock
NT-CMV
p= 5 × 10-8, n = 62
1hr
NahG-Mock
NT-CMV
p= 0.01, n = 38
24hrs
NahG-Mock
NahG-Mock
p= 1, n = 40
1hr
NahG-Mock
NahG-Mock
p= 0.10, n = 37
1.0 0.8 0.6 0.4 0.2 0.0 0.2 0.4 0.6 0.8 1.0
Proportion of aphids settling
Experiment 2
24hrs
NT-Mock
NahG-CMV
p= 1 × 10-6 n = 53
1hr
NT-Mock
NahG-CMV
p= 1 × 10-8, n = 52
24hrs
NahG-Mock
NT-CMV
p= 3 × 10-9, n = 43
1hr
NahG-Mock
NT-CMV
p= 2 × 10-4, n = 34
24hrs
NahG-Mock
NahG-Mock
p= 0.32, n = 50
1hr
NahG-Mock
NahG-Mock
p= 0.86, n = 34
1.0 0.8 0.6 0.4 0.2 0.0 0.2 0.4 0.6 0.8 1.0
Proportion of aphids settling

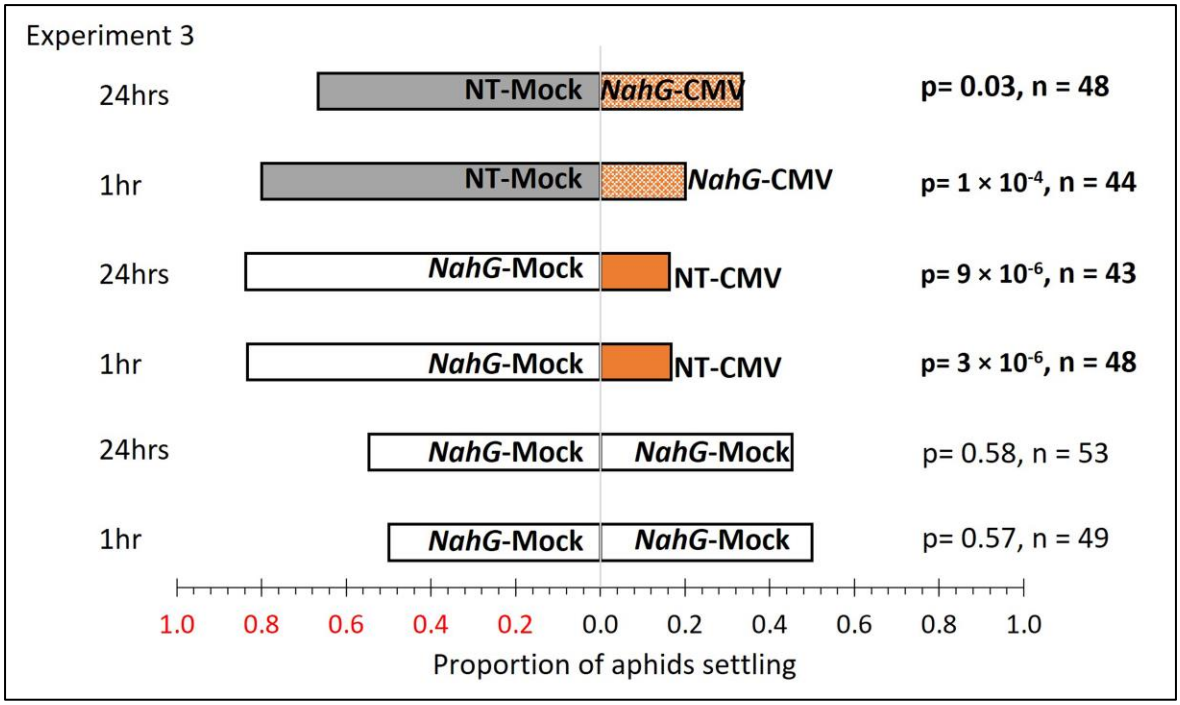


Figure 6.4. The settling preference of *Myzus persicae* on additional treatment combinations of *NahG*-transgenic and non-transgenic plants. Plot bar labels NT-Mock indicate non-transgenic plants inoculated with milli-Q water. *NahG*-CMV represent *NahG*-transgenic plants infected with Fny-CMV, *NahG*-Mock are *NahG*-transgenic tomato plants inoculated with milli-Q water, and NT-CMV are non-transgenic plants infected with Fny-CMV. Aphid counts in each treatment comparison were recorded at 1 hour and 24 hours post-release in three independent experiments denoted in the plot as experiment 1, experiment 2 and experiment 3. The p-values against each control/treatment comparison indicate a binomial test for statistical significance at $\alpha = 0.05$, and n represents the total number of aphids that settled per treatment pair. The p-values in bold indicate treatments that aphid settling preferences were altered significantly.

When aphids were allowed to choose between *NahG*-transgenic plants infected with Fny-CMV and non-transgenic mock-inoculated plants, aphids settled in markedly greater numbers on mock-inoculated plants (Fig.6.4). A similar settling preference (i.e. for the mock-inoculated plants) was recorded in non-transgenic tomato plants infected with Fny-CMV versus *NahG*-transgenic mock-inoculated plants (Fig. 6.4). These findings suggest that SA-dependent signalling in tomato does not play a major role in determining the preference of *Myzus persicae* settling preference for settling on mock-inoculated plants over those infected with Fny-CMV.

6.2.3 Host SA levels have no role in influencing *Macrosiphum euphorbiae* settling preferences between mock-inoculated tomato plants and plants infected with Fny-CMV

No experiments comparing the settling preference of the solanaceous specialist aphid *Macrosiphum euphorbiae* on *NahG*-transgenic and non-transgenic tomato plants infected with Fny-CMV revealed any effect of decreasing SA accumulation (Fig.6.5). Likewise, aphids of this species showed no preferential settlement on mock-inoculated non-transgenic versus mock-inoculated *NahG*-transgenic plants. These findings suggest that SA does not influence CMV-induced changes in the settling preference of *Macrosiphum euphorbiae* on tomato. Observations of *NahG*-transgenic plants infected with Fny-CMV and mock-inoculated non-transgenic plants, showed that *Macrosiphum euphorbiae* settled preferentially on mock-inoculated plants. Similarly, these aphids preferred to settle on mock-inoculated *NahG*-transgenic plants rather than on non-transgenic plants infected with Fny-CMV.

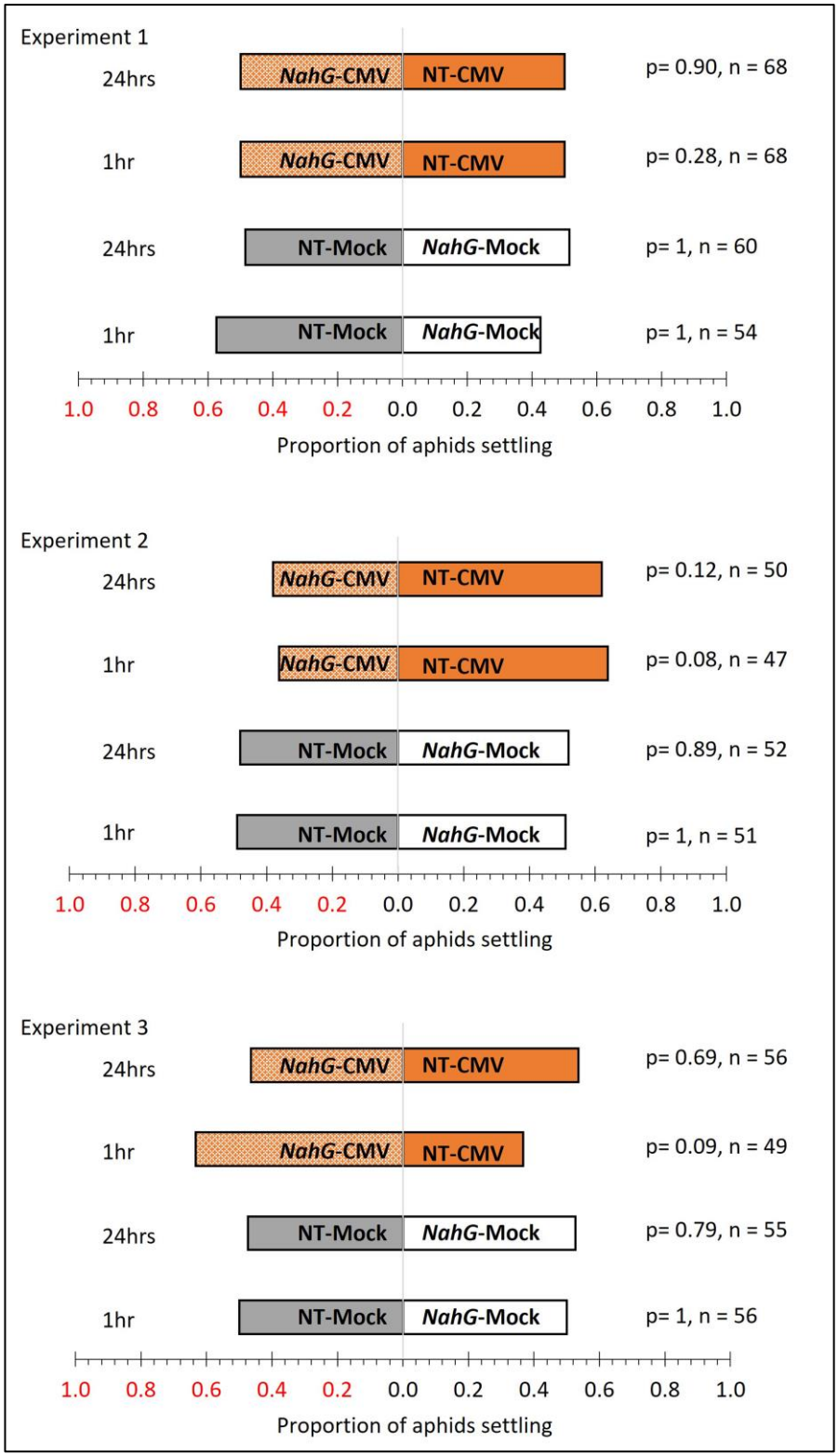
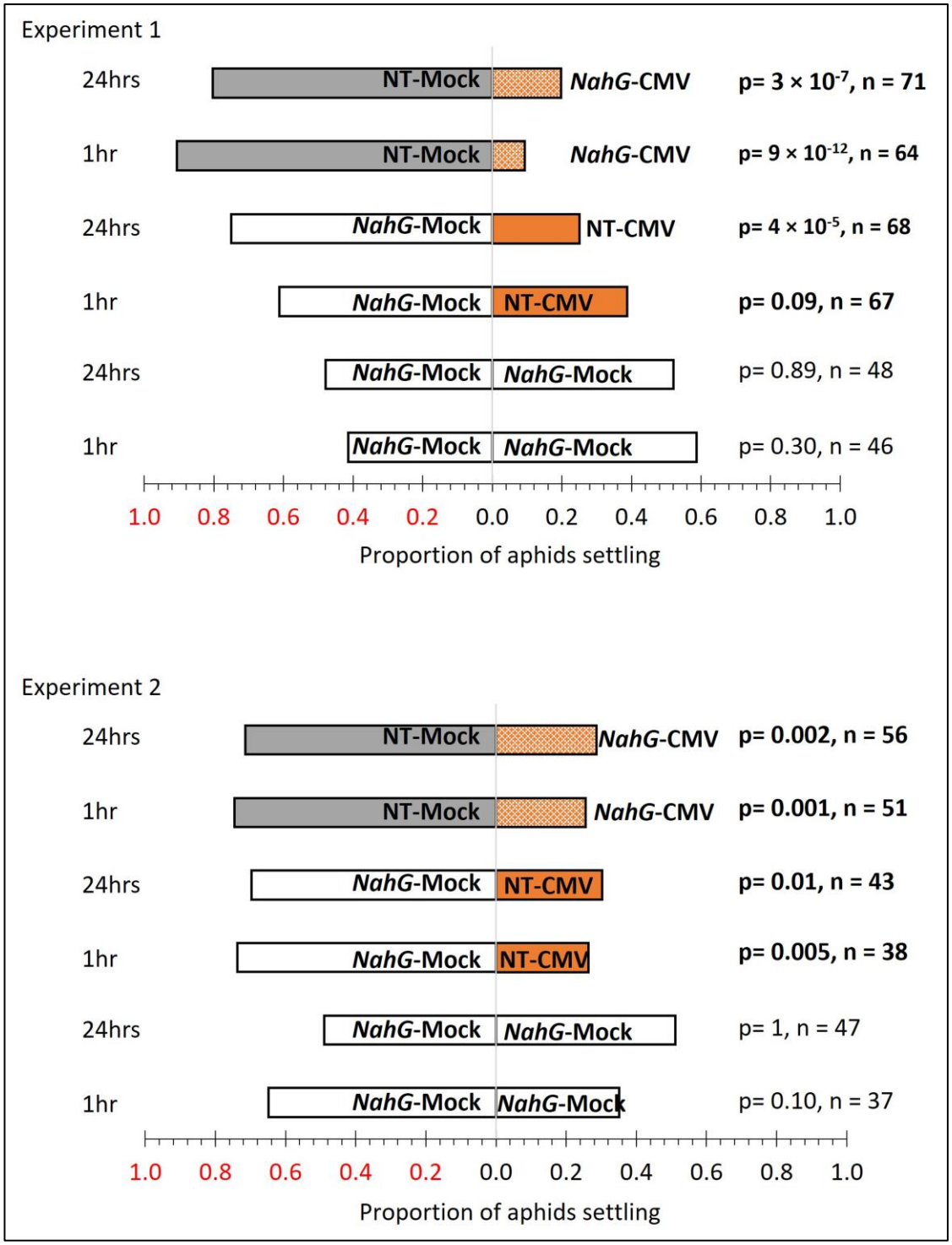


Figure 6.5. Settling preference of *Macrosiphum euphorbiae* on tomato plants infected with CMV is not affected by SA accumulation in the host plant. The *NahG*-CMV label indicates *NahG*-transgenic tomato plants infected with Fny-CMV; NT-CMV represents non-transgenic tomato plants infected with Fny-CMV. NT-Mock and *NahG*-Mock, respectively, indicate non-transgenic plants and *NahG*-transgenic plants inoculated with sterile water. Aphid settling preference at 1 hour and 24 hours post-release in each treatment comparison in three independent experiments denoted in the plot as experiment 1, experiment 2 and experiment 3. The p-values indicate a binomial test for statistical significance at $\alpha = 0.05$, and n represents the total number of aphids that settled per treatment pair.


Experiment 1
24hrs
NT-Mock
NahG-CMV
p= 3 × 10-7, n = 71
1hr
NT-Mock
NahG-CMV
p= 9 × 10-12, n = 64
24hrs
NahG-Mock
NT-CMV
p= 4 × 10-5, n = 68
1hr
NahG-Mock
NT-CMV
p= 0.09, n = 67
24hrs
NahG-Mock
NahG-Mock
p= 0.89, n = 48
1hr
NahG-Mock
NahG-Mock
p= 0.30, n = 46
1.0 0.8 0.6 0.4 0.2 0.0 0.2 0.4 0.6 0.8 1.0
Proportion of aphids settling
Experiment 2
24hrs
NT-Mock
NahG-CMV
p= 0.002, n = 56
1hr
NT-Mock
NahG-CMV
p= 0.001, n = 51
24hrs
NahG-Mock
NT-CMV
p= 0.01, n = 43
1hr
NahG-Mock
NT-CMV
p= 0.005, n = 38
24hrs
NahG-Mock
NahG-Mock
p= 1, n = 47
1hr
NahG-Mock
NahG-Mock
p= 0.10, n = 37
1.0 0.8 0.6 0.4 0.2 0.0 0.2 0.4 0.6 0.8 1.0
Proportion of aphids settling

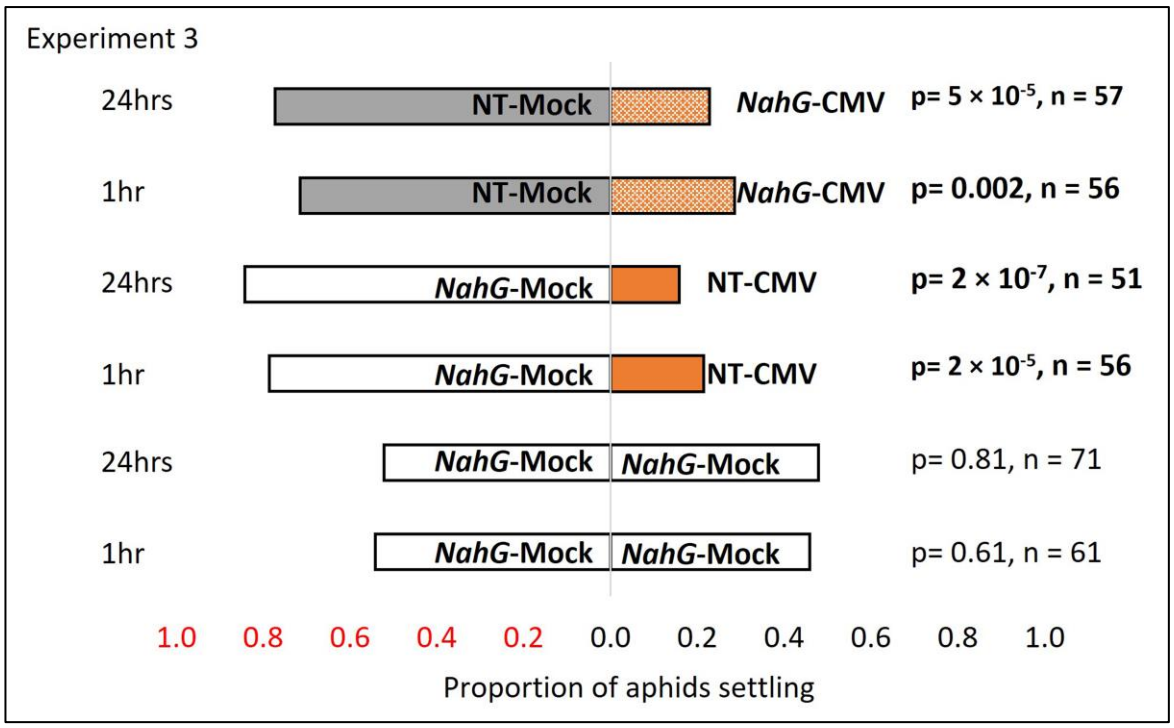


Figure 6.6. Disruption of SA accumulation in host plants did not affect the settling preference of *Macrosiphum euphorbiae* for mock-inoculated plants. Plot bar labels NT-Mock indicate non-transgenic plants inoculated with milli-Q water. *NahG*-CMV represents *NahG*-transgenic plants infected with Fny-CMV. *NahG*-Mock are *NahG-transgenic* tomato plants inoculated with milli-Q water, and NT-CMV are non-transgenic plants infected with Fny-CMV. Aphid counts in each treatment comparison were recorded at 1 h and 24 h post-release in three independent experiments denoted in the plot as experiment 1, experiment 2 and experiment 3. The p-values against each control/treatment comparison indicate a binomial test for statistical significance at $\alpha = 0.05$, and n represents the total number of aphids that settled per treatment pair. The p-values in bold indicate treatments in which settling preferences were significantly altered.

6.3 Discussion

Fny-CMV infection increased the attractiveness of *NahG*-transgenic tomato plants to *Myzus persicae* but not to *Macrosiphum euphorbiae*. However, aphids preferred to settle on non-transgenic mock-inoculated plants irrespective of whether or not virus-infected plants were *NahG*-transgenic or non-transgenic. This indicates that the induction by CMV of SA accumulation (and consequent effects on SA-dependent gene expression) cannot fully explain CMV-induced deterrence against aphids. The results suggest that in tomato, CMV can induce SA-dependent and SA-independent mechanisms that influence aphid-host interactions but that whilst the interaction of the generalist aphid *Myzus persicae* with tomato is somewhat influenced by the SA-dependent mechanism(s), SA has no effect on this host's interaction with *Macrosiphum euphorbiae*. These data suggest that SA may be required to induce some aspects of deterrence against *Myzus persicae* but not against *Macrosiphum euphorbiae*.

Before conducting choice experiments, and while characterising the properties of the *NahG*-transgenic tomato line I noticed a phenotype of sudden wilting in *NahG*-transgenic tomato plants infected with Fny-CMV. Their leaf canopy first exhibited a palm tree-like appearance, i.e., leaves curled downwards, and in severe cases, older leaves fell off plant stems. A similar dramatic phenotype was previously described in *NahG*-transgenic potato plants infected with PVY (Baebler et al., 2011). Though many viruses, including CMV, are known to activate or suppress many host genes, which may drastically change the plant phenotype, the molecular mechanisms underpinning symptomology remain primarily unclear (García-Marcos et al., 2009; Hanssen et al., 2011; Whitham et al., 2006). This study showed that depletion of SA in *NahG*-transgenic tomato plants enhanced CMV-induced disease development and symptom expression. This result suggests that the SA defence signalling pathway might be involved in the tolerant-like response of the tomato cv. Moneymaker to Fny-CMV infection. The role of SA in viral accumulation and symptom expression was investigated previously in tobacco by SA treatment, which resulted in delayed symptom appearance in tobacco plants infected with TMV (Naylor et al., 1998). Gene expression studies using the *NahG* transgene have shown that SA is required for PVY symptom development in potato cv. Désirée (Baebler et al., 2011).

In summary, it appears that SA has at best a minor role in influencing aphid settling preferences towards or away from tomato plants infected with Fny-CMV. However, the work showed that SA is important in moderating the severity of CMV-induced disease in tomato. Quantitative transcriptomic studies in tomato during Fny-CMV infection could reveal host genes controlling disease severity. Unfortunately, due to the Covid-19 laboratory shutdown there was insufficient time to determine if increased disease severity in *NahG*-transgenic tomato correlated with increased CMV titre.

Chapter 7. CMVΔ2b-induced aphid resistance in tobacco relies on JA-dependent defensive signalling

7.1 Introduction

Myzus persicae survival and reproduction rates are often enhanced on tobacco infected with Fny-CMV and more consistently on tobacco plants infected with LS-CMV (Tungadi et al., 2020; Ziebell et al., 2011). Interestingly, aphid survival and reproduction are drastically reduced on tobacco plants infected with the mutant Fny-CMVΔ2b, which cannot express the 2b protein (Ziebell et al., 2011). A recent study established that it is the Fny-CMV 1a protein that is inducing strong resistance to aphids in tobacco plants infected with Fny-CMVΔ2b, and in the same work, it was shown that 1a-induced resistance could be counteracted by the 2b proteins encoded by either Fny-CMV or LS-CMV (Tungadi et al., 2020). A recent publication by Watt et al. (2020), showing that the 2b and the 1a protein of Fny-CMV interact directly *in vivo*, suggests a possible mechanism by which the 2b protein might inhibit the resistance-inducing activity of the 1a protein in tobacco.

CMV-induced effects on aphid performance, such as changing settling preferences and feeding behaviours, might be due to virus-induced alterations in the JA-dependent defensive signalling pathway (Westwood et al., 2013, 2014; Wu et al., 2017). Lewsey *et al*. (2010) and Westwood *et al*. (2014) showed that the 2b protein and certain other VSRs inhibit the induction of JA-regulated gene expression, and in the case of the 2b protein they proposed that this was effected through interference with miRNA-regulated gene expression, as suggested by earlier work by Pandey *et al*. (2008). However, Wu *et al*. (2017) found that the 2b protein directly interacts with specific JAZ proteins leading to increased emission of VOCs attractive to *Myzus persicae*, suggesting another means of interference with JA-mediated signalling. JAZ proteins bind COI1, a co-receptor that mediates the perception of JA-Ile (the biologically active derivative of JA) (see references in Section 1.6.1). At the start of my study, it was not known if the JA-dependent signalling pathway is required for the resistance to *Myzus persicae* that is engendered in tobacco by infection with the mutant Fny-CMVΔ2b (Ziebell *et al*., 2011). In this chapter, it was investigated whether JA-dependent signalling influences CMV-induced and CMVΔ2b-induced changes in host-aphid interactions in tobacco. To do this, I decreased the expression of the *COI1* gene by transforming tobacco (cv. Xanthi) plants with a gene silencing construct, and assessed the performance of *Myzus persicae* on these plants following infection with Fny-CMV or Fny-CMVΔ2b.

7.2 Results

7.2.1 Down-regulation of *COI1* gene expression by RNA silencing affected tobacco phenotypes and JA-regulated gene expression

I generated twenty-four independent transgenic tobacco lines, and fourteen of these lines were confirmed by PCR to harbour a T-DNA construct designed to decrease the expression of COI1 (Fig. 7.1). Using RT-PCR to analyse plants of the T_0 generation, it was found that the accumulation of the *COI1* RNA transcript in tobacco was diminished in twelve out of fourteen independent transgenic tobacco lines tested (Lines CIA, C3A, C291SR, C283yLR, C26-4y-SR3, C2825LR, Cpb1, C273LR, C273LR2, C2825LR3, C1A3 and C28-3y-LR2: Table 7.1; Fig. 7.2), indicating a high success rate in knocking down accumulation of the target transcript. Regenerated (T_0) plants were allowed to self-fertilise, and the T_1 seed was collected. This process was repeated for up to the T_3 generation seed, which were used for experiments. In each generation, putative *COI1*-silenced seedlings were grown on nutrient media plates containing the antibiotic kanamycin to further select plants harbouring T-DNA.

Anthers of four out of fourteen T_0 *COI1*-silenced tobacco lines (C291SR, C283yLR, C273LR2 and C273LR: Table 7.1) were indehiscent, i.e., could not release pollen grains (Fig.7.3A), compared to anthers of untransformed plants and those of the other transgenic lines (Fig.7.3B). Plants with the indehiscent anther flower phenotype did not produce viable seeds, suggesting that COI1 is required for maternal control of seed maturation in tobacco.

To further verify whether transformation was successful, *COI1*-silenced tobacco plants and untransformed plants were treated with either a 250 µM MeJA solution dissolved in 0.05% ethanol or 0.05% ethanol as a control. Relative expression of transcript accumulation for *LOX2,* a JA-induced gene, was quantified by RT-qPCR (Section 2.5) in five transformed lines and untransformed tobacco (Fig. 7.4). *LOX2* expression was markedly reduced in four *COI1*-silenced transgenic lines (C1A, C2825LR, Cpb1, and C3A: Table 7.1) compared to untransformed tobacco plants, confirming that *COI1* is required for JA induction in tobacco. This result further proves that *COI1* gene expression was successfully silenced in plants of these transgenic lines.

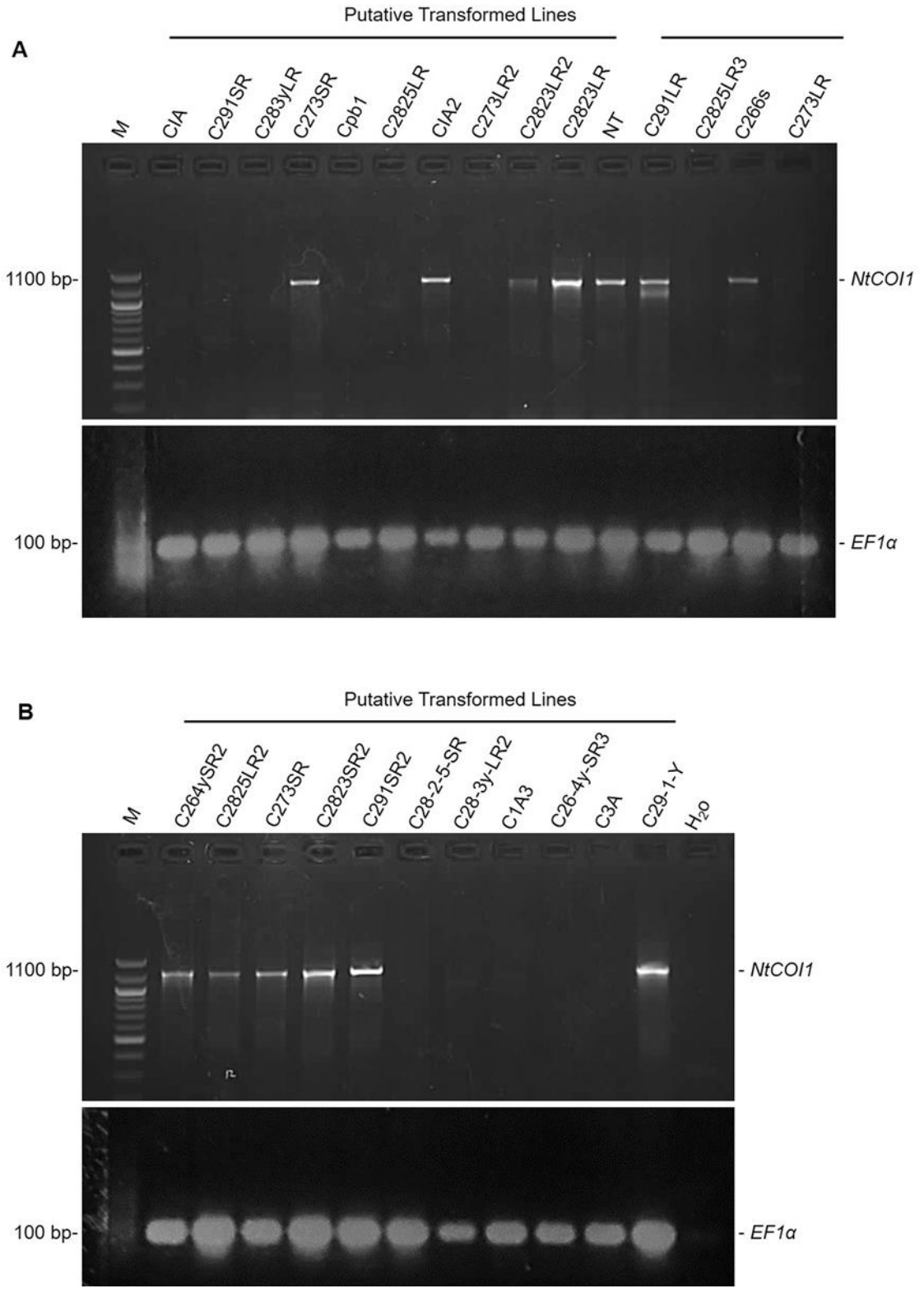


Figure 7.1. Detection of the *NtCOI1* silencing construct in independently generated putatively *COI1*-silenced tobacco lines. PCR was performed on genomic DNA extracted from leaves of non-transformed (NT) tobacco plants and all T_o generation plants. Identities of tobacco lines in lanes 1-10; 12-25 appear in column 2 of Table 7.1. A and B show gel analysis of PCR amplicons of 25 transgenic tobacco lines. *EF1α* was used as an internal control gene. The PCR amplicons of *NtCOI1* and *EF1α* and their band sizes in base pairs (bp) are indicated alongside DNA markers (lanes M). H_2O shows a PCR reaction that contained no DNA template. Samples in which the *NtCOI1*-silencing transgene amplicon was undetectable were selected for RT-PCR to verify the level of *COI1* transcript accumulation (Fig. 7.2).

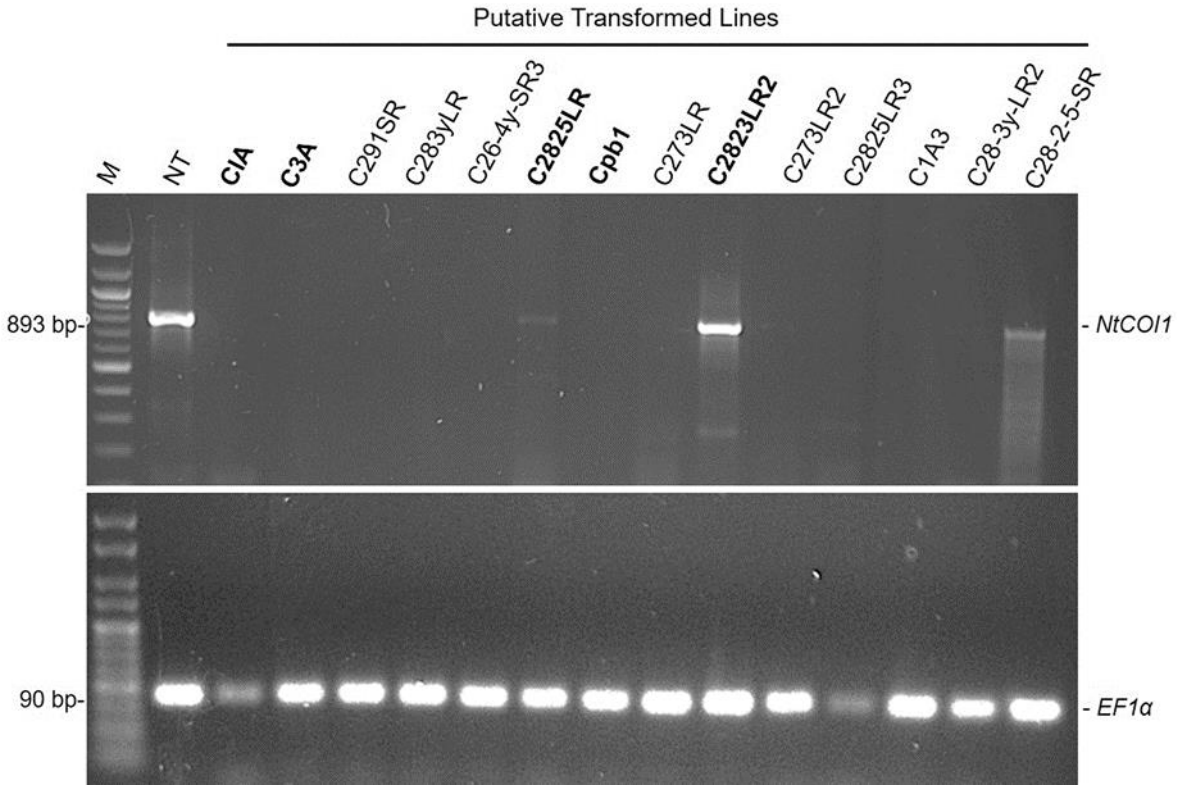


Figure 7.2. *COI1* transcript accumulation in putative *COI1*-silenced transgenic tobacco lines. RT-PCR was used to detect the accumulation of the NtCOI1 transcript in total RNA extracted from newly emerged leaves of non-transformed (NT) tobacco plants, and T_0 generation plants from 14 independent tobacco lines regenerated following the transformation procedure and selected for further work (Table 7.1). Of the 14 putative *COI1*-silenced lines chosen for further study, plants of two lines (9 and 14) were found to accumulate detectable amounts of *COI1* transcript and were not used for further experiments. Putative lines in bold were advanced for *LOX2* expression studies (see Fig. 7.4). *EF1α* transcript accumulation was used as an internal control. Bands corresponding to the PCR amplicons of *COI1* and *EF1α* are indicated along with their sizes in base pairs (bp), and adjacently running DNA size markers (lanes M) are shown.

Figure 7.3. Floral structure variations between transformed tobacco and untransformed tobacco plants. A shows impaired floral anther dehiscence observed in plants germinated from lines C291SR, C283yLR, C273LR2 and C273LR. **B** indicates normal flower anther dehiscence in non-transgenic plants and a significant proportion of *COI1*-silenced tobacco lines, e.g., C2825LR and C2823LR2 (see Table 7.1). The lines with defective pollen production were not taken on to T1 for further characterisation.

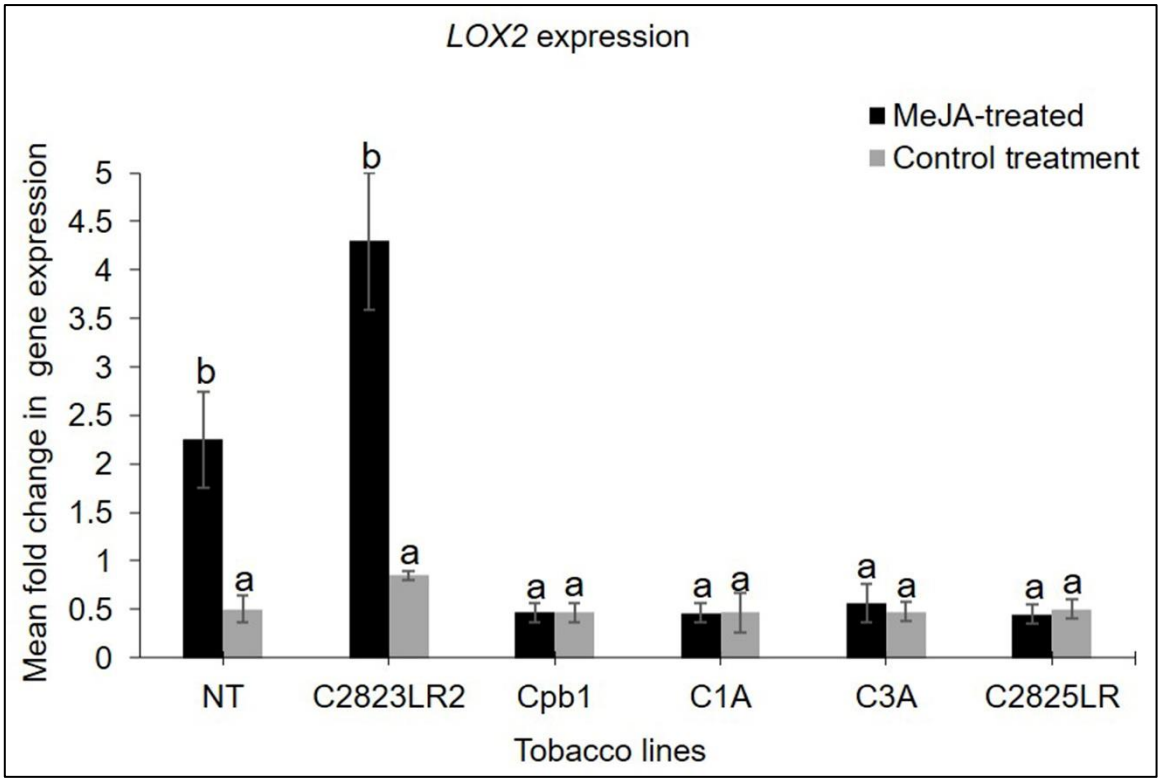


Figure 7.4. Decreased *COI1* expression in transgenic tobacco plants suppresses induction of the JA-responsive gene *LOX2* in plants sprayed with MeJA. C2823LR2, Cpb1, C1A, C3A, and C2825LR are independent transgenic tobacco lines (Table 7.1). Plants of NT and *COI1*-silenced tobacco lines were treated with MeJA or control solution as described in Section 2.7.1. After 24 hours, the accumulation of the JA-inducible *LOX2* transcript from control or JA-treated plants was quantified by RT-qPCR. Histogram bars represent a measure of accumulation of dsDNA amplicon by qPCR, relative to that for *EF1α* transcripts. Error bars represent SEM for three technical repeats. Different lower-case letters (a or b) indicate statistically significant (α= 0.05) differences in *LOX2* transcript accumulation (t-test). This is one of three independent experiments carried out.

Table 7.1. Summary of *COI1* silencing transformation workflow. The table indicates phenotypic and molecular differences between plants of transformed lines and non-transformed (NT) tobacco plants. Tobacco lines in grey rows were selected for aphid experiments.

Line ID	Kanamycin resistance confirmed	T-DNA detected at T_0 generation[1]	Knockdown of *COI1* expression[2]	Anther dehiscence	Effects on JA-responsive gene expression[3]
C1A	✓	✓	✓	**Normal**	**Reduced**
C291SR	✓	✓	✓	Impaired	Not tested
C283yLR	✓	✓	✓	Impaired	Not tested
C273SR	✓	×			
Cpb1	✓	✓	✓	**Normal**	**Reduced**
C2825LR	✓	✓	✓	**Normal**	**Reduced**
C1A2	✓	×		Normal	Not tested
C273LR2	✓	✓	✓	Impaired	Not tested
C2823LR2	✓	✓	✓	Normal	No change
C2823LR	✓	×			
C291LR	✓	×			
C2825LR3	✓	✓	✓	Normal	Not tested
C266s	✓	×			
C273LR	✓	✓	✓	Impaired	Not tested
C264ySR2	✓	×			
C2825LR2	✓	×			
C273SR	✓	×			
C2823SR2	✓	×			
C291SR2	✓	×			
28-2-5-SR	✓	✓	✓	Normal	Not tested
28-3y-LR2	✓	✓	✓	Normal	Not tested
C1A3	✓	✓	✓	Normal	Not tested
C3A	✓	✓	✓	**Normal**	**Reduced**
26-4y-SR3	✓	✓	✓	Normal	Not tested

Notes

[1] Confirmed by PCR (Fig 7.1)

[2] Decreased *COI1* transcript accumulation confirmed by RT-PCR at T_0 generation (Fig. 7.2)

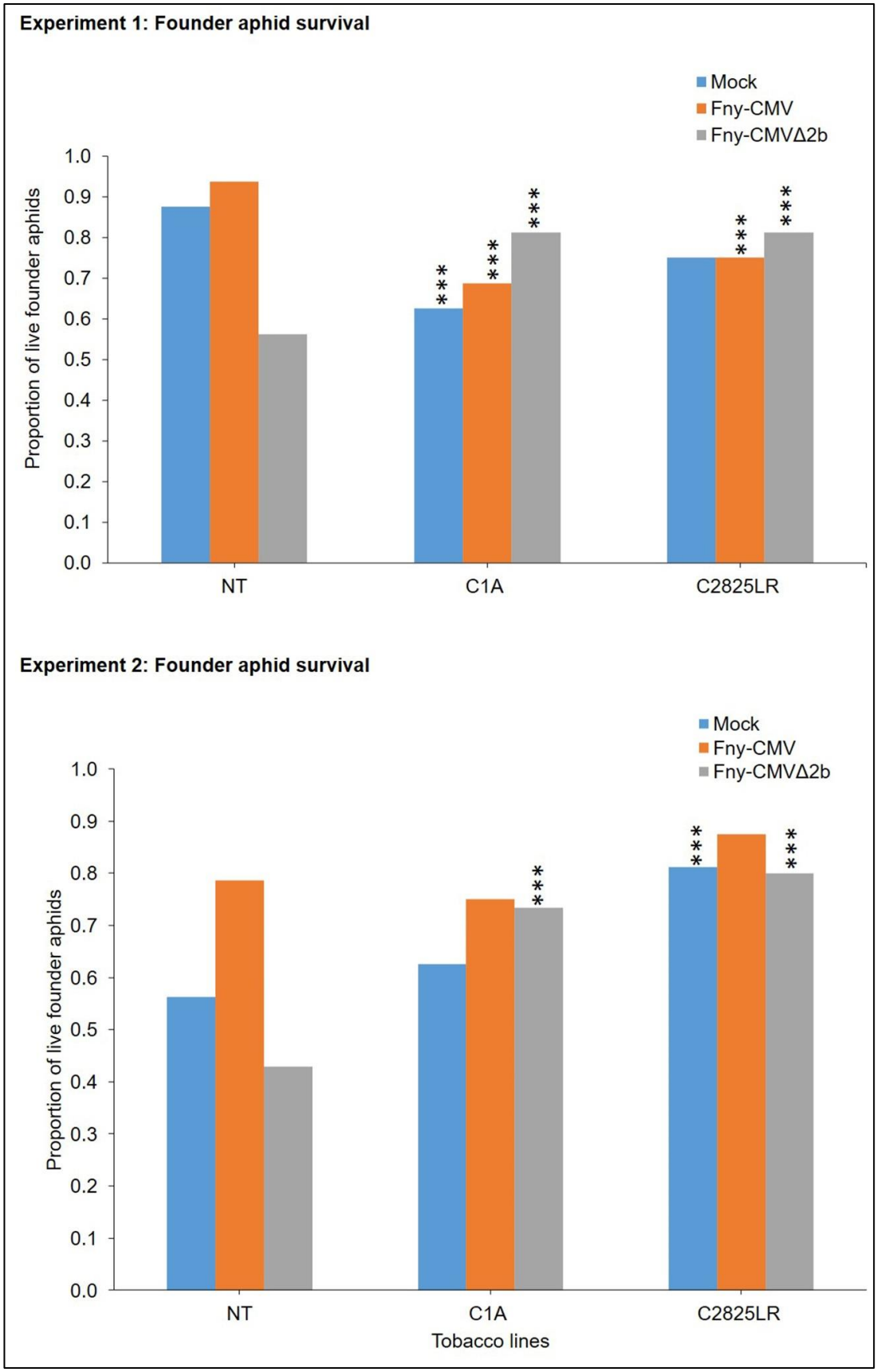
[3] Transcript accumulation of *NtLOX2* measured by RT-qPCR (Fig. 7.4)

7.2.2 JA plays a role in CMVΔ2b-induced resistance to aphids in tobacco

Using non-transgenic plants and plants of transgenic lines C1A and C2825LR, I examined the effect of Fny-CMV and Fny-CMVΔ2b on aphid survival rate and reproduction on tobacco plants with normal or diminished JA perception (see Section 2.8.4 for detailed methods). Founder aphid survival and the number of offspring produced by each aphid were recorded after fourteen days. Each experiment was carried out at least three times. In agreement with Ziebell *et al*. (2011) and Tungadi *et al*. (2020), *Myzus persicae* survival was significantly reduced on untransformed tobacco plants infected with Fny-CMVΔ2b compared to mock-inoculated plants and plants infected with Fny-CMV (Fig 7.5). Remarkably, Fny-CMVΔ2b infection rescued aphid survival on *COI1*-silenced tobacco plants to levels comparable to those on uninfected plants. This effect was observed in all experiments with transgenic lines C1A and C2825LR.

Myzus persicae reproduction on *COI1*-silenced transgenic tobacco plants was not affected by Fny-CMVΔ2b (Fig. 7.6). Aphids confined on *COI1*-silenced transgenic plants infected with Fny-CMVΔ2b produced significantly more offspring when compared to aphids on untransformed tobacco plants infected with this mutant virus (Fig.7.6). This suggests that Fny-CMVΔ2b induces resistance to *Myzus persicae* reproduction on tobacco by induction of JA-dependent host defences.

To validate whether the normal levels of survival and reproduction displayed by aphids placed on Fny-CMVΔ2b-infected plants of *COI1*-silenced tobacco lines C1A and C2825LR were not artefacts of these two particular lines, I further examined aphid performance on plants of two other independently generated transgenic lines, Cpb1 and C3A, together with untransformed plants. Founder aphids lived longer and produced more offspring on plants of lines Cpb1 and C3A than on untransformed tobacco plants infected with Fny-CMVΔ2b (Fig.7.7). The consistent results obtained with four independent transgenic lines of plants with diminished *COI1* expression (C1A, C2825LR, Cpb1, and C3A) showed that decreased survival and reproduction of aphids on tobacco plants infected with Fny-CMVΔ2b is due to activation of JA-dependent defensive signalling.


Experiment 1: Founder aphid survival
Mock
Fny-CMV
Fny-CMVΔ2b
Proportion of live founder aphids
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
***
NT
C1A
C2825LR
Experiment 2: Founder aphid survival
Mock
Fny-CMV
Fny-CMVΔ2b
Proportion of live founder aphids
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
***
NT
C1A
C2825LR
Tobacco lines

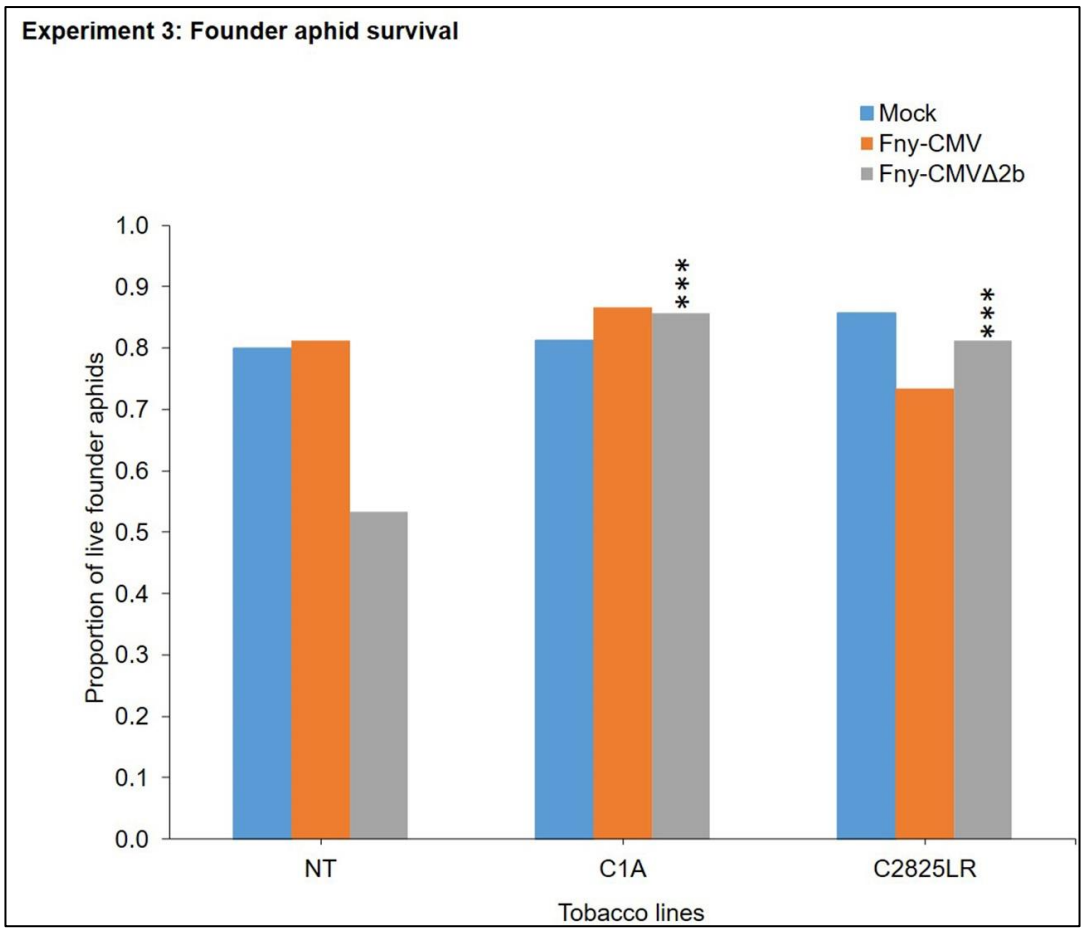
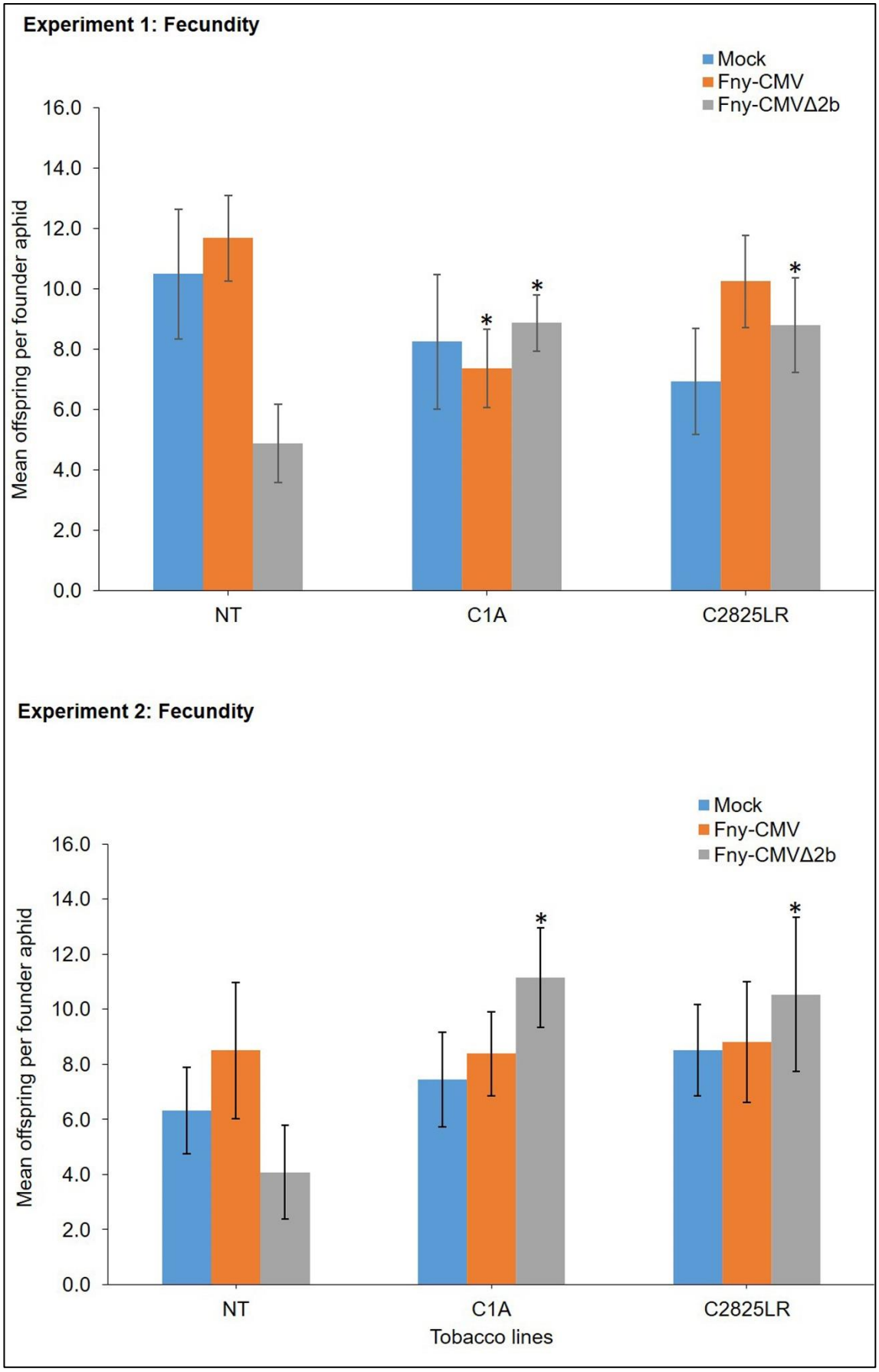


Figure 7.5. The survival of founder aphids was improved on *COI1*-silenced transgenic tobacco lines infected with Fny-CMVΔ2b. Tobacco plants were inoculated with purified virions of Fny-CMV, Fny-CMVΔ2b or milli-Q water as a control (Mock: mock-inoculated). At 10 dpi, a single one-day-old nymph was confined on the abaxial surface of a tobacco plant leaf that had been either virus-infected or mock-inoculated (Section 2.8.4). After fourteen days of confinement, the number of founder aphids remaining alive was recorded. Three independent experiments were performed. In each experiment, 16 nymphs were confined in each treatment group per line, i.e., NT, C1A and C2825LR (Table 7.1). Survival data marked with asterisks *** p<0.001 (Chi-square test) differ significantly from corresponding NT values.


Experiment 1: Fecundity
Mock
Fny-CMV
Fny-CMVΔ2b
Mean offspring per founder aphid
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
*
*
*
NT
C1A
C2825LR
Experiment 2: Fecundity
Mock
Fny-CMV
Fny-CMVΔ2b
Mean offspring per founder aphid
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
*
*
NT
C1A
C2825LR
Tobacco lines

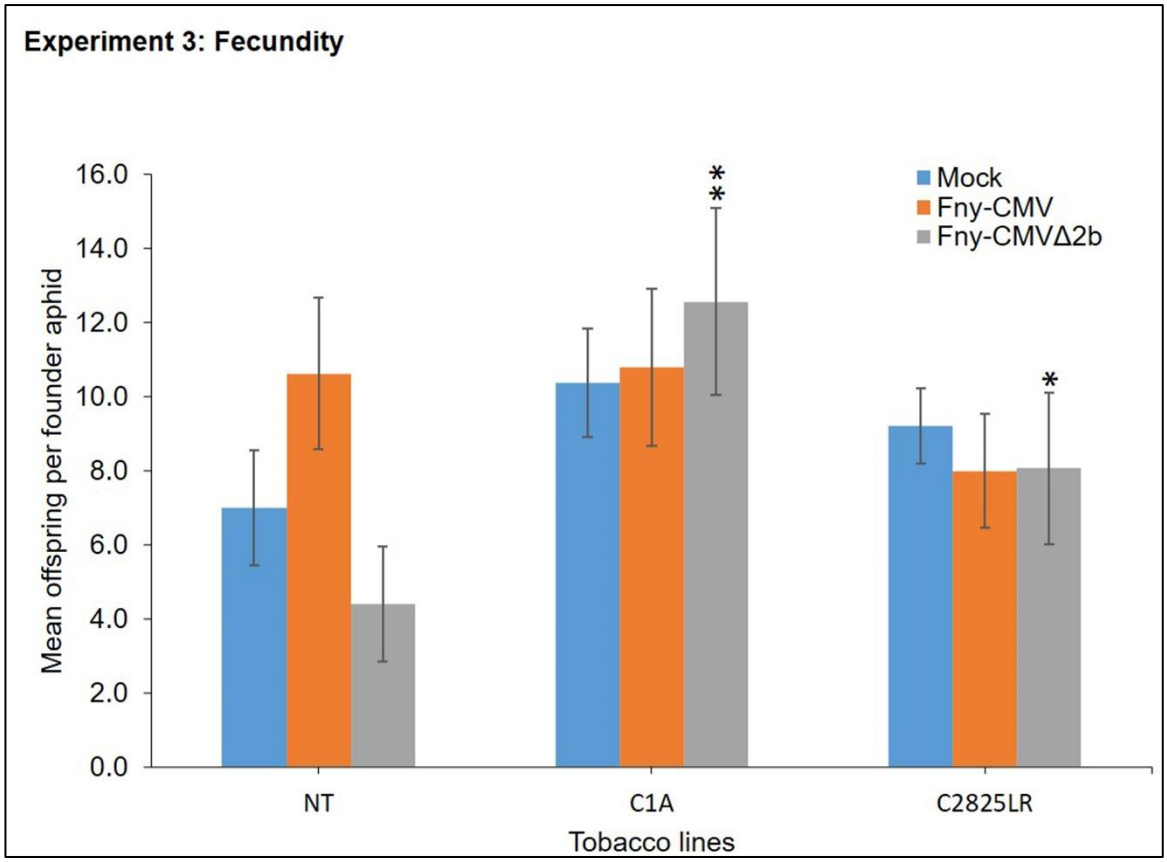


Figure 7.6. Fny-CMVΔ2b induced resistance to aphid reproduction on tobacco was abolished on *COI1*-silenced transgenic lines. The setup for the survival assay described in Fig.7.5 was maintained in this experiment. After fourteen days of confinement, the number of progenies per founder aphid were recorded per each experiment for the three independent experiments. Significant differences in fecundity between aphids placed on non-transgenic plants (mock-inoculated, Fny-CMV infected, or Fny-CMVΔ2b infected) and correspondingly treated plants of the *COI1*-silenced lines C1A and C2825LR are indicated by * $p < 0.05$ and ** $p<0.01$ (Kruskal-Wallis test). The error bars indicate SEM.

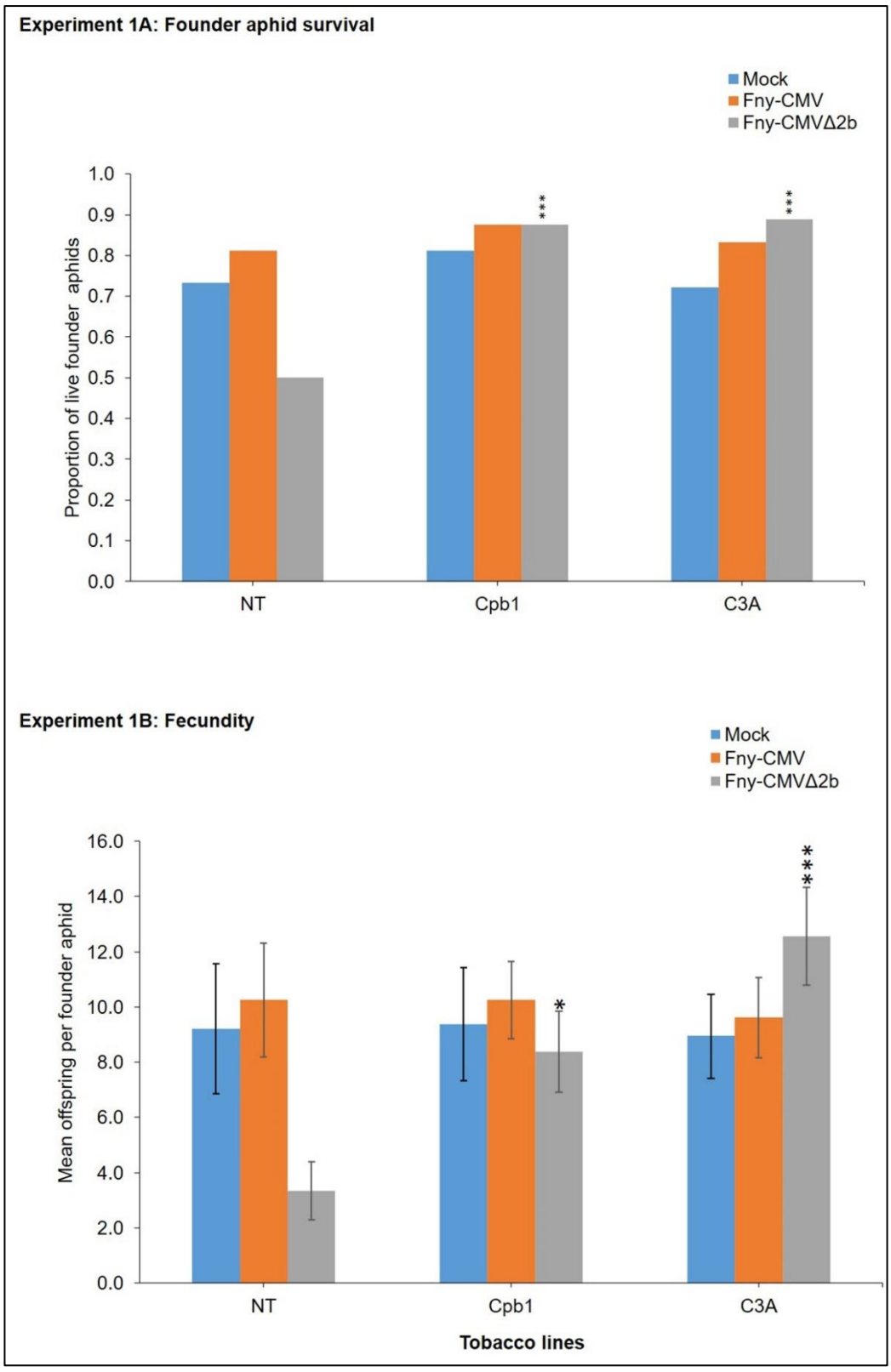


Figure 7.7. Fny-CMVΔ2b induced resistance to aphid reproduction and survival on tobacco was abolished on two additional *COI1*-silenced transgenic lines. Cbp1 and C3A are COI1-silenced tobacco lines (Table 7.1). Significant differences in survival (experiment 1A) and fecundity (Experiment 1B) between aphids placed on non-transgenic plants (mock-inoculated, Fny-CMV infected, or Fny-CMVΔ2b infected) and correspondingly treated plants of the *COI1*-silenced lines C*pb1* and C*3A* are indicated by * $p < 0.05$ and *** $p < 0.001$.

7.3 Discussion

The main goal of this study was to determine if the resistance against aphids induced in tobacco by Fny-CMVΔ2b is dependent upon JA-dependent defensive signal transduction. Using transgenic tobacco plants diminished in their response to JA, mortality and reproduction rates of aphids confined on virus-infected or mock-inoculated plants were evaluated. I showed the resistance to aphids induced in tobacco by Fny-CMVΔ2b, which was recently shown to be due to the activity of the Fny-CMV 1a protein (Tungadi et al., 2020), requires JA-dependent defensive signalling. Aphid survival and reproduction on *COI1*-silenced tobacco plants were not affected by Fny-CMVΔ2b infection. Founder aphids lived longer on *COI1*-silenced tobacco plants infected with Fny-CMVΔ2b compared to untransformed tobacco plants infected with this mutant virus. Similarly, aphids reproduced better on Fny-CMVΔ2b-infected *COI1*-silenced tobacco plants than on untransformed plants infected with Fny-CMVΔ2b. These results suggests that in plants of the Xanthi and Xanthi-nc tobacco varieties the 1a protein encoded by Fny-CMV triggers JA-dependent defensive signalling that results in production of as yet unidentified plant metabolite(s) toxic to *Myzus persicae* (Tungadi et al., 2020; Ziebell et al., 2011b). Previous work has shown that nicotine is unlikely to be one of these hypothesised aphid-toxic substances (Ziebell et al., 2011).

Several *COI1*-silenced tobacco lines were impaired in floral anther dehiscence and seed maturation. Defects in floral development were previously reported in different JA-deficient mutants of Arabidopsis (Xie et al., 1998), *Zea mays* (Yan et al., 2014) and tomato (L. Li et al., 2004). A similar observation in *COI1*-silenced Xanthi plants (this study) strongly suggests that the functions of COI1 in the development of floral structures and in seed maturation are highly conserved across plant species, both dicots and monocots. To my knowledge, this study is the first to demonstrate the importance of a functional *COI1* gene in tobacco reproduction.

Chapter 8. General discussion

8.1 The effects of CMV infection on aphid settling preference in tomato depend on the stage of infection

The choice of insect vectors to settle on virus-infected plants or uninfected plants significantly influences the epidemiology of insect-vectored plant viruses (Carr et al., 2020; Donnelly et al., 2019). A vector preference for infected plants dramatically affects how viruses spread between plant populations and landscapes (Carr et al., 2020; Gandon, 2018). Donnelly et al. (2019) showed that, for aphids, increased vector preference for settling on virus-infected plants is likely to encourage crowding and consequent birth of winged morphs, which could be important in the long-distance spread of nonpersistently transmitted viral pathogens to new hosts. Though several examples of modelling the relationship between viruses, insect vectors and host plants exist in the literature, most of these are based on a fixed stage of infection, usually a week post-infection (Cunniffe et al., 2015; Madden et al., 2000; Shaw et al., 2017). Yet we know that the infection process of many disease-causing pathogens progresses dynamically, and involves complex changes in the biochemistry of infected plants, which eventually influence insect vector behaviour (R. Hull, 2014; Zaitlin & Hull, 1987). For instance, squash plants infected with zucchini yellow mosaic virus (ZYMV) at 21 dpi were less attractive to aphids (*Aphis gossypii*), but at 7 dpi, aphids did not show any bias for either ZYMV-infected squash plants or uninfected plants (Blua & Perring, 1992). In tobacco, *Myzus persicae* did not differentiate between the odours of PVY-infected tobacco plants and those of uninfected plants at an early stage of infection (5 dpi). However, by 12 dpi aphids showed a statistically significant preference for the odours of infected tobacco plants compared to mock-inoculated plants (Liu et al., 2019).

To my knowledge, nobody has previously investigated the effects of CMV on tomato-aphid interactions and how they change as infection progressed. Results presented in Chapter 3 show that as CMV infection progresses, aphids find infected tomato plants increasingly repellent. As early as 3 dpi, CMV-infected tomato plants and mock-inoculated plants were equally attractive to *Myzus persicae* and *Macrosiphum euphorbiae*. But by 9 dpi and 21 dpi, aphids of both species preferred to settle on mock-inoculated plants compared to plants infected with CMV. The neutral response by *Myzus persicae* and *Macrosiphum euphorbiae* towards virus-infected plants and mock-inoculated plants during the early days of infection is likely to increase the population of infected plants sufficiently enough to launch or sustain a viral epidemic. In farming systems, especially in developing countries where planting time is not synchronised, an abundance of asymptomatic plants in newly established fields will act as inoculum reservoir for spread to new host plants by insect vectors (and other transmission vehicles).

Cryptic infection has been shown to drive disease outbreaks such as Witches' Broom disease of lime (Donkersley et al., 2019) and, most recently, COVID-19 in humans (Moghadas et al., 2020). As infection develops, the increasing repellence of infected plants will encourage migration of viruliferous aphids to uninfected plants. In agricultural contexts, the increasingly aphid-repellent properties of CMV-infected plants in later stages of infection may facilitate transmission within and between farms, driving epidemics of CMV.

CMV accumulated to high titres as early as 3 dpi and viral titres at different time points (3, 9, and 21 dpi) were similar even though symptoms progressed from no visible symptoms at 3 dpi to strong mosaic, stunting and shoestring-like leaves at 9 and 21 dpi. Though delayed viral symptom expression has been reported elsewhere (Lee et al., 2016; Schwach et al., 2005), a high titre of Fny-CMV at 3 dpi is fascinating. As shown in Section 3.2.2, at 3 dpi, infected tomato plants were desirable to aphids of both species at 1 and 24 h post-release. This finding suggests that Fny-CMV-infected tomato plants may be more "infectious" before any significant physiological changes in the tomato plant, influencing aphid choices within a system of infected tomato plants and healthy plants. Evidence of high viral titres in asymptomatic young plants raises fresh clues crucial in improving existing CMV monitoring programmes in greenhouses and open fields. Previously, most epidemiological models heavily relied on the incidence of the first discovery of visible symptoms (Parnell et al., 2012) and time to first discovery (Metz et al., 1983) with a few exceptions, especially among bacterial pathogens, where asymptomatic stage has been addressed (Chavez et al., 2016). Chavez and others (2016) showed that inclusion of the cryptic stage of Psyllid-transmitted citrus greening disease in forecasting greatly improved citrus greening disease tracking and minimised its impact. Since CMV can accumulate rapidly before symptom expression, investments in accurate early disease diagnostic methods such as ELISA and polymerase chain reaction may deliver significant gains in containing future CMV outbreaks.

Furthermore, preferential settling of *Macrosiphum euphorbiae* on CMV-infected plants at 21 dpi increased while that of *Myzus persicae* declined further (see Fig. 3.5). Several studies have shown that plant-related factors such as plant age and stressors, including pests and disease, influence gene expression. For instance, *Myzus persicae* infestation and foliage maturity were positively correlated in potato (Alvarez et al., 2014). Using choice bioassays, these authors showed that more aphids preferred to settle on old leaves pre-infested with aphids than young leaves, also pre-infested with aphids. Likewise, *Myzus persicae* fed and reproduced more on old potato leaves compared to young leaves. Alvarez and others attributed this behaviour to activation of plant defence. *Myzus persicae* infestation elicited a substantially higher proportion of differentially regulated genes in young leaves than in old

leaves. Examples of induced genes included several *PR* genes, *PR-1* and *PR-2*. *PR* genes have been observed to be induced in many pathogen-induced aphid-host interactions (Alvarez et al., 2014; Coppola et al., 2018; De Vos et al., 2005), hence their widespread use as markers in plant-pest and plant-pathogen studies. This finding may imply that as virus-infected tomato plants grow older, Fny-CMV induces specific changes in gene expression, thereby improving *Macrosiphum euphorbiae* response more than *Myzus persicae* to act as a transmission bridge between seasons, especially in open-field agriculture. In future, it will be essential to investigate whether there are substantial differences in feeding and virus transmission rates between the two aphid species between 9 and 21 dpi.

Finally, a likely tipping point for CMV transmission by *Myzus persicae* was established (Chapter 3: Fig. 3.5). The number of *Myzus persicae* migrating to and settling on virus-infected plants and mock-inoculated plants reached equilibrium at 5 dpi. After that, infected tomato plants became repellent to the vectors. *Macrosiphum euphorbiae* did not show any point of convergence in settling between virus-infected plants and mock-inoculated tomato plants. This is new information that could support timely implementation of scouting and aphid population management strategies for *Myzus persicae* within and between tomato gardens. Although efforts were not made to understand further the mechanism behind tipping point attainment for *Myzus persicae* but not *Macrosiphum euphorbiae*, differences in evolutionary adaption between generalist and specialist aphids could be responsible for this distinct behaviour between the two aphid species.

8.2 Visual cues are key drivers of aphid settling preference in the CMV-tomato system

Vision plays a central role for many insects when searching for suitable host plants (Döring & Chittka, 2007; Egelhaaf & Kern, 2002). Insect vision can be classified into achromatic or chromatic. The former is associated with perception of moving objects such as predators in the absence of light (Giurfa & Menzelt, 1997). Chromatic vision is particularly important to insects in perception of colour differences between flowers of different plant species (Döring & Chittka, 2007; Dyer et al., 2012; Imafuku, 2008; Osorio & Vorobyev, 2008). Chromatic and achromatic visual systems are important during host selection by many insects (Schnaitmann et al., 2020).

Findings in Chapter 3 suggest that CMV influences aphid phototactic responses towards tomato. In experiments performed under normal illumination, *Myzus persicae* and *Macrosiphum euphorbiae* aphids significantly chose mock-inoculated tomato plants over virus-infected plants at 9 dpi.

Remarkably, aphids of both species did not discriminate between virus-infected plants and mock-inoculated plants in trapping assays performed in the dark (Section 3.2.3; Fig.3.6 & Fig. 3.7). The aphid response in the dark agrees with the hypothesis that aphids cannot resolve objects at distances greater than 1.5 times their body length and have extremely low visual acuity (Döring et al., 2008), so their inherent capability to discriminate host plants is further compromised by the dark environment where these experiments were conducted. This suggests that light plays a significant role in the ability of aphids to distinguish between CMV-infected and non-infected tomato plants. To verify this further, I performed choice assays under different monochromatic light filters. It was found that particular lights influence the settling preference of aphids on tomato (Section 3.2.3; Fig.3.9 & Fig. 3.10). Aphids of both species preferentially chose mock-inoculated over virus-infected plants under blue and green light compared to when test plants were placed under yellow and red light. Aphids of both species did not show any bias for either infected or non-infected plants under yellow and red light. To this end, the mechanism(s) responsible for this spectral-specific aphid behaviour in CMV-tomato interaction remains unknown.

8.3 The CMV 2b protein influences aphid interactions with CMV-infected tomato plants

In Chapter 5, I determined that one of the two proteins encoded by RNA2 of Fny-CMV determines induction of aphid deterrence in tomato. Tomato plants infected with pseudorecombinants $L_1F_2L_3$ and $F_1F_2L_3$ discouraged aphid settling at 9 dpi. Further experiment with RNA 2 recombinant having the 2b protein from LS-CMV and Fny-CMVΔ2b deletion mutant showed that the 2b protein of Fny-CMV but not the LS-CMV 2b can induce strong resistance against aphids in virus-infected tomato plants. Taken together, these findings suggest that the properties of 2b proteins determines the ability of CMV strains to induce aphid deterrence in tomato.

The various effects of the multifunctional 2b protein on aphid-host interactions has been shown in several different plant species. Westwood *et al*. (2013) showed that the Fny-CMV 2b triggers antibiosis against aphids in Arabidopsis (ecotype Col-0) plants. If this was the resistance mechanism induced in an actual CMV infection, this would be deleterious for the virus since its vectors would be poisoned, and the virus could not be spread onwards. However, Fny-CMV prevents antibiosis induction via the interplay of the 2b, 1a and 2a proteins. The authors further demonstrated that Fny-CMV deters aphid feeding on plants infected with CMV by inducing biosynthesis of an anti-feedant substance. A similar anti-aphid feeding mechanism was found in CMV-infected squash plants (Carmo-Sousa et al., 2014; Mauck et al., 2010b). *Aphis gossypii* exhibited decreased phloem-feeding on virus-infected plants leading to migration of aphids to mock-inoculated plants (Carmo-Sousa et al., 2014). This particular

example of virus-induced deterrence to feeding was linked to an unidentified anti-feeding deterrent in squash plants. In tobacco, the 2b protein enhanced the emission of VOCs, but interestingly, aphids were not influenced by this change in VOC emission (Tungadi et al., 2017). Although it was found that Fny-CMV infection increased the emission of VOCs in both dark and light conditions (Chapter 4), it is less probable that olfactory cues drive aphid settling behaviour in tomato because aphids of neither species tested showed any bias between virus-infected and mock-inoculated, as discussed in Section 8.2. Possibly, in tomato, the Fny-CMV 2b protein induces the production of other unknown metabolites that diminish the ability of aphids to feed on the phloem of virus-infected tomato plants, which may encourage CMV transmission to healthy plants.

8.4 CMV-induced SA accumulation is unlikely to be influencing aphid settling on tomato

Before this study, the effect of SA on aphid preferential settling on tomato plants infected with CMV had not been explored. However, a recent study showed that CMV diminishes *Myzus persicae* fecundity and survival by enhancing SA accumulation in virus-infected tobacco plants (X. Shi et al., 2016c). In tomato, three different PVY strains (PVY^{O}, PVY^{NO}, and PVY^{NTN}) broadly encouraged *Macrosiphum euphorbiae* to settle on virus-infected tomato plants compared to control plants (Kersch-Becker et al., 2014). Those authors found that SA levels were significantly higher in virus-infected tomato plants compared to uninfected plants. Kersch-Becker and others (2014) concluded that strong virus-induced aphid resistance in PVY-infected plants was SA-dependent.

NahG-transgenic tomato plants, which are deficient in SA accumulation, were used to determine if SA influences settling preferences properties of *Myzus persicae* or *Macrosiphum euphorbiae* (Chapter 6). Aphids (*Myzus persicae*) consistently preferred to settle on *NahG*-transgenic tomato plants infected with Fny-CMV compared to untransformed plants infected with Fny-CMV (Fig 6.4; Chapter 6). In contrast, aphids of *Macrosiphum euphorbiae* did not show any bias in settlement on virus-infected *NahG*-transgenic tomato plants versus virus-infected untransformed plants (Fig. 6.5; Chapter 6). However, for neither aphid species did the ability of host plants to accumulate SA alter the tendency of aphids to migrate towards and settle on mock-inoculated plants rather than on infected plants. Thus, this signal molecule is unlikely play a primary role in conditioning the induction of repellence to aphids by Fny-CMV in tomato. In contrast, SA appears to play an important role in ameliorating the disease symptoms engendered by infection with CMV.

8.5 JA-dependent signalling is required for induction of aphid resistance in tobacco plants infected with Fny-CMVΔ2b

In many experiments Fny-CMV infection of Xanthi tobacco plants improves aphid performance, but infection with Fny-CMVΔ2b infection consistently induces strong resistance to *Myzus persicae* (Tungadi et al., 2020; Ziebell et al., 2011b). Consistent with this finding, I found that aphids of *Myzus persicae* produced more offspring and survived better on Fny-CMV-infected tobacco plants (cv. Xanthi) than plants infected with Fny-CMVΔ2b, as has been noted in other work reported from this laboratory (Tungadi et al., 2020; Ziebell et al., 2011). This finding is consistent with previous work showing that the CMV 2b protein and infection by Fny-CMV and LS-CMV inhibit responses to JA, a hormone that is important for resistance to insects (Lewsey et al., 2010; Westwood et al., 2013, 2014; Wu et al., 2017). This suggested that the induction by the 1a protein of anti-aphid resistance during infection with Fny-CMVΔ2b might be a JA-dependent process but that during infection by wild-type Fny-CMV, the 2b protein inhibits JA-dependent signalling, preventing 1a-induced aphid resistance from being triggered. My work with transgenic tobacco plants with diminished accumulation of the *COI1* transcript support this idea. However, it does not exclude the possibility that direct interactions between the 2b and 1a proteins recently demonstrated by Watt et al. (2020), might also play a role in suppressing the induction of resistance to aphids during infection of Xanthi tobacco by Fny-CMV. In Figure 8.1, I have summarised three possibilities in which the 1a and 2b proteins might be interacting to affect aphid resistance in tobacco plants (cv. Xanthi) infected with Fny-CMV or its mutant virus, Fny-CMVΔ2b.

However, it is noteworthy that the effects of Fny-CMVΔ2b on aphid survival and reproduction seen in Xanthi tobacco are not seen in all tobacco cultivars. Previous data by my lab colleagues (Joyce, Tungadi and others) showed that Fny-CMVΔ2b infection does not induce strong aphid resistance in plants of the tobacco cultivar Petit Havana SR1. Knock-down of *COI1* transcript accumulation in transgenic Petit Havana SR1 plants did not significantly improve *Myzus persicae* survival or reproduction. It will be interesting in the future to look at these host-specific differences, as well as virus strain-specific differences in the outcomes of CMV-host-vector interactions, since they may provide both a window to the mechanisms underlying these interactions and provide epidemiological insights.

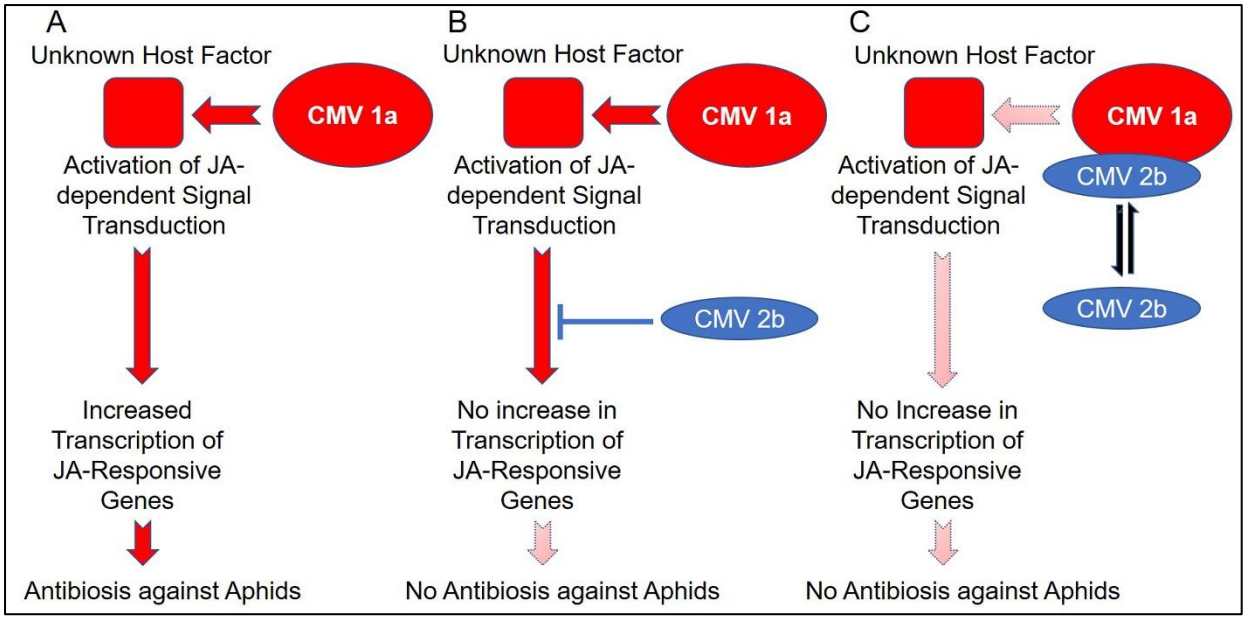


Figure 8.1. Models for the effects on aphid resistance of the cucumber mosaic virus (CMV) proteins 1a and 2b in Xanthi tobacco plants infected with Fny-CMV or Fny-CMVΔ2b. In tobacco (cv. Xanthi) plants infected with Fny-CMVΔ2b, a strong resistance is induced against aphids that decreases their survival and reproduction (Tungadi et al., 2020; Ziebell et al., 2011). Tungadi et al. (2020) showed that in plants infected with Fny-CMVΔ2b, it is likely the 1a protein that stimulates the induction of aphid resistance, and in this study (Chapter 7), it was shown that induction of resistance requires a functional pathway for jasmonate (JA) perception and signal transduction. This suggests the model shown in A, in which the CMV 1a protein interacts with a host factor that triggers the induction of JA-dependent signalling and the induction of JA-responsive genes, including those required for the synthesis of (unknown) anti-aphid metabolites by the plant. Antibiosis is not induced in Fny-CMV-infected plants, suggesting that the 2b protein inhibits induction of this form of resistance against aphids (as suggested originally by Ziebell et al., 2011). It is proposed here that the 2b protein can inhibit the induction of antibiosis by the 1a protein in two ways. In B, the 2b protein inhibits JA-mediated signal transduction and gene expression through its effects on JAZ proteins and/or on the turnover of JA-regulated transcripts, and in C, the recently discovered direct interaction between the 2b and 1a proteins (Watt et al., 2020), diminishes the amount of free 1a protein available for interaction with the proposed host factor. It should be noted that the models depicted in B and C are not mutually exclusive.

8.6 Future work

8.6.1 Transcriptomic analysis of tomato at different timepoints post-infection with CMV

I showed that tomato plants infected with Fny-CMV are more attractive to aphids at 3 dpi but repellent at 9 dpi and beyond (see Chapter 3). Assumptions that odours of virus-infected tomato plants drove the change in aphid preference between the two timepoints were proved untrue by trapping experiments in the dark. Aphids of both species in the dark do not distinguish the odours of infected plants from those of mock-inoculated plants. In literature, examples exist showing that viruses induce significant changes in the expression of host genes, which may alter insect behaviour. Genome-wide transcriptomic analysis in the tobacco-CMV system found over 700 genes functionally linked to defence signalling transduction, reactive oxygen species and stress resistance were either induced or repressed between 1 dpi and 5 dpi (Liu et al., 2019). The authors also reported variations in CMV-induced gene expression between resistant and susceptible tobacco genotypes: more genes were activated by CMV in resistant tobacco plants than susceptible plants. A microarray study of the CMV-Arabidopsis interaction by Westwood and others (2013) found that Fny-CMV significantly changed the expression of 920 genes, many of which were essential in defence-related signalling against aphids (Westwood et al., 2013). These findings provide a basis for testing whether the differential aphid settling preference on virus-infected tomato plants at 3 dpi and 9 dpi results from sequential variations in gene expression during Fny-CMV infection.

8.6.2 Investigate the effect of CMV on the feeding behaviour of *Myzus persicae* and *Macrosiphum euphorbiae*

CMV infection influenced aphid feeding behaviour in a species-specific manner (Wamonje et al., 2020). Wamonje and others showed that *Myzus persicae*, a generalist aphid, experienced difficulties probing phloem of CMV- infected common bean plants. Phloem feeding by *Aphis fabae*, a specialist aphid, was also sharply decreased. Results in Chapter 3 show that very few *Macrosiphum euphorbiae* (a solanaceous specialist) aphids, compared to *Myzus persicae* (a generalist aphid), settled on CMV-infected tomato plants at 9 dpi. Though aphid feeding was not analysed by EPG (due to time constraints), reduced aphid settling on virus-infected plants may indicate indirect evidence for inhibited aphid feeding. It would be of interest to examine the feeding behaviour of both aphid species on CMV-infected tomato plants using EPG. Such data would provide additional information on the possible mechanisms used by CMV to foster its transmission in space and time.

8.6.3 Examine the rate of CMV transmission by aphids under light and dark conditions

I have shown that aphid settling patterns between CMV infected tomato plants and mock-inoculated plants vary greatly between light and dark treatments. CMV infected tomato plants are repellent to aphids in the light, but this changed in the dark. The inherent ability of aphids to choose between infected and mock-inoculated plants was abolished in the dark. This raises the question of whether CMV acquisition and transmission by aphids also differ between light and dark conditions. The effect of light on CMV acquisition and transmission by aphids in tomato has not been studied before. It would be of epidemiological significance to test CMV transmission efficiency by aphids under light and dark treatment in microcosm, macrocosm and field-level experiments.

8.6.4 Explore whether hormonal crosstalk between SA and JA modulates the interaction between CMV, aphids and tomato plants

Increased SA accumulation does not fully explain enhanced resistance against aphids in tomato plants infected with Fny-CMV (Chapter 6), suggesting that other host factors may be 'collaborating' with SA to induce strong resistance. Recent data indicate that JA is required for anti-feeding on CMV-infected Arabidopsis plants (Tungadi et al., 2021). In many ways, CMV-triggered effects on aphid-Arabidopsis interactions are similar to those in tomato. For instance, *Myzus persicae* settling data showed that Arabidopsis plants infected with Fny-CMV changed from being attractive to aphids at 3 dpi to repellence at 9 dpi and 21 dpi (Murphy et al., *Unpublished*). I found similar CMV-induced effects on aphid settling preference in tomato. Therefore, future studies should examine if JA-related defence signalling is required to induce strong resistance against aphids in virus-infected tomato plants. Such research will contribute towards a complete understanding of the mechanisms devised by CMV to shape aphid behaviour that promotes its transmission.

8.6.5 Investigate how the CMV 1a protein interacts with the tobacco host factors to induce resistance against *Myzus persicae*

Previous studies showed that on Fny-CMVΔ2b infected tobacco plants, the survival and reproduction of aphids (*Myzus persicae*) are adversely affected (Tungadi et al., 2020; Ziebell et al., 2011). In Chapter 8, transgenic tobacco plants diminished in JA perception by knock-down of *COI1* transcript accumulation were used to determine if JA-dependent signalling played a role in induction of this resistance.

It was found that aphid reproduction and survival on Fny-CMVΔ2b-infected transgenic plants were higher than on Fny-CMVΔ2b-infected non-transgenic tobacco plants. This result shows that JA-dependent signalling is required for CMVΔ2b-induced resistance to aphids in tobacco.

Tungadi and colleagues (2020) showed that the RNA1 of Fny-CMV encodes the elicitor that activates the induction of resistance to aphids in plants infected with Fny-CMVΔ2b. They concluded, therefore that the 1a protein, the sole translation product of RNA1, was the elicitor, although they could not entirely rule out the less likely possibility that some characteristic of the RNA1 molecule itself might play a role. Recently, protein-protein pull-down assays combined with confocal scanning laser microscopy of fluorescently labelled proteins were used to investigate the interactions of the 1a and 2b proteins of CMV. The results of this study suggested that the 1a protein regulates the 2b-AGO1 interaction by limiting the amount of the 2b protein available to bind AGO1 (Watt et al., 2020). Other authors have shown that the 1a protein modulates CMV-aphid interaction in different tobacco genotypes (Tungadi et al., 2020; Yoon & Palukaitis, 2021). Transgenic Samsun tobacco plants expressing CMV 1a protein showed increased expression of a resistance-inducing transcription factor called signalling hub effector 1 (SHE1) (Yoon & Palukaitis, 2021). SHE1 had previously been reported to activate host resistance against TMV, PVX and PVY, but SHE1 induction is independent of SA, JA and ethylene (Fischer & Dröge-Laser, 2004). It would be interesting to determine whether the interaction between the 1a protein and host factors in tobacco (e.g., SHE1) is essential in inducing CMV-induced resistance against aphids.

8.7 Conclusions

I reported changes over time in the effects of CMV on aphid-host interactions in tomato. *Myzus persicae* aphids are preferentially attracted to CMV-infected plants at 3 dpi but prefer to settle on mock-inoculated plants rather than on CMV-infected plants by 9 dpi and at subsequent time points. *Macrosiphum euphorbiae* aphids showed no bias for settling on mock-inoculated over CMV-infected plants at 3 dpi (in contrast to *Myzus persicae*) but behaved similarly to *Myzus persicae* (i.e., preferred mock-inoculated plants) from 9 dpi onwards.

Visual cues affected aphid behaviour in the CMV-tomato system more than volatile cues, in contrast to what has been discovered in the CMV-Arabidopsis and CMV-cucurbit systems. Using light filters of different wavelengths, it was found that blue and green light, but not yellow and red light, enhances aphid settling preference between CMV-infected tomato plants and mock-inoculated plants, indicating

that specific lights of a particular wavelength could act as stimuli CMV-aphid-tomato interactions. However, the mechanism that drives light-induced aphid behaviour remains to be determined.

I also found that the Fny-CMV 2b protein is the most plausible candidate viral factor regulating tomato-aphid interactions, and aphid settling data from *NahG*-transgenic tomato plants infected with CMV imply that SA-associated defence pathway as a possible mechanism of modulating CMV transmission by specific aphid species, in this case *Myzus persicae*. Finally, this study demonstrated that the CMVΔ2b-induced inhibition of aphid fecundity and survival on Xanthi tobacco plants is dependent on JA-mediated signalling. However, this is not conserved between all tobacco cultivars; aphid performance on *NahG*-transgenic tobacco plants of the Samsun cultivar was unaffected (Tungadi et al., *Unpublished*). This suggests that other host defensive signalling pathways might regulate CMV-aphid-tobacco interactions.

In summary, this study demonstrated novel wide-ranging effects of CMV on the preference and settling properties of two aphid species with distinct host ranges, which could profoundly affect CMV acquisition and transmission in tomato. The study also provides evidence that JA rather than SA influences aphid-plant interactions. This study further increases understanding of CMV-aphid interactions in tomato, which could help develop future epidemiological models and design timely integrated disease management measures.

References

Abe, H., Tomitaka, Y., Shimoda, T., Seo, S., Sakurai, T., Kugimiya, S., Tsuda, S. & Kobayashi, M. (2012). Antagonistic plant defence system regulated by phytohormones assists interactions among vector insect, thrips and a tospovirus. *Plant and Cell Physiology*, *53*(1), 204-212.

Agbogba, B. C., & Powell, W. (2007). Effect of the presence of a nonhost herbivore on the response of the aphid parasitoid *Diaeretiella rapae* to host-infested cabbage plants. *Journal of Chemical Ecology*, *33*(12), 2229-2235.

Åhman, I., Kim, S. Y., & Zhu, L. H. (2019). Plant genes benefitting aphids—potential for exploitation in resistance breeding. *Frontiers in Plant Science*, *10*, 1452.

Ahn, I. P., Kim, S., Lee, Y. H., & Suh, S. C. (2007). Vitamin B1-induced priming is dependent on hydrogen peroxide and the NPR1 gene in Arabidopsis. *Plant Physiology*, *143*(2), 838-848.

Ajayi, B. O., & Dewar, A. M. (1983). The effect of barley yellow dwarf virus on field populations of the cereal aphids, *Sitobion avenae* and *Metopolophium dirhodum*. *Annals of Applied Biology*, *103*(1), 1-11.

Alexander, H. M., Mauck, K. E., Whitfield, A. E., Garrett, K. A., & Malmstrom, C. M. (2014). Plant-virus interactions and the agro-ecological interface. *European Journal of Plant Pathology*, *138*(3), 529–547.

Ali, A., & Kobayashi, M. (2010). Seed transmission of cucumber mosaic virus in pepper. *Journal of Virological Methods*, *163*(2), 234–237.

Alonso Chavez, V., Parnell, S., & Van Den Bosch, F. (2016). Monitoring invasive pathogens in plant nurseries for early-detection and to minimise the probability of escape. *Journal of Theoretical Biology*, *407*, 290–302.

Alvarez, A. E., Alberti D'Amato, A. M., Tjallingii, W. F., Dicke, M., & Vosman, B. (2014). Response of *Solanum tuberosum* to *Myzus persicae* infestation at different stages of foliage maturity. *Insect Science*, *21*(6), 727–740.

Anderson, P. K., Cunningham, A. A., Patel, N. G., Morales, F. J., Epstein, P. R., & Daszak, P. (2004). Emerging infectious diseases of plants: Pathogen pollution, climate change and agrotechnology drivers. *Trends in Ecology and Evolution*, *19*(10), 535–544.

Andret-Link, P., Schmitt-Keichinger, C., Demangeat, G., Komar, V., & Fuchs, M. (2004). The specific transmission of Grapevine fanleaf virus by its nematode vector *Xiphinema index* is solely determined by the viral coat protein. *Virology*, *320*(1), 12–22.

Archetti, M. (2000). The origin of autumn colours by coevolution. *Journal of Theoretical Biology*, *205*(4), 625–630.

Archetti, M., & Brown, S. P. (2004). The coevolution theory of autumn colours. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(1545), 1219-1223.

Archetti, M., & Leather, S. R. (2005). A test of the coevolution theory of autumn colours: Colour preference of *Rhopalosiphum padi* on *Prunus padus*. *Oikos*, *110*(2), 339–343.

Arie, T., Takahashi, H., Kodama, M., & Teraoka, T. (2007). Tomato as a model plant for plant-pathogen interactions. *Plant Biotechnology*, *24*(1), 135–147.

Arimura, G. I., Kost, C., & Boland, W. (2005). Herbivore-induced, indirect plant defences. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, *1734*(2), 91–111.

Atamian, H. S., Eulgem, T., & Kaloshian, I. (2012). *SlWRKY70* is required for *Mi-1*-mediated resistance to aphids and nematodes in tomato. *Planta*, *235*(2), 299-309.

Auclair, J. L. (1969). Nutrition of plant-sucking insects on chemically defined diets. *Entomologia Experimentalis et Applicata*, *12*(5), 623-641.

Auclair, Jacques L. (1963). Aphid Feeding and Nutrition. *Annual Review of Entomology*, *8*(1), 439–490.

Baebler, Š., Stare, K., Kovač, M., Blejec, A., Prezelj, N., Stare, T., Kogovšek, P., Pompe-Novak, M., Rosahl, S., Ravnikar, M. and Gruden, K. (2011). Dynamics of responses in compatible potato-Potato virus Y interaction are modulated by salicylic acid. *PloS One*, *6*(12), e29009.

Banerjee, S., Hess, D., Majumder, P., Roy, D., & Das, S. (2004). The interactions of *Allium sativum* leaf agglutinin with a chaperonin group of unique receptor protein isolated from a bacterial endosymbiont of the mustard aphid. *Journal of Biological Chemistry*, *279*(22), 23782–23789.

Banik, M. T., & Zitter, T. A. (1990). Determination of cucumber mosaic virus titer in muskmelon by enzyme-linked immunosorbent assay and correlation with aphid transmission. *Plant Disease*, *74*(11), 857-859.

Bashir, M., & Hampton, R. O. (1996). Detection and identification of seed-borne viruses from cowpea (*Vigna unguiculata* (L.) Walp.) germplasm. *Plant Pathology*, *45*(1), 54-58.

Beale, M. H., Birkett, M. A., Bruce, T. J. A., Chamberlain, K., Field, L. M., Huttly, A. K., Martin, J. L., Parker, R., Phillips, A. L., Pickett, J. A., Prosser, I. M., Shewry, P. R., Smart, L. E., Wadhams, L. J., Woodcock, C. M., & Zhang, Y. (2006). Aphid alarm pheromone produced by transgenic plants affects aphid and parasitoid behaviour. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(27), 10509–10513.

Blackman, R. L., & Eastop, V. F. (2000). *Aphids on the world's crops: an identification and information guide* (No. Ed. 2). John Wiley & Sons Ltd, New York, USA.

Blanc, S., Ammar, E. D., Garcia-Lampasona, S., Dolja, V. V., Llave, C., Baker, J., & Pirone, T. P. (1998). Mutations in the potyvirus helper component protein: Effects on interactions with virions and aphid stylets. *Journal of General Virology*, *79*(12), 3119–3122.

Blevins, T., Rajeswaran, R., Shivaprasad, P. V., Beknazariants, D., Si-Ammour, A., Park, H.-S., Vazquez, F., Robertson, D., Meins, F., Hohn, T., & Pooggin, M. M. (2006). Four plant Dicers mediate viral small RNA biogenesis and DNA virus-induced silencing. *Nucleic Acids Research*, *34*(21), 6233–6246.

Blua, M. J., & Perring, T. M. (1992). Alatae production and population increase of aphid vectors on virus-infected host plants. *Oecologia*, *92*(1), 65–70.

Boissot, N., Schoeny, A., & Vanlerberghe-Masutti, F. (2016). *Vat*, an amazing gene conferring resistance to aphids and viruses they carry: from molecular structure to field effects. *Frontiers in Plant Science*, *7*, 1420.

Boonham, N., Kreuze, J., Winter, S., van der Vlugt, R., Bergervoet, J., Tomlinson, J., & Mumford, R. (2014). Methods in virus diagnostics: From ELISA to next generation sequencing. *Virus Research*, *186*, 20–31.

Boonham, N., Tomlinson, J., & Mumford, R. (2016). *Molecular methods in plant disease diagnostics: Principles and protocols*. Wallingford: CABI

Boquel, S., Ameline, A., & Giordanengo, P. (2011). Assessing aphids potato virus Y-transmission efficiency: A new approach. *Journal of Virological Methods*, *178*(1–2), 63–67.

Boquel, S., Delayen, C., Couty, A., Giordanengo, P., & Ameline, A. (2012). Modulation of aphid vector activity by potato virus Y on *in vitro* potato plants. *Plant Disease*, *96*(1), 82–86.

Bouvaine, S., Boonham, N., & Douglas, A. E. (2011). Interactions between a luteovirus and the GroEL chaperonin protein of the symbiotic bacterium Buchnera aphidicola of aphids. *Journal of General Virology*, *92*(6), 1467-1474.

Bos, L. (1983). *Introduction to plant virology*. Longman, London, UK.

Braam, J. (2004). In touch: plant responses to mechanical stimuli. *New Phytologist*, *165*(2), 373–389.

Braendle, C., Davis, G. K., Brisson, J. A., & Stern, D. L. (2006). Wing dimorphism in aphids. *Heredity*, *97*, 192–199.

Bragard, C., Caciagli, P., Lemaire, O., Lopez-Moya, J. J., Macfarlane, S., Peters, D., Susi, P., & Torrance, L. (2013). Status and prospects of plant virus control through interference with vector transmission. *Annual Review of Phytopathology*, *51*, 177–201.

Brault, V., Uzest, M., Monsion, B., Jacquot, E., & Blanc, S. (2010). Aphids as transport devices for plant viruses. *Comptes Rendus Biologies*, *333*(6-7), 524-538.

Bravo-Cazar, A. L. (2018). *Disrupting insect-mediated transmission of plant viruses.* Doctoral thesis. University of Cambridge. https://doi.org/10.17863/CAM.40681

Bricchi, I., Bertea, C. M., Occhipinti, A., Paponov, I. A., & Maffei, M. E. (2012). Dynamics of membrane potential variation and gene expression induced by *Spodoptera littoralis*, *Myzus persicae*, and *Pseudomonas syringae* in Arabidopsis. *PLoS One*, *7*(10), e46673.

Burgyán, J., & Havelda, Z. (2011). Viral suppressors of RNA silencing. *Trends in Plant Science*, *16*(5), 265–272.

Cao, H., Bowling, S. A., Gordon, A. S., & Dong, X. (1994). Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. *The Plant Cell*, *6*(11), 1583-1592.

Carmo-Sousa, M., Moreno, A., Garzo, E., & Fereres, A. (2014). A non-persistently transmitted-virus induces a pull-push strategy in its aphid vector to optimize transmission and spread. *Virus Research*, *186*, 38–46.

Carr, J. P., Gal-On, A., Palukaitis, P., & Zaitlin, M. (1994). Replicase-mediated resistance to cucumber mosaic virus in transgenic plants involves suppression of both virus replication in the inoculated leaves and long-distance movement. *Virology*, *199*(2), 439-447.

Carr, J. P., Lewsey, M. G., & Palukaitis, P. (2010). Signalling in induced resistance. *Advances in Virus Research*, *76*, 57-121.

Carr, J. P., Murphy, A. M., Tungadi, T., & Yoon, J. Y. (2019). Plant defence signals: Players and pawns in plant-virus-vector interactions. *Plant Science*, *279*, 87-95.

Carr, J.P., Tungadi, T., Donnelly, R., Bravo-Cazar, A., Rhee, S.J., Watt, L.G., Mutuku, J.M., Wamonje, F.O., Murphy, A.M., Arinaitwe, W., & Pate, A.E. (2020). Modelling and manipulation of aphid-mediated spread of non-persistently transmitted viruses. *Virus Research*, *277*,197845.

Carvalho, F. P. (2017). Pesticides, environment, and food safety. *Food and Energy Security*, *6*(2), 48–60.

Castle, S. J., & Berger, P. H. (1993). Rates of growth and increase of *Myzus persicae* on virus-infected potatoes according to type of virus-vector relationship. *Entomologia Experimentalis et Applicata*, *69*(1), 51–60.

Chen, B., & Francki, R. I. B. (1990). Cucumovirus transmission by the aphid *Myzus persicae* is determined solely by the viral coat protein. *Journal of General Virology*, *71*(4), 939–944.

Chikh-Rouhou, H., Ben Belgacem, A. M., Sta-Baba, R., Tarchoun, N., & Gómez-Guillamón, M. L. (2019). New source of resistance to *Aphis gossypii* in Tunisian melon accessions using phenotypic and molecular marker approaches. *Phytoparasitica*, *47*(3), 405–413.

Chinchilla, D., Bauer, Z., Regenass, M., Boller, T., & Felix, G. (2006). The Arabidopsis receptor kinase FLS2 binds flg22 and determines the specificity of flagellin perception. *Plant Cell*, *18*(2), 465–476.

Chini, A., Fonseca, S., Fernández, G., Adie, B., Chico, J. M., Lorenzo, O., García-Casado, G., López-Vidriero, I., Lozano, F. M., Ponce, M. R., Micol, J. L., & Solano, R. (2007). The JAZ family of repressors is the missing link in jasmonate signalling. *Nature*, *448*(7154), 666–671.

Chini, A., Gimenez-Ibanez, S., Goossens, A., & Solano, R. (2016). Redundancy and specificity in jasmonate signalling. *Current Opinion in Plant Biology*, *33*, 147–156.

Choi, S. K., Palukaitis, P., Min, B. E., Lee, M. Y., Choi, J. K., & Ryu, K. H. (2005). Cucumber mosaic virus 2a polymerase and 3a movement proteins independently affect both virus movement and the timing of symptom development in zucchini squash. *Journal of General Virology*, *86*(4), 1213–1222.

Chomczynski, P., & Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analytical Biochemistry*, *162*(1), 156–159.

Chong, J., Wishart, D. S., & Xia, J. (2019). Using MetaboAnalyst 4.0 for Comprehensive and integrative metabolomics data analysis. *Current Protocols in Bioinformatics*, *68*(1), 1–128.

Chung, H. S., Niu, Y., Browse, J., & Howe, G. A. (2009). Top hits in contemporary JAZ: An update on jasmonate signaling. *Phytochemistry*, *70*(13–14), 1547–1559.

Cillo, F., Mascia, T., Pasciuto, M. M., & Gallitelli, D. (2009). Differential effects of mild and severe Cucumber mosaic virus strains in the perturbation of microRNA-regulated gene expression in tomato map to the 3' sequence of RNA 2. *Molecular plant-microbe interactions*, *22*(10), 1239-1249.

Cillo, F., Roberts, I. M., & Palukaitis, P. (2002). *In situ* localization and tissue distribution of the replication-associated proteins of cucumber mosaic virus in tobacco and cucumber. *Journal of Virology*, *76*(21), 10654–10664.

Cipollini, D., Enright, S., Traw, M. B., & Bergelson, J. (2004). Salicylic acid inhibits jasmonic acid-induced resistance of *Arabidopsis thaliana* to *Spodoptera exigua*. *Molecular Ecology*, *13*(6), 1643–1653.

Clark, M. F., & Adams, A. N. (1977). Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology*, *34*(3), 475–483.

Coombe, P. E. (1982). Visual behaviour of the greenhouse whitefly, *Trialeurodes vaporariorum*. *Physiological Entomology*, *7*(3), 243-251.

Coppola, M., Manco, E., Vitiello, A., Di Lelio, I., Giorgini, M., Rao, R., Pennacchio, F., & Digilio, M. C. (2018). Plant response to feeding aphids promotes aphid dispersal. *Entomologia Experimentalis et Applicata*, *166*(5), 386–394.

Coppola, V., Coppola, M., Rocco, M., Digilio, M. C., D'Ambrosio, C., Renzone, G., Martinelli, R., Scaloni, A., Pennacchio, F., Rao, R., & Corrado, G. (2013). Transcriptomic and proteomic analysis of a compatible tomato-aphid interaction reveals a predominant salicylic acid-dependent plant response. *BMC Genomics*, *14*(1).

Csorba, T., Kontra, L., & Burgyán, J. (2015). Viral silencing suppressors: Tools forged to fine-tune host-pathogen coexistence. *Virology*, *479*, 85-103.

Cunniffe, N. J., Koskella, B., E. Metcalf, C. J., Parnell, S., Gottwald, T. R., & Gilligan, C. A. (2015). Thirteen challenges in modelling plant diseases. *Epidemics*, *10*, 6–10.

De Bon, H., Huat, J., Parrot, L., Sinzogan, A., Martin, T., Malézieux, E., & Vayssières, J. F. (2014). Pesticide risks from fruit and vegetable pest management by small farmers in sub-Saharan Africa. A review. *Agronomy for Sustainable Development*, *34*(4), 723-736.

De Moraes, C. M., Mescher, M. C., & Tumlinson, J. H. (2001). Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature*, *410*(6828), 577–579.

De Vos, M., Van Oosten, V.R., Van Poecke, R.M., Van Pelt, J.A., Pozo, M.J., Mueller, M.J., Buchala, A.J., Métraux, J.P., Van Loon, L.C., Dicke, M., & Pieterse, C.M. (2005). Signal signature and transcriptome changes of Arabidopsis during pathogen and insect attack. *Molecular Plant-Microbe Interactions*, *18*(9), 923-937.

Delaney, T.P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kessmann, H., Ward, E., & Ryals, J. (1994). A central role of salicylic acid in plant disease resistance. *Science*, *266*(5188),1247-1250.

Delp, G., Gradin, T., Åhman, I., & Jonsson, L. M. V. (2009). Microarray analysis of the interaction between the aphid *Rhopalosiphum padi* and host plants reveals both differences and similarities between susceptible and partially resistant barley lines. *Molecular Genetics and Genomics*, *281*(3), 233–248.

Devonshire, A. L., & Sawicki, R. M. (1979). Insecticide-resistant *Myzus persicae* as an example of evolution by gene duplication. *Nature*, *280*(5718), 140-141.

Dietzgen, R. G., Callaghan, B., & Campbell, P. R. (2007). Biology and genomics of lettuce necrotic yellows virus. *Plant Viruses*, *1*(1), 85-92.

Dietzgen, R. G., Mann, K. S., & Johnson, K. N. (2016). Plant virus–insect vector interactions: Current and potential future research directions. *Viruses*, *8*(11), 303.

Dixon, A. F. G. (1977). Aphid ecology: life cycles, polymorphism, and population regulation. *Annual Review of Ecology and Systematics*, *8*(1), 329-353.

Dogimont, C., Bendahmane, A., Chovelon, V., & Boissot, N. (2010). Host plant resistance to aphids in cultivated crops: genetic and molecular bases, and interactions with aphid populations. *Comptes Rendus Biologies*, *333*(6-7), 566-573.

Dombrecht, B., Gang, P. X., Sprague, S. J., Kirkegaard, J. A., Ross, J. J., Reid, J. B., Fitt, G. P., Sewelam, N., Schenk, P. M., Manners, J. M., & Kazana, K. (2007). MYC2 differentially modulates diverse jasmonate-dependent functions in Arabidopsis. *Plant Cell*, *19*(7), 2225–2245.

Donkersley, P., Silva, F. W., Alves, M. S., Carvalho, C. M., Al-Sadi, A. M., Elliot, S. L., & Topolovec-Pintarić, S. (2019). Asymptomatic phytoplasma reveal a novel and troublesome infection. *Plant Diseases-Current Threats and Management Trends*, Topolovec-Pintarić, S., Ed, pp. 1–20. IntechOpen.

Donnelly, R., Cunniffe, N. J., Carr, J. P., & Gilligan, C. A. (2019). Pathogenic modification of plants enhances long-distance dispersal of nonpersistently transmitted viruses to new hosts. *Ecology*, *100*(7), e02725.

Doring, T. F., & Kirchner, S. M. (2007). Preliminary characterisation of the spectral sensitivity in the cabbage aphid with electroretinogram recordings (Hemiptera: Aphididae). *Entomologia Generalis*, *30*(3), 233–234.

Döring, T. F., & Röhrig, K. (2016). Behavioural response of winged aphids to visual contrasts in the field. *Annals of Applied Biology*, *168*(3), 421–434.

Döring, Thomas F. (2014). How aphids find their host plants, and how they don't. *Annals of Applied Biology*, *165*(1), 3–26.

Döring, T. F., Archetti, M., & Hardie, J. (2009). Autumn leaves seen through herbivore eyes. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1654), 121-127.

Döring, Thomas Felix, & Chittka, L. (2007). Visual ecology of aphids—a critical review on the role of colours in host finding. *Arthropod-Plant Interactions*, *1*(1), 3–16.

Döring, Tnghomas F., Hardie, J., Leather, S., Spaethe, J., & Chittka, L. (2008). Can aphids play football? *Antenna*, *32*(3), 146–148.

Drucker, M., Froissart, R., Hébrard, E., Uzest, M., Ravallec, M., Espérandieu, P., Mani, J.C., Pugniere, M., Roquet, F., Fereres, A., & Blanc, S. (2002). Intracellular distribution of viral gene products regulates a complex mechanism of cauliflower mosaic virus acquisition by its aphid vector. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(4), 2422-2427.

Du, M., Zhao, J., Tzeng, D. T. W., Liu, Y., Deng, L., Yang, T., Zhai, Q., Wu, F., Huang, Z., Zhou, M., Wang, Q., Chen, Q., Zhong, S., Li, C. B., & Li, C. (2017). MYC2 orchestrates a hierarchical transcriptional

cascade that regulates jasmonate-mediated plant immunity in tomato. *Plant Cell*, *29*(8), 1883–1906.

Du, Z., Chen, A., Chen, W., Liao, Q., Zhang, H., Bao, Y., Roossinck, M.J., & Carr, J.P. (2014a). Nuclear-cytoplasmic partitioning of cucumber mosaic virus protein 2b determines the balance between its roles as a virulence determinant and an RNA-silencing suppressor. *Journal of Virology*, *88*(10), 5228-5241.

Du, Z., Chen, A., Chen, W., Westwood, J. H., Baulcombe, D. C., & Carr, J. P. (2014b). Using a viral vector to reveal the role of microRNA159 in disease symptom induction by a severe strain of Cucumber mosaic virus. *Plant Physiology*, *164*(3), 1378-1388.

Durrant, W. E., & Dong, X. (2004). Systemic acquired resistance. *Annual Review of Phytopathology*, *42*, 185-209.

Dyer, A. G., Boyd-Gerny, S., McLoughlin, S., Rosa, M. G., Simonov, V., & Wong, B. B. (2012). Parallel evolution of angiosperm colour signals: common evolutionary pressures linked to hymenopteran vision. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1742), 3606-3615.

Edwardson, J. R., & Corbett, M. K. (1962). A virus-like syndrome in tomato caused by a mutation. *American Journal of Botany*, *49*, 571–575.

Egelhaaf, M., & Kern, R. (2002). Vision in flying insects. *Current Opinion in Neurobiology*, *12*, 699–706.

Ertl, H. M., Mora, M. A., Brightsmith, D. J., & Navarro-Alberto, J. A. (2018). Potential impact of neonicotinoid use on northern bobwhite (*Colinus virginianus*) in Texas: A historical analysis. *PloS One*, *13*(1), e0191100.

Faoro, F., & Gozzo, F. (2015). Is modulating virus virulence by induced systemic resistance realistic? *Plant Science*, *234*, 1–13.

Farmer, E. E. (2007). Jasmonate perception machines. Nature, *448*(7154), 659-660.

Fennell, J. T., Fountain, M. T., & Paul, N. D. (2019). Direct effects of protective cladding material on insect pests in crops. *Crop Protection*, *121*, 147-156.

Fennell, J. T., Wilby, A., Sobeih, W., & Paul, N. D. (2020). New understanding of the direct effects of spectral balance on behaviour in *Myzus persicae*. *Journal of Insect Physiology*, *126*, 104096.

Fereres, A., Kampmeier, G. E., & Irwin, M. E. (1999). Aphid attraction and preference for soybean and pepper plants infected with Potyviridae. *Annals of the Entomological Society of America*, *92*(4), 542–548.

Fericean, L. M., Palagesiu, I., Palicica, R., Varteiu, A. M., & Prunar, S. (2011). The behaviour, life cycle and biometrical measurements of *Myzus persicae*. *Research Journal of Agricultural Science*, *43*(2), 34–39.

Fericean, M. L. (2015). Research regarding external anatomy of *Phorodon humuli*. *Research Journal of Agricultural Science*, *47*(2), 52–55.

Fischer, U., & Dröge-Laser, W. (2004). Overexpression of *NtERF5*, a new member of the tobacco ethylene response transcription factor family enhances resistance to tobacco mosaic

virus. *Molecular Plant-Microbe Interactions*, *17*(10), 1162-1171.

Flor, H. H. (1955). Host-parasite interactions in flax rust - Its genetics and other implications. *Phytopathology*, *45*, 680-685.

Fonseca, S., Chini, A., Hamberg, M., Adie, B., Porzel, A., Kramell, R., Miersch, O., Wasternack, C., & Solano, R. (2009). (+)-7-iso-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. *Nature Chemical Biology*, *5*(5), 344–350.

Gadhave, K. R., Dutta, B., Coolong, T., & Srinivasan, R. (2019). A non-persistent aphid-transmitted Potyvirus differentially alters the vector and non-vector biology through host plant quality manipulation. *Scientific Reports*, *9*, 2503

Gallitelli, D., Vovlas, C., Martelli, G., Montasser, M. S., Tousignant, M. E., & Kaper, J. M. (1991). Satellite-mediated protection of tomato against cucumber mosaic virus: II. Field test under natural epidemic conditions in southern Italy. *Plant Disease*, *75*(1), 93-95.

Gandon, S. (2018). Evolution and manipulation of vector host choice. *American Naturalist*, *192*(1), 23–34.

García-Arenal, F., & McDonald, B. A. (2003). An analysis of the durability of resistance to plant viruses. *Phytopathology*, *93*(8), 941-952.

García-Marcos, A., Pacheco, R., Martiáñez, J., González-Jara, P., Díaz-Ruíz, J. R., & Tenllado, F. (2009). Transcriptional changes and oxidative stress associated with the synergistic interaction between potato virus X and potato virus Y and their relationship with symptom expression. *Molecular Plant-Microbe Interactions*, *22*(11), 1431–1444.

Garret, A., Kerlan, C., & Thomas, D. (1996). Ultrastructural study of acquisition and retention of potato leafroll luteovirus in the alimentary canal of its aphid vector, *Myzus persicae* Sulz. *Archives of Virology*, *141*(7), 1279–1292.

Geering, A. D. W., & Randles, J. W. (1994). Interactions between a seed-borne strain of cucumber mosaic cucumovirus and its lupin host. *Annals of Applied Biology*, *124*(2), 301-314.

Ghanim, M., & Czosnek, H. (2000). Tomato yellow leaf curl geminivirus (TYLCV-Is) is transmitted among whiteflies (*Bemisia tabaci*) in a sex-related manner. *Journal of Virology*, *74*(10), 4738-4745.

Gildow, F. E., Shah, D. A., Sackett, W. M., Butzler, T., Nault, B. A., & Fleischer, S. J. (2008). Transmission efficiency of cucumber mosaic virus by aphids associated with virus epidemics in snap bean. *Phytopathology*, *98*, 1233–1241.

Gitelson, A. A., Chivkunova, O. B., & Merzlyak, M. N. (2009). Nondestructive estimation of anthocyanins and chlorophylls in anthocyanic leaves. *American Journal of Botany*, *96*(10), 1861–1868.

Giurfa, M., & Menzelt, R. (1997). Insect visual perception: complex abilities of simple nervous systems. *Current Opinion in Neurobiology*, *7*, 505–513.

González, I., Martínez, L., Rakitina, D. V., Lewsey, M. G., Atencio, F. A., Llave, C., Kalinina, N. O., Carr, J. P., Palukaitis, P., & Canto, T. (2010). Cucumber mosaic virus 2b protein subcellular targets and interactions: their significance to RNA silencing suppressor activity. *Molecular Plant-Microbe Interactions 23*(3), 294–303.

Gonzalez, I., Rakitina, D., Semashko, M., Taliansky, M., Praveen, S., Palukaitis, P., Carr, J. P., Kalinina, N., & Canto, T. (2012). RNA binding is more critical to the suppression of silencing function of Cucumber mosaic virus 2b protein than nuclear localization. *RNA*, *18*(4), 771–782.

Groen, S. C., Jiang, S., Murphy, A. M., Cunniffe, N. J., Westwood, J. H., Davey, M. P., Bruce, T. J. A., Caulfield, J. C., Furzer, O. J., Reed, A., Robinson, S. I., Miller, E., Davis, C. N., Pickett, J. A., Whitney, H. M., Glover, B. J., & Carr, J. P. (2016). Virus infection of plants alters pollinator preference: A payback for susceptible hosts? *PLoS Pathogens*, *12*(8), 1–28, e1005790.

Groen, S. C., Wamonje, F. O., Murphy, A. M., & Carr, J. P. (2017). Engineering resistance to virus transmission. *Current Opinion in Virology*, *26*, 20–27.

Hamada, A. M., & Jonsson, L. M. V. (2013). Thiamine treatments alleviate aphid infestations in barley and pea. *Phytochemistry*, *94*, 135–141.

Hamera, S., Song, X., Su, L., Chen, X., & Fang, R. (2012). Cucumber mosaic virus suppressor 2b binds to AGO4-related small RNAs and impairs AGO4 activities. *Plant Journal*, *69*(1), 104-115.

Hampton, R. O., & Francki, R. I. B. (1992). RNA-1 dependent seed transmissibility of cucumber mosaic virus in *Phaseolus vulgaris*. *Phytopathology*, *82*(2), 127-130.

Hanssen, I. M., van Esse, H. P., Ballester, A. R., Hogewoning, S. W., Parra, N. O., Paeleman, A., Lievens, B., Bovy, A. G., & Thomma, B. P. H. J. (2011). Differential tomato transcriptomic responses induced by pepino mosaic virus isolates with differential aggressiveness. *Plant Physiology*, *156*(1), 301–318.

Hardie, J. (1989). Spectral specificity for targeted flight in the black bean aphid, *Aphis fabae*. *Journal of Insect Physiology*, *35*(8), 619–626.

Harris, K. F. (1977). An ingestion-egestion hypothesis of noncirculative virus transmission. *Aphids as virus vectors; Harris, K. F., Maramorosch, K., Eds,* pp. 165-220. Academic Press, New York, USA.

Harrison, B. D., & Cadman, C. H. (1959). Role of a dagger nematode (*Xiphinema* sp.) in outbreaks of plant diseases caused by Arabis mosaic virus. *Nature*, *184*(4699), 1624-1626.

Hewer, A., Will, T., & Van Bel, A. J. E. (2010). Plant cues for aphid navigation in vascular tissues. *Journal of Experimental Biology*, *213*(23), 4030–4042.

Hily, J. M., Garcia, A., Moreno, A., Plaza, M., Wilkinson, M. D., Fereres, A., Fraile, A., & Garcia-Arenal, F. (2014). The relationship between host lifespan and pathogen reservoir potential: an analysis in the system *Arabidopsis thaliana*-cucumber mosaic virus. *PLoS Pathogens*, *10*(11), e1004492.

Hodge, S., & Powell, G. (2008). Do plant viruses facilitate their aphid vectors by inducing symptoms that alter behaviour and performance? *Environmental Entomology*, *37*(6), 1573–1581.

Hogenhout, S. A., Ammar, E. D., Whitfield, A. E., & Redinbaugh, M. G. (2008). Insect vector interactions with persistently transmitted viruses. *Annual. Review of Phytopathology*, *46*, 327-359.

Hogenhout, S. A., & Bos, J. I. (2011). Effector proteins that modulate plant–insect interactions. *Current Opinion in Plant Biology*, *14*(4), 422-428.

Hoh, F., Uzest, M., Drucker, M., Plisson-Chastang, C., Bron, P., Blanc, S., & Dumas, C. (2010). Structural insights into the molecular mechanisms of cauliflower mosaic virus transmission by its insect

vector. *Journal of Virology*, *84*(9), 4706-4713.

Hohenstein, J. D., Studham, M. E., Klein, A., Kovinich, N., Barry, K., Lee, Y. J., & MacIntosh, G. C. (2019). Transcriptional and chemical changes in soybean leaves in response to long-term aphid colonization. *Frontiers in Plant Science*, *10*, 310.

Horsch, R. B., Fry, J. E., Hoffmann, N. L., Eichholtz, D., Rogers, S. G., & Fraley, R. T. (1985). A simple and general method for transferring genes into plants. *Science*, *227*, 1229–1231.

Hu, J. S. (1995). Comparison of dot blot, ELISA, and RT-PCR Assays for detection of two cucumber mosaic virus isolates infecting banana in Hawaii. *Plant Disease*, *79*(9), 903.

Hull, A. K., Vij, R., & Celenza, J. L. (2000). Arabidopsis cytochrome P450s that catalyse the first step of tryptophan-dependent indole-3-acetic acid biosynthesis. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(5), 2379-2384.

Hull, R. (2014). Chapter 14 - Ecology, Epidemiology and Control of Plant Viruses. pp. 809-876 in *Plant Virology* 5th Edition, Elsevier.

Imafuku, M. (2008). Variation in UV light reflected from the wings of *Favonius* and *Quercusia* butterflies. *Entomological Science*, *11*(1), 75-80.

Insam, H., & Seewald, M. S. A. (2010). Volatile organic compounds (VOCs) in soils. *Biology and Fertility of Soils*, *46*(3), 199–213.

Jacquemond, M. (2012). Cucumber mosaic virus. *Advances in Virus Research*, *84*, 439–504.

Ji, L. H., & Ding, S. W. (2001). The suppressor of transgene RNA silencing encoded by cucumber mosaic virus interferes with salicylic acid-mediated virus resistance. *Molecular Plant-Microbe Interactions*, *14*(6), 715-724.

Jones, J. D., & Dangl, J. L. (2006). The plant immune system. *Nature*, *444*(7117), 323-329.

Jones, M. G. (1944). The structure of the antenna of *Aphis (Doralis) fabae* Scopoli, and of *Melanoxantherium salicis* L. (Hemiptera), and some experiments on olfactory responses. *Proceedings of the Royal Entomological Society of London. Series A, General Entomology*, *19*,13-22.

Jones, R. A. C. (2009). Plant virus emergence and evolution: Origins, new encounter scenarios, factors driving emergence, effects of changing world conditions, and prospects for control. *Virus Research*, *141*(2), 113–130.

Jones, R. A. C. (2018). Plant and insect viruses in managed and natural environments: novel and neglected transmission pathways. *Advances in Virus Research*, *101*, 149-187.

Jones, R. A., & Naidu, R. A. (2019). Global dimensions of plant virus diseases: Current status and future perspectives. *Annual Review of Virology*, *6*, 387-409.

Jones, R. A., Salam, M. U., Maling, T. J., Diggle, A. J., & Thackray, D. J. (2010). Principles of predicting plant virus disease epidemics. *Annual Review of Phytopathology*, *48*, 179-203.

Jordá, C., Alfaro, A., Aranda, M. A., Moriones, E., & García-Arenal, F. (1992). Epidemic of cucumber mosaic virus plus satellite RNA in tomatoes in eastern Spain. *Plant Disease*, *76*(4), 363-366.

Kaloshian, I., Kinsey, M. G., Ullman, D. E., & Williamson, V. M. (1997). The impact of *Meu1*-mediated resistance in tomato on longevity, fecundity and behaviour of the potato aphid, *Macrosiphum euphorbiae*. *Entomologia Experimentalis et Applicata*, *83*(2), 181-187.

Kaloshian, I., Kinsey, M. G., Williamson, V. M., & Ullman, D. E. (2000). *Mi*-mediated resistance against the potato aphid *Macrosiphum euphorbiae* (Hemiptera: Aphididae) limits sieve element ingestion. *Environmental Entomology*, *29*(4), 690-695.

Kaloshian, I., & Walling, L. L. (2016). Plant immunity: Connecting the dots between microbial and hemipteran immune responses. *Management of Insect Pests to Agriculture: Lessons Learned From Deciphering Their Genome, Transcriptome and Proteome; Czosnek, H., Ghanim, M., Eds*, pp. 217-243. Springer, Cham.

Kang, J. H., Liu, G., Shi, F., Jones, A. D., Beaudry, R. M., & Howe, G. A. (2010). The tomato *odourless-2* mutant is defective in trichome-based production of diverse specialized metabolites and broad-spectrum resistance to insect herbivores. *Plant Physiology*, *154*(1), 262–272.

Kant, M. R., Ament, K., Sabelis, M. W., Haring, M. A., & Schuurink, R. C. (2004). Differential timing of spider mite-induced direct and indirect defences in tomato plants. *Plant Physiology*, *135*(1), 483–495.

Katis, N. I., Tsitsipis, J. A., Stevens, M., & Powell, G. (2007). Transmission of plant viruses. *Aphids as Crop Pests; van Emden, H., Harrington, R., Eds*. CAB International, Wallingford, UK.

Kennedy, J. S. (1951). Benefits to aphids from feeding on galled and virus-infected leaves. *Nature*, *168*(4280), 825-826.

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

Kennedy, J. S., Day, M. F., & Eastop, V. F. (1962). *A conspectus of aphids as vectors of plant viruses.* Commonwealth Institute of Entomology, London.

Kersch-Becker, M. F., & Thaler, J. S. (2014). Virus strains differentially induce plant susceptibility to aphid vectors and chewing herbivores. *Oecologia*, *174*(3), 883–892.

Kidner, C. A., & Martienssen, R. A. (2005). The developmental role of microRNA in plants. *Current Opinion in Plant Biology*, *8*(1), 38-44.

Kim, J. H., & Jander, G. (2007). *Myzus persicae* (green peach aphid) feeding on Arabidopsis induces the formation of a deterrent indole glucosinolate. *Plant Journal*, *49*(6), 1008–1019.

Kim, J. H., Lee, B. W., Schroeder, F. C., & Jander, G. (2008). Identification of indole glucosinolate breakdown products with antifeedant effects on *Myzus persicae* (green peach aphid). *Plant Journal*, *54*(6), 1015–1026.

Kirchner, S. M., Döring, T. F., & Saucke, H. (2005). Evidence for trichromacy in the green peach aphid, *Myzus persicae* (Sulz.) (Hemiptera: Aphididae). *Journal of Insect Physiology*, *51*(11), 1255–1260.

Klingler, J. P., Nair, R. M., Edwards, O. R., & Singh, K. B. (2009). A single gene, *AIN*, in *Medicago truncatula* mediates a hypersensitive response to both bluegreen aphid and pea aphid but confers resistance only to bluegreen aphid. *Journal of Experimental Botany*, *60*(14), 4115–4127.

Kloth, K. J., Busscher-Lange, J., Wiegers, G. L., Kruijer, W., Buijs, G., Meyer, R. C., Albrectsen, B. R., Bouwmeester, H. J., Dicke, M., & Jongsma, M. A. (2017). SIEVE ELEMENT-LINING CHAPERONE1 restricts aphid feeding on *Arabidopsis* during heat stress. *Plant Cell*, *29*(10), 2450–2464.

Köhler, H. R., & Triebskorn, R. (2013). Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond? *Science*, *341*(6147), 759-765.

Koornneef, A., Leon-Reyes, A., Ritsema, T., Verhage, A., Den Otter, F. C., Van Loon, L. C., & Pieterse, C. M. J. (2008). Kinetics of salicylate-mediated suppression of jasmonate signalling reveal a role for redox modulation. *Plant Physiology*, *147*(3), 1358–1368.

Kroes, A., Weldegergis, B. T., Cappai, F., Dicke, M., & Loon, J. J. A. Van. (2017). Terpenoid biosynthesis in *Arabidopsis* attacked by caterpillars and aphids : effects of aphid density on the attraction of a caterpillar parasitoid. *Oecologia*, *185*(4), 699–712.

Kruskal, W. H., & Wallis, W. A. (1952). Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association*, *47*(260), 583–621.

Kunert, G., & Weisser, W. W. (2005). The importance of antennae for pea aphid wing induction in the presence of natural enemies. *Bulletin of Entomological Research*, *95*(2), 125–131.

Lamb, R. J., & MacKay, P. A. (1997). Photoperiodism and life cycle plasticity of an aphid, *Macrosiphum euphorbiae* (Thomas), from central North America. *Canadian Entomologist*, *129*(6), 1035–1048.

Leather, S. R. (1993). Overwintering in six arable aphid pests: a review with particular relevance to pest management. *Journal of Applied Entomology*, *116*(1-5), 217-233.

Leckstein, P. M., & Llewellyn, M. (1973). Effect of dietary amino acids on the size and alary polymorphism of *Aphis fabae*. *Journal of Insect Physiology*, *19*(5), 973–980.

Lee, W. S., Fu, S. F., Li, Z., Murphy, A. M., Dobson, E. A., Garland, L., Chaluvadi, S. R., Lewsey, M. G., Nelson, R. S., & Carr, J. P. (2016). Salicylic acid treatment and expression of an *RNA-dependent RNA polymerase 1* transgene inhibit lethal symptoms and meristem invasion during tobacco mosaic virus infection in *Nicotiana benthamiana*. *BMC Plant Biology*, *16*(1), 1–14.

Lees, A. D. (1967). The production of the apterous and alate forms in the aphid *Megoura viciae* Buckton, with special reference to the role of crowding. *Journal of Insect Physiology*, *13*(2), 289-318.

Lefeuvre, P., Martin, D. P., Elena, S. F., Shepherd, D. N., Roumagnac, P., & Varsani, A. (2019). Evolution and ecology of plant viruses. *Nature Reviews Microbiology*, *17*(10), 632–644.

Lei, L. (2017). Plant volatiles: Active emission. *Nature Plants*, *3*(8), 1-1.

Lerdau, M., & Gray, D. (2003). Ecology and evolution of light-dependent and light-independent phytogenic volatile organic carbon. *New Phytologist*, *157*(2), 199–211.

Lewsey, M. G., Murphy, A. M., Maclean, D., Dalchau, N., Westwood, J. H., Macaulay, K., Bennett, M. H., Moulin, M., Hanke, D. E., Powell, G., Smith, A. G., & Carr, J. P. (2010). Disruption of two defensive signalling pathways by a viral RNA silencing suppressor. *Molecular Plant-Microbe Interactions*, *23*(7), 835–845.

Lewsey, M., Robertson, F. C., Canto, T., Palukaitis, P., & Carr, J. P. (2007). Selective targeting of miRNA-

regulated plant development by a viral counter-silencing protein. *Plant Journal*, *50*(2), 240–252.

Lewsey, M., Surette, M., Robertson, F. C., Ziebell, H., Choi, S. H., Ryu, K. H., Canto, T., Palukaitis, P., Payne, T., Walsh, J. A., & Carr, J. P. (2009). The role of the cucumber mosaic virus 2b protein in viral movement and symptom induction. *Molecular Plant-Microbe Interactions*, *22*(6), 642–654.

Li, B., Bickel, R.D., Parker, B.J., Ziabari, O.S., Liu, F., Vellichirammal, N.N., Simon, J.C., Stern, D.L. and Brisson, J.A., 2020. A large genomic insertion containing a duplicated follistatin gene is linked to the pea aphid male wing dimorphism. *eLife*, 9:e50608

Li, L., Zhao, Y., McCaig, B.C., Wingerd, B.A., Wang, J., Whalon, M.E., Pichersky, E., & Howe, G.A.(2004). The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signalled defence responses, and glandular trichome development. *Plant Cell*, *16*(1), pp.126-143.

Li, R., Weldegergis, B. T., Li, J., Jung, C., Qu, J., Sun, Y., Qian, H., Tee, C., Van Loon, J. J. A., Dicke, M., Chua, N. H., Liu, S. S., & Ye, J. (2014). Virulence factors of geminivirus interact with MYC2 to subvert plant resistance and promote vector performance. *Plant Cell*, *26*(12), 4991–5008.

Li, Y., Hill, C. B., Carlson, S. R., Diers, B. W., & Hartman, G. L. (2007). Soybean aphid resistance genes in the soybean cultivars Dowling and Jackson map to linkage group M. *Molecular Breeding*, *19*(1), 25–34.

Liao, Q., Tu, Y., Carr, J. P., & Du, Z. (2015). An improved cucumber mosaic virus-based vector for efficient decoying of plant microRNAs. *Scientific Reports*, *5*, 13187.

Liu, D., Zhao, Q., Cheng, Y., Li, D., Jiang, C., Cheng, L., Wang, Y., & Yang, A. (2019). Transcriptome analysis of two cultivars of tobacco in response to cucumber mosaic virus infection. *Scientific Reports*, *9*, 3124.

Liu, J., Liu, Y., Donkersley, P., Dong, Y., Chen, X., Zang, Y., Xu, P., & Ren, G. (2019). Preference of the aphid *Myzus persicae* (Hemiptera: Aphididae) for tobacco plants at specific stages of potato virus Y infection. *Archives of Virology*, *164*(6), 1567–1573.

Liu, S., He, X., Park, G., Josefsson, C., & Perry, K. L. (2002). A conserved capsid protein surface domain of cucumber mosaic virus is essential for efficient aphid vector transmission. *Journal of Virology*, *76*(19), 9756–9762.

Liu, S. R., Zhou, J. J., Hu, C. G., Wei, C. L., & Zhang, J. Z. (2017). MicroRNA-mediated gene silencing in plant defence and viral counter-defence. *Frontiers in Microbiology*, *8*, 1801.

Liu, Y., Schiff, M., Marathe, R., & Dinesh-Kumar, S. P. (2002). Tobacco *Rar1*, *EDS1* and *NPR1/NIM1* like genes are required for N-mediated resistance to tobacco mosaic virus. *Plant Journal*, *30*(4), 415–429.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, *25*(4), 402–408.

Llave, C., Xie, Z., Kasschau, K. D., & Carrington, J. C. (2002). Cleavage of scarecrow-like mRNA targets directed by a class of Arabidopsis miRNA. *Science*, *297*(5589), 2053–2056.

López-Gresa, M. P., Lisón, P., Yenush, L., Conejero, V., Rodrigo, I., & Bellés, J. M. (2016). Salicylic acid is involved in the basal resistance of tomato plants to citrus exocortis viroid and tomato spotted

wilt virus. *PLoS One*, *11*(11), e0166938.

Losvik, A., Beste, L., Glinwood, R., Ivarson, E., Stephens, J., Zhu, L. H., & Jonsson, L. (2017). Overexpression and down-regulation of barley lipoxygenase *LOX2. 2* affects jasmonate-regulated genes and aphid fecundity. *International Journal of Molecular Sciences*, *18*(12), 2765.

Lot, H., & Kaper, J. M. (1976). Physical and chemical differentiation of three strains of cucumber mosaic virus and peanut stunt virus. *Virology*, *74*(1), 209-222.

Ma, K. W., & Ma, W. (2016). Phytohormone pathways as targets of pathogens to facilitate infection. Plant Molecular Biology, *91*(6), 713-725.

MacGillivray, M. E., & Anderson, G. B. (1958). Development of four species of aphids (Homoptera) on potato. *The Canadian Entomologist*, *90*(3), 148–155.

MacGillivray, M. E., & Anderson, G. B. (1964). The effect of photoperiod and temperature on the production of gamic and agamic forms in *Macrosiphum euphorbiae* (Thomas). *Canadian Journal of Zoology*, *42*(3), 491-510.

Madden, L. V., Jeger, M. J., & Van Den Bosch, F. (2000). A theoretical assessment of the effects of vector-virus transmission mechanism on plant virus disease epidemics. *Phytopathology*, *90*(6), 576–594.

Makkouk, K. M., Kumari, S. G., van Leur, J. A., & Jones, R. A. (2014). Control of plant virus diseases in cool-season grain legume crops. *Advances in Virus Research*, *90*, 207-253.

Malamy, J., & Klessig, D. F. (1992). Salicylic acid and plant disease resistance. *Plant Journal*, *2*(5), 643-654.

Martin, D. M., Gershenzon, J., & Bohlmann, J. (2003). Induction of volatile terpene biosynthesis and diurnal emission by methyl jasmonate in foliage of Norway spruce. *Plant Physiology*, *132*(3), 1586-1599.

Matsuo, Y., Novianti, F., Takehara, M., Fukuhara, T., Arie, T., & Komatsu, K. (2019). Acibenzolar-s-methyl restricts infection of *Nicotiana benthamiana* by plantago asiatica mosaic virus at two distinct stages. *Molecular Plant-Microbe Interactions*, *32*(11), 1475–1486.

Mauck, K. E., De Moraes, C. M., & Mescher, M. C. (2010a). Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(8), 3600-3605.

Mauck, K. E., De Moraes, C. M., & Mescher, M. C. (2010b). Effects of cucumber mosaic virus infection on vector and non-vector herbivores of squash. *Communicative & Integrative Biology*, *3*(6), 579–582.

Mauck, K. E., Smyers, E., De Moraes, C. M., & Mescher, M. C. (2015). Virus infection influences host plant interactions with non-vector herbivores and predators. *Functional Ecology*, *29*(5), 662–673.

Megahed, E. S., & Pirone, T. P. (1966). Comparative transmission of cucumber mosaic virus acquired by aphids from plants or through a membrane. *Phytopathology*, *56*(12), 1420.

Meissner, R., Jacobson, Y., Melamed, S., Levyatuv, S., Shalev, G., Ashri, A., Elkind, Y., & Levy, A. (1997). A new model system for tomato genetics. *Plant Journal*, *12*(6), 1465–1472.

Metz, J. A. J., Wedel, M., & Angulo, A. F. (1983). Discovering an epidemic before it has reached a certain level of prevalence. *Biometrics*, 39(3), 765-770.

Mhlanga, N. M. (2020). *Effects of plant viral pathogens on plant-pollinator relationships.* Doctoral Thesis, University of Cambridge. https://www.repository.cam.ac.uk/handle/1810/301488.

Mikkelsen, M. D., Hansen, C. H., Wittstock, U., & Halkier, B. A. (2000). Cytochrome P450 CYP79B2 from Arabidopsis catalyzes the conversion of tryptophan to indole-3-acetaldoxime, a precursor of indole glucosinolates and indole-3-acetic acid. *Journal of Biological Chemistry*, *275*(43), 33712–33717.

Mikkelsen, M. D., Petersen, B. L., Glawischnig, E., Jensen, A. B., Andreasson, E., & Halkier, B. A. (2003). Modulation of CYP79 genes and glucosinolate profiles in Arabidopsis by defence signalling pathways. *Plant Physiology*, *131*(1), 298-308.

Mittler, T. E. (1967). Effect of amino acid and sugar concentrations on the food uptake of the aphid *Myzus persicae*. *Entomologia Experimentalis et Applicata*, *10*(1), 39-51.

Moghadas, S. M., Fitzpatrick, M. C., Sah, P., Pandey, A., Shoukat, A., Singer, B. H., & Galvani, A. P. (2020). The implications of silent transmission for the control of COVID-19 outbreaks. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(30), 17513-17515.

Moran, N. A. (1992). The evolution of aphid life cycles. *Annual Review of Entomology*, *37*(1), 321-348.

Muhammad, T., Zhang, F., Zhang, Y., & Liang, Y. (2019). RNA interference: a natural immune system of plants to counteract biotic stressors. *Cells*, *8*(1), 38.

Murphy, A. M., Zhou, T., & Carr, J. P. (2020). An update on salicylic acid biosynthesis, its induction and potential exploitation by plant viruses. *Current Opinion in Virology*, *42*, 8–17.

Mutuku, J.M., Wamonje, F.O., Mukeshimana, G., Njuguna, J., Wamalwa, M., Choi, S.K., Tungadi, T., Djikeng, A., Kelly, K., Domelevo Entfellner, J.B., & Ghimire, S.R. (2018). Metagenomic analysis of plant virus occurrence in common bean (*Phaseolus vulgaris*) in Central Kenya. *Frontiers in Microbiology*, *9*, 2939.

Nalam, V. J., Keeretaweep, J., Sarowar, S., & Shah, J. (2012). Root-derived oxylipins promote green peach aphid performance on *Arabidopsis* foliage. *Plant Cell*, *24*(4), 1643–1653.

Nalam, V., Louis, J., & Shah, J. (2019). Plant defence against aphids, the pest extraordinaire. *Plant Science*, *279*, 96-107.

Naylor, M., Murphy, A. M., Berry, J. O., & Carr, J. P. (1998). Salicylic acid can induce resistance to plant virus movement. *Molecular Plant-Microbe Interactions*, *11*(9), 860–868.

Ndamukong, I., Abdallat, A. A., Thurow, C., Fode, B., Zander, M., Weigel, R., & Gatz, C. (2007). SA-inducible Arabidopsis glutaredoxin interacts with TGA factors and suppresses JA-responsive *PDF1.2* transcription. *Plant Journal*, *50*(1), 128-139.

Ng, J. C. K., Josefsson, C., Clark, A. J., Franz, A. W. E., & Perry, K. L. (2005). Virion stability and aphid vector transmissibility of cucumber mosaic virus mutants. *Virology*, *332*(1), 397–405.

Ng, J. C., & Perry, K. L. (2004). Transmission of plant viruses by aphid vectors. *Molecular Plant*

Pathology, *5*(5), 505-511.

Ng, J. C. K., Tian, T., & Falk, B. W. (2004). Quantitative parameters determining whitefly (*Bemisia tabaci*) transmission of lettuce infectious yellows virus and an engineered defective RNA. *Journal of General Virology*, *85*(9), 2697–2707.

Nguyen, V. C., Villate, L., Gutierrez-Gutierrez, C., Castillo, P., Van Ghelder, C., Plantard, O., & Esmenjaud, D. (2019). Phylogeography of the soil-borne vector nematode *Xiphinema index* highly suggests Eastern origin and dissemination with domesticated grapevine. *Scientific Reports*, *9*, 7313.

Norkute, M., Olsson, U., & Ninkovic, V. (2020). Aphids-induced plant volatiles affect diel foraging behaviour of a ladybird beetle *Coccinella septempunctata*. *Insect Science*, *27*(6), 1266-1275.

Osorio, D., & Vorobyev, M. (2008). A review of the evolution of animal colour vision and visual communication signals. *Vision Research*, *48*, 2042–2051.

Oedraogo, R. S., & Roossinck, M. J. (2018). Molecular evolution. *Cucumber mosaic virus*; *Palukaitis, P., García-Arenal, F., Eds*, pp. 207–215. APS Press, Minneapolis, MN

Owen, J., & Palukaitis, P. (1988). Characterization of cucumber mosaic virus I. Molecular heterogeneity mapping of RNA 3 in eight CMV strains. *Virology*, *166*(2), 495-502.

Pagán, I. (2019). Movement between plants: Vertical transmission. *Cucumber mosaic virus; Palukaitis, P., García-Arenal, F., Eds*, pp. 185-198. APS Press, Washington, USA.

Pagán, Israel, González-Jara, P., Moreno-Letelier, A., Rodelo-Urrego, M., Fraile, A., Piñero, D., & García-Arenal, F. (2012). Effect of biodiversity changes in disease risk: Exploring disease emergence in a plant-virus system. *PLoS Pathogens*, *8*(7), e1002796.

Palukaitis, P., Roossinck, M. J., Dietzgen, R. G., & Francki, R. I. (1992). Cucumber mosaic virus. *Advances in Virus Research*, *41*, 281-348.

Palukaitis, P, & Zaitlin, M. (1997). Replicase-mediated resistance to plant virus disease. *Advances in Virus Research*, *48*, 349–377.

Palukaitis, P., & García-Arenal, F. (2003). Cucumoviruses. *Advances in Virus Research*, *62*, 241-323.

Palukaitis, P., Groen, S. C., & Carr, J. P. (2013). The Rumsfeld paradox: Some of the things we know that we don't know about plant virus infection. *Current Opinion in Plant Biology*, *16*(4), 513–519.

Palukaitis, P., Roossinck, M. J., Dietzgen, R. G., & Francki, R. I. (1992). Cucumber mosaic virus. *Advances in Virus Research*, *41*, 281-348.

Palukaitis, P., Yoon, J. Y., Choi, S. K., & Carr, J. P. (2017). Manipulation of induced resistance to viruses. *Current Opinion in Virology*, *26*, 141-148.

Pandey, S. P., & Baldwin, I. T. (2007). RNA-directed RNA polymerase 1 (RdR1) mediates the resistance of *Nicotiana attenuata* to herbivore attack in nature. *Plant Journal*, *50*(1), 40-53.

Park, W., Li, J., Song, R., Messing, J., & Chen, X. (2002). CARPEL FACTORY, a Dicer homolog, and HEN1, a novel protein, act in microRNA metabolism in *Arabidopsis thaliana*. *Current Biology*, *12*(17), 1484-1495.

Parnell, S., Gottwald, T. R., Gilks, W. R., & Van den Bosch, F. (2012). Estimating the incidence of an epidemic when it is first discovered and the design of early detection monitoring. *Journal of Theoretical Biology*, *305*, 30-36.

Paschold, A., Bonaventure, G., Kant, M. R., & Baldwin, I. T. (2008). Jasmonate perception regulates jasmonate biosynthesis and JA-Ile metabolism: The case of COI1 in *Nicotiana attenuata*. *Plant and Cell Physiology*, *49*(8), 1165–1175.

Pauwels, L., & Goossens, A. (2011). The JAZ proteins: A crucial interface in the jasmonate signaling cascade. *Plant Cell*, *23*(9), 3089–3100.

Pauwels, L., Morreel, K., De Witte, E., Lammertyn, F., Van Montagu, M., Boerjan, W., Inzé, D., & Goossens, A. (2008). Mapping methyl jasmonate-mediated transcriptional reprogramming of metabolism and cell cycle progression in cultured *Arabidopsis* cells. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(4), 1380–1385.

Peiffer, M., Tooker, J. F., Luthe, D. S., & Felton, G. W. (2009). Plants on early alert: glandular trichomes as sensors for insect herbivores. *New Phytologist*, *184*(3), 644–656.

Peter, A. J., Shanower, T. G., & Romeis, J. (1995). The role of plant trichomes in insect resistance: a selective review. *Phytophaga*, *7*, 41-63.

Picazo-Aragonés, J., Terrab, A., & Balao, F. (2020). Plant volatile organic compounds evolution: Transcriptional regulation, epigenetics and polyploidy. *International Journal of Molecular Sciences*, *21*(23), 1–18.

Pickett, J.A., Aradottir, G.I., Birkett, M.A., Bruce, T.J., Chamberlain, K., Khan, Z.R., Midega, C.A., Smart, L.E., & Woodcock, C.M. (2012). Aspects of insect chemical ecology: exploitation of reception and detection as tools for deception of pests and beneficial insects. *Physiological Entomology*, *37*(1), 2-9.

Pita, J. S., & Roossinck, M. J. (2013). Fixation of emerging interviral recombinants in cucumber mosaic virus populations. *Journal of Virology*, *87*(2), 1264–1269.

Pleydell, D. R., Soubeyrand, S., Dallot, S., Labonne, G., Chadœuf, J., Jacquot, E., & Thébaud, G. (2018). Estimation of the dispersal distances of an aphid-borne virus in a patchy landscape. *PLoS Computational Biology*, *14*(4), e1006085.

Ploeg, A. T., Brown, D. J. F., & Robinson, D. J. (1989). Transmission of tobraviruses by trichodorid nematodes 1. *EPPO Bulletin*, *19*(3), 605-610.

Ponder, K. L., Pritchard, J., Harrington, R., & Bale, J. S. (2000). Difficulties in location and acceptance of phloem sap combined with reduced concentration of phloem amino acids explain lowered performance of the aphid *Rhopalosiphum padi* on nitrogen deficient barley (*Hordeum vulgare*) seedlings. *Entomologia Experimentalis et Applicata*, *97*(2), 203–210.

Powell, G., & Hardie, J. (2001). The chemical ecology of aphid host alternation: How do return migrants find the primary host plant? *Applied Entomology and Zoology*, *36*(3), 259–267.

Prokopy, R. J., & Owens, E. D. (1983). Visual detection of plants by herbivorous insects. *Annual Review of Entomology. 28*, 337–364.

Provvidenti, R., Robinson, R. W., & Shail, J. W. (1980). A source of resistance to a strain of cucumber

mosaic virus in *Lactuca saligna* L. *Hortscience*, *15*(4), 528–529.

Rabideau, G. S., French, C. S., & Holt, A. S. (1946). The absorption and reflection spectra of leaves, chloroplast suspensions, and chloroplast fragments as measured in an Ulbricht sphere. *American Journal of Botany*, 769-777.

Rashid, U. J., Hoffmann, J., Brutschy, B., Piehler, J., & Chen, J. C. H. (2008). Multiple targets for suppression of RNA interference by tomato aspermy virus protein 2B. *Biochemistry*, *47*(48), 12655-12657.

Rawlins, T.E., Tompkins, C. M. (1934). The use of carborundum as an abrasive in plant-virus inoculation. *Phytopathology*, *26*, 578-587.

Rayapuram, C., & Baldwin, I. T. (2007). Increased SA in *NPR1*-silenced plants antagonizes JA and JA-dependent direct and indirect defences in herbivore-attacked *Nicotiana attenuata* in nature. *Plant Journal*, *52*(4), 700–715.

Reeves, J. L. (2011). Vision should not be overlooked as an important sensory modality for finding host plants. *Environmental Entomology*, *40*(4), 855–863.

Reina-Pinto, J. J., & Yephremov, A. (2009). Surface lipids and plant defences. *Plant Physiology and Biochemistry 47*(6), 540–549).

Reinhart, B. J., Weinstein, E. G., Rhoades, M. W., Bartel, B., & Bartel, D. P. (2002). MicroRNAs in plants. *Genes & Development*, *16*(13), 1616–1626.

Retnakaran, A., & Beck, S. D. (1968). Amino acid requirements and sulfur amino acid metabolism in the pea aphid, *Acyrthosiphon pisum* (Harris). *Comparative Biochemistry And Physiology*, *24*(2), 611–619.

Rhee, S. J., Watt, L. G., Bravo, A. C., Murphy, A. M., & Carr, J. P. (2020). Effects of the cucumber mosaic virus 2a protein on aphid–plant interactions in *Arabidopsis thaliana*. *Molecular Plant Pathology*, *21*(9), 1248–1254.

Rizzo, T. M., & Palukaitis, P. (1990). Construction of full-length cDNA clones of cucumber mosaic virus RNAs 1, 2 and 3: generation of infectious RNA transcripts. *Molecular and General Genetics 222*(2), 249-256.

Rodriguez-Saona, C. R., Musser, R. O., Vogel, H., Hum-Musser, S. M., & Thaler, J. S. (2010). Molecular, biochemical, and organismal analyses of tomato plants simultaneously attacked by herbivores from two feeding guilds. *Journal of Chemical Ecology*, *36*(10), 1043-1057.

Roossinck, M J, Zhang, L., & Hellwald, K. H. (1999). Rearrangements in the 5' nontranslated region and phylogenetic analyses of cucumber mosaic virus RNA 3 indicate radial evolution of three Subgroups. *Journal of Virology*, *73*(8), 6752–6758.

Roossinck, M. J., & García-Arenal, F. (2015). Ecosystem simplification, biodiversity loss and plant virus emergence. *Current Opinion in Virology*, *10*, 56–62.

Roossinck, M. J. (2002). Evolutionary history of Cucumber mosaic virus deduced by phylogenetic analyses. *Journal of Virology*, *76*(7), 3382-3387.

Ryabov, E. V., Fraser, G., Mayo, M. A., Barker, H., & Taliansky, M. (2001). Umbravirus gene expression

helps potato leafroll virus to invade mesophyll tissues and to be transmitted mechanically between plants. *Virology*, *286*(2), 363-372.

Saad, K. A., Mohamad Roff, M. N., Hallett, R. H., & Abd-Ghani, I. B. (2019). Effects of cucumber mosaic virus-infected chilli plants on non-vector *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Insect Science*, *26*(1), 76–85.

Salem, N. M., Ehlers, J. D., Roberts, P. A., & Ng, J. C. K. (2010). Biological and molecular diagnosis of seed-borne viruses in cowpea germplasm of geographically diverse sub-Saharan origins. *Plant Pathology*, *59*(4), 773–784.

Sarria, E., Palomares-Rius, F. J., López-Sesé, A. I., Heredia, A., & Gómez-Guillamón, M. L. (2010). Role of leaf glandular trichomes of melon plants in deterrence of *Aphis gossypii* Glover. *Plant Biology*, *12*(3), 503–511.

Sastry, K.S. Seed-borne plant virus diseases, 1st ed.; Springer: New Delhi, India, 2013; pp. 1–145

Sastry, K.S. & Zitter, T.A. Plant virus and viroid diseases in the tropics: Epidemiology and management, 1st ed.; Springer: Dordrecht, The Netherlands, 2014; Volume 2, 1–111.

Scala, A., Allmann, S., Mirabella, R., Haring, M. A., & Schuurink, R. C. (2013). Green leaf volatiles: a plant's multifunctional weapon against herbivores and pathogens. *International Journal of Molecular Sciences*, 14(9), 17781–17811.

Schaeffer, S., Koepke, T., & Dhingra, A. (2012). Tobacco: A Model Plant for Understanding the Mechanism of Abiotic Stress Tolerance. *Improving Crop Resistance to Abiotic Stress; Tuteja, N., Gill, S. S., Tiburcio, A. F., Tuteja, R.,* pp. 1169–1201. Wiley-VCH Verlag GmbH & Co. KGaA.

Schnaitmann, C., Pagni, M., & Reiff, D. F. (2020). Colour vision in insects : insights from Drosophila. *Journal of Comparative Physiology A*, *206*(2), 183–198.

Schnee, C., Köllner, T. G., Held, M., Turlings, T. C., Gershenzon, J., & Degenhardt, J. (2006). The products of a single maize sesquiterpene synthase form a volatile defence signal that attracts natural enemies of maize herbivores. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(4), 1129-1134.

Schröder, M. L., Glinwood, R., Ignell, R., & Krüger, K. (2017). The role of visual and olfactory plant cues in aphid behaviour and the development of non-persistent virus management strategies. *Arthropod-Plant Interactions 11*(1), 1–13).

Schwach, F., Vaistij, F. E., Jones, L., & Baulcombe, D. C. (2005). An RNA-dependent RNA polymerase prevents meristem invasion by potato virus X and is required for the activity but not the production of a systemic silencing signal. *Plant Physiology*, *138*(4), 1842–1852.

Seo, J. K., Kwon, S. J., Choi, H. S., & Kim, K. H. (2009). Evidence for alternate states of cucumber mosaic virus replicase assembly in positive- and negative-strand RNA synthesis. *Virology*, *383*(2), 248–260.

Shaw, A. K., Peace, A., Power, A. G., & Bosque-Pérez, N. A. (2017). Vector population growth and condition-dependent movement drive the spread of plant pathogens. *Ecology*, *98*(8), 2145–2157.

Sheahan, M., Barrett, C. B., & Goldvale, C. (2017). The unintended consequences of agricultural input

intensification : Human health implications of pesticide use in Sub-Saharan Africa. *8*(4), 1–8.

Shepherd, R. W., Bass, W. T., Houtz, R. L., & Wagner, G. J. (2005). Phylloplanins of tobacco are defencive proteins deployed on aerial surfaces by short glandular trichomes. *Plant Cell*, *17*(6), 1851–1861.

Shi, B.-J., Symons, R. H., & Palukaitis, P. (2008). The cucumovirus 2b gene drives selection of inter-viral recombinants affecting the crossover site, the acceptor RNA and the rate of selection. *Nucleic Acids Research*, *36*(4), 1057–1071.

Shi, X., Gao, Y., Yan, S., Tang, X., Zhou, X., Zhang, D., & Liu, Y. (2016). Aphid performance changes with plant defence mediated by cucumber mosaic virus titer. *Virology Journal*, *13*(1), 1-6.

Shingleton, A. W., Sisk, G. C., & Stern, D. L. (2003). Diapause in the pea aphid (*Acyrthosiphon pisum*) is a slowing but not a cessation of development. *BMC Developmental Biology*, *12*, 1–12.

Shoji, T., Ogawa, T., & Hashimoto, T. (2008). Jasmonate-induced nicotine formation in tobacco is mediated by tobacco COI1 and JAZ genes. *Plant and Cell Physiology*, *49*(7), 1003-1012.

Shrestha, D., McAuslane, H. J., Adkins, S. T., Smith, H. A., Dufault, N., Colee, J., & Webb, S. E. (2017). Host-mediated effects of semipersistently transmitted squash vein yellowing virus on sweetpotato whitefly (Hemiptera: Aleyrodidae) behaviour and fitness. *Journal of Economic Entomology*, *110*(4), 1433–1441.

Shrestha, D., McAuslane, H.J., Ebert, T.A., Cervantes, F.A., Adkins, S.T., Smith, H.A., Dufault, N., & Webb, S.E. (2019). Assessing the temporal effects of squash vein yellowing virus infection on settling and feeding behaviour of *Bemisia tabaci* (MEAM1) (Hemiptera: Aleyrodidae). *Journal of Insect Science*, *19*(3), 5.

Silva, R., & Clarke, A. R. (2020). The “sequential cues hypothesis”: a conceptual model to explain host location and ranking by polyphagous herbivores. *Insect Science*, *27*(6), 1136-1147.

Simpson, S. J., Abisgold, J. D., & Douglas, A. E. (1995). Response of the pea aphid (*Acyrthosiphon pisum*) to variation in dietary levels of sugar and amino acids: the significance of amino acid quality. *Journal of Insect Physiology*, *41*(1), 71–75.

Sinisterra, X. H., McKenzie, C. L., Hunter, W. B., Powell, C. A., & Shatters, R. G. (2005). Differential transcriptional activity of plant-pathogenic begomoviruses in their whitefly vector (*Bemisia tabaci*, Gennadius: Hemiptera Aleyroadidae). *Journal of General Virology*, *86*(5), 1525–1532.

Snoeren, T. A. L., Mumm, R., Poelman, E. H., Yang, Y., Pichersky, E., & Dicke, M. (2010). The herbivore-induced plant volatile methyl salicylate negatively affects attraction of the parasitoid *Diadegma semiclausum*. *Journal of Chemical Ecology*, *36*(5), 479–489.

Soards, A. J., Murphy, A. M., Palukaitis, P., & Carr, J. P. (2002). Virulence and differential local and systemic spread of cucumber mosaic virus in tobacco are affected by the CMV 2b protein. *Molecular Plant-Microbe Interactions*, *15*(7), 647–653.

Srinivasan, D. G., & Brisson, J. A. (2012). Aphids: A model for polyphenism and epigenetics. *Genetics Research International*, *2012*, Article ID 431531.

Srinivasan, R., & Alvarez, J. M. (2007). Effect of mixed viral infections (potato virus Y-potato leafroll virus) on biology and preference of vectors *Myzus persicae* and *Macrosiphum euphorbiae*

(Hemiptera: Aphididae). *Journal of Economic Entomology*, *100*(3), 646–655.

Stout, M. J., Fidantsef, A. L., Duffey, S. S., & Bostock, R. M. (1999). Signal interactions in pathogen and insect attack: Systemic plant-mediated interactions between pathogens and herbivores of the tomato, *Lycopersicon esculentum*. *Physiological and Molecular Plant Pathology*, *54*(3–4), 115–130.

Sun, Y., Guo, H., Yuan, E., & Ge, F. (2018). Elevated CO_2 increases *R* gene-dependent resistance of *Medicago truncatula* against the pea aphid by up-regulating a heat shock gene. *New Phytologist*, *217*(4), 1696–1711.

Suzuki, M., Kuwata, S., Kataoka, J., Masuta, C., Nitta, N., & Takanami, Y. (1991). Functional analysis of deletion mutants of cucumber mosaic virus RNA3 using an *in vitro* transcription system. *Virology*, *183*(1), 106-113.

Sylvester, E. S. (1956). Beet yellows virus transmission by the green peach aphid. *Journal of Economic Entomology*, *49*(6), 789-800.

Takahashi, K., Tanaka, T., Iida, W., & Tsuda, Y. (1980). Studies on virus diseases and causal viruses of soybean in Japan. *Bulletin of the Tohoku National Agricultural Experiment Station*, *62*, 1–130.

ten Broeke, C. J. M., Dicke, M., & van Loon, J. J. A. (2013). Resistance to a new biotype of the lettuce aphid *Nasonovia ribisnigri* in *Lactuca virosa* accession IVT280. *Euphytica*, *193*(2), 265–275.

Tepfer, M., Girardot, G., Fénéant, L., Ben Tamarzizt, H., Verdin, E., Moury, B., & Jacquemond, M. (2016). A genetically novel, narrow-host-range isolate of cucumber mosaic virus (CMV) from rosemary. *Archives of Virology*, *161*(7), 2013–2017.

Thaler, J. S., Humphrey, P. T., & Whiteman, N. K. (2012). Evolution of jasmonate and salicylate signal crosstalk. *Trends in Plant Science*, *17*, 260–270.

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K., He, S. Y., Howe, G. A., & Browse, J. (2007). JAZ repressor proteins are targets of the SCFCOI1 complex during jasmonate signalling. *Nature*, *448*(7154), 661–665.

Thipyapong, P., Joel, D. M., & Steffens, J. C. (1997). Differential expression and turnover of the tomato polyphenol oxidase gene family during vegetative and reproductive development. *Plant Physiology*, *113*(3), 707–718.

Tian, D., Tooker, J., Peiffer, M., Chung, S. H., & Felton, G. W. (2012). Role of trichomes in defence against herbivores: Comparison of herbivore response to woolly and hairless trichome mutants in tomato (*Solanum lycopersicum*). *Planta*, *236*(4), 1053–1066.

Tóbiás, I., Szabo, B., Sálanki, K., Sari, L., Kuhlmann, H., Palkovics, L., & Pitrat, M. (2008). Seedborne transmission of zucchini yellow mosaic virus and cucumber mosaic virus in Styrian Hulless group of *Cucurbita pepo. Cucurbitaceae 2008, Proceedings of the IXth EUCARPIA meeting on the genetics and breeding of Cucurbitaceae, Pitrat, M., ed,* pp. 189-197. INRA, Avignon, France, May 21–24, 2008.

Trionnaire, G., Hardie, J., Jaubert-Possamai, S., Simon, J.-C., & Tagu, D. (2008). Shifting from clonal to sexual reproduction in aphids: physiological and developmental aspects. *Biology of the Cell*, *100*(8), 441–451.

Trivedi, P., Trivedi, C., Grinyer, J., Anderson, I. C., & Singh, B. K. (2016). Harnessing host-vector microbiome for sustainable plant disease management of phloem-limited bacteria. *Frontiers in Plant Science*, *7*, 1423.

Truniger, V., & Aranda, M. (2009). Advances in virus research. *Advances in Virus Research*, *75*, 119-159.

Tungadi, T., Donnelly, R., Qing, L., Iqbal, J., Murphy, A. M., Pate, A. E., Cunniffe, N. J., & Carr, J. P. (2020). Cucumber mosaic virus 2b proteins inhibit virus-induced aphid resistance in tobacco. *Molecular Plant Pathology*, *21*(2), 250–257.

Tungadi, T., Groen, S.C., Murphy, A.M., Pate, A.E., Iqbal, J., Bruce, T.J., Cunniffe, N.J. and Carr, J.P., 2017. Cucumber mosaic virus and its 2b protein alter emission of host volatile organic compounds but not aphid vector settling in tobacco. *Virology Journal*, *14*(1), 1-9.

Tungadi, T., Watt, L. G., Groen, S. C., Murphy, A. M., Du, Z., Pate, A. E., Westwood, J. H., Fennell, T. G., Powell, G., Carr, J. P., & John Carr, C. P. (2021). Infection of Arabidopsis by cucumber mosaic virus triggers jasmonate-dependent resistance to aphids that relies partly on the pattern-triggered immunity factor BAK1. *Molecular Plant Pathology*, 22,1082–1091.

Turlings, T. C., & Erb, M. (2018). Tritrophic interactions mediated by herbivore-induced plant volatiles: mechanisms, ecological relevance, and application potential. *Annual Review of Entomology*, *63*, 433-452.

Uzest, M., Gargani, D., Drucker, M., Hébrard, E., Garzo, E., Candresse, T., Fereres, A., & Blanc, S.(2007). A protein key to plant virus transmission at the tip of the insect vector stylet. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(46),17959-17964.

Van Emden, H. F., Eastop, V. F., Hughes, R. D., & Way, M. J. (1969). The ecology of *Myzus persicae*. *Annual Review of Entomology*, *14*(1), 197-270.

Varsani, S., Grover, S., Zhou, S., Koch, K. G., Huang, P. C., Kolomiets, M. V, Williams, W. P., Heng-Moss, T., Sarath, G., Luthe, D. S., Jander, G., & Louisa, J. (2019). 12-oxo-phytodienoic acid acts as a regulator of maize defence against corn leaf aphid. *Plant Physiology*, *179*(4), 1402–1415.

Verma, D. P. S., & Hong, Z. (2001). Plant callose synthase complexes. *Plant Molecular Biology*, *47*(6), 693–701.

Vespermann, A., Kai, M., & Piechulla, B. (2007). Rhizobacterial volatiles affect the growth of fungi and *Arabidopsis thaliana*. *Applied and Environmental Microbiology*, *73*(17), 5639–5641.

Wamonje, F.O., Donnelly, R., Tungadi, T.D., Murphy, A.M., Pate, A.E., Woodcock, C., Caulfield, J., Mutuku, J.M., Bruce, T.J., Gilligan, C.A., & Pickett, J.A. (2020). Different plant viruses induce changes in feeding behaviour of specialist and generalist aphids on common bean that are likely to enhance virus transmission. *Frontiers in Plant Science*, *10*, 1811.

Wasternack, C. (2007). Jasmonates: An update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Annals of Botany*, *100*(4), 681–697.

Watson, M. A., & Roberts, F. M. (1939). A comparative study of the transmission of Hyoscyamus virus 3, potato virus Y and cucumber virus 1 by the vectors *Myzus persicae* (Sulz), *Myzus circumflexus* (Buckton), and *Macrosiphum gei* (Koch). *Proceedings of the Royal Society of London. Series B-Biological Sciences*, *127*(849), 543-576.

Watt, A. D., & Dixon, A. F. G. (1981). The role of cereal growth stages and crowding in the induction of alatae in *Sitobion avenae* and its consequences for population growth. *Ecological Entomology*, *6*(4), 441-447.

Watt, L. G., Crawshaw, S., Rhee, S. J., Murphy, A. M., Canto, T., & Carr, J. P. (2020). The cucumber mosaic virus 1a protein regulates interactions between the 2b protein and ARGONAUTE 1 while maintaining the silencing suppressor activity of the 2b protein. *PLoS Pathogens*, *16*(12), 1-24, e1009125.

Weber, K. A., and Hampton, R.O. (1980). Transmission of two purified carlaviruses by the pea aphid. *Phytopathology*, *70*(7), 631-633.

Webster, B. (2012). The role of olfaction in aphid host location. *Physiological Entomology*, *37*(1), 10–18.

Webster, B., Bruce, T., Dufour, S., Birkemeyer, C., Birkett, M., Hardie, J., & Pickett, J. (2008). Identification of volatile compounds used in host location by the black bean aphid, *Aphis fabae*. *Journal of Chemical Ecology*, *34*(9), 1153–1161.

Westwood, J. H., Groen, S. C., Du, Z., Murphy, A. M., Anggoro, D. T., Tungadi, T., Luang-In, V., Lewsey, M. G., Rossiter, J. T., Powell, G., Smith, A. G., & Carr, J. P. (2013). A trio of viral proteins tunes aphid-plant interactions in *Arabidopsis thaliana*. *PLoS One*, *8*(12), 1–18, e 0083066.

Westwood, J. H., Lewsey, M. G., Murphy, A. M., Tungadi, T., Bates, A., Gilligan, C. A., & Carr, J. P. (2014). Interference with jasmonic acid-regulated gene expression is a general property of viral suppressors of RNA silencing but only partly explains virus-induced changes in plant-aphid interactions. *Journal of General Virology*, *95*, 733–739.

Westwood, J. H., & Stevens, M. (2010). Resistance to aphid vectors of virus disease. *Advances in Virus Research*, *76*, 179-210.

White, R. F. (1979). Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. *Virology*, *99*(2), 410–412.

Whitfield, A. E., Falk, B. W., & Rotenberg, D. (2015). Insect vector-mediated transmission of plant viruses. *Virology*, *479*, 278-289.

Whitfield, A. E., Huot, O. B., Martin, K. M., Kondo, H., & Dietzgen, R. G. (2018). Plant rhabdoviruses—their origins and vector interactions. *Current Opinion in Virology*, *33*, 198–207.

Whitham, S. A., Yang, C., & Goodin, M. M. (2006). Global impact: Elucidating plant responses to viral infection. *Molecular Plant-Microbe Interactions*, *19*(11), 1207–1215.

Wilkinson, T. L., & Douglas, A. E. (2003). Phloem amino acids and the host plant range of the polyphagous aphid, *Aphis fabae*. *Entomologia Experimentalis et Applicata*, *106*(2), 103–113.

Will, T., & Van Bel, A. J. E. (2006). Physical and chemical interactions between aphids and plants. *Journal of Experimental Botany*, *57*(4), 729–737.

Winde, I., & Wittstock, U. (2011). Phytochemistry Insect herbivore counteradaptations to the plant glucosinolate – myrosinase system. *Phytochemistry*, *72*(13), 1566–1575.

Wu, D., Qi, T., Li, W. X., Tian, H., Gao, H., Wang, J., Ge, J., Yao, R., Ren, C., Wang, X. B., Liu, Y., Kang, L.,

Ding, S. W., & Xie, D. (2017). Viral effector protein manipulates host hormone signalling to attract insect vectors. *Cell Research*, *27*(3), 402–415.

Wylie, S. J., Zhang, C., Long, V., Roossinck, M. J., Koh, S. H., Jones, M. G. K., Iqbal, S., & Li, H. (2015). Differential responses to virus challenge of laboratory and wild accessions of Australian species of *Nicotiana*, and comparative analysis of *RDR1* gene sequences. *PLoS One*, *10*(3), 1–22.

Xie, D. X., Feys, B. F., James, S., Nieto-Rostro, M., & Turner, J. G. (1998). COI1: An Arabidopsis gene required for jasmonate-regulated defence and fertility. *Science*, *280*(5366), 1091–1094.

Xiu, C., Dai, W., Pan, H., Zhang, W., Luo, S., Wyckhuys, K. A. G., Yang, Y., & Lu, Y. (2019). Herbivore-induced plant volatiles enhance field-level parasitism of the mirid bug *Apolygus lucorum*. *Biological Control*, *135*, 41–47.

Xu, P., Chen, F., Mannas, J. P., Feldman, T., Sumner, L. W., & Roossinck, M. J. (2008). Virus infection improves drought tolerance. *New Phytologist*, *180*(4), 911–921.

Yi, X. U. (2020). Aphids and their transmitted potato viruses: A continuous challenges in potato crops. *Journal of Integrative Agriculture*, *19*(2), 367-375.

Yan, Y., Huang, P. C., Borrego, E., & Kolomiets, M. (2014). New perspectives into jasmonate roles in maize. *Plant Signaling and Behavior*, *9*(10), 1–5.

Yang, J. Y., Lee, S. M., & Lee, H. S. (2015). Phototactic behaviour 6: behavioural responses of *Myzus persicae* (Hemiptera: Aphididae) to light-emitting diodes. *Journal of the Korean Society for Applied Biological Chemistry*, *58*(1), 9–12.

Yang, Y., Kim, K. S., & Anderson, E. J. (1997). Seed transmission of cucumber mosaic virus in spinach. *Phytopathology*, *87*(9), 924-931.

Yang, Z., Ebright, Y. W., Yu, B., & Chen, X. (2006). HEN1 recognizes 21-24 nt small RNA duplexes and deposits a methyl group onto the 2′ OH of the 3′ terminal nucleotide. *Nucleic Acids Research*, *34*(2), 667–675.

Yoon, J. Y., & Palukaitis, P. (2021). Cucumber mosaic virus 1a protein interacts with the tobacco she1 transcription factor and partitions between the nucleus and the tonoplast membrane. *Plant Pathology Journal*, *37*(2), 182–193.

Yoshii, M., Nishikiori, M., Tomita, K., Yoshioka, N., Kozuka, R., Naito, S., & Ishikawa, M. (2004). The Arabidopsis cucumovirus multiplication 1 and 2 loci encode translation initiation factors 4E and 4G. *Journal of Virology*, *78*(12), 6102-6111.

Yoshii, M., Yoshioka, N., Ishikawa, M., & Naito, S. (1998). Isolation of an *Arabidopsis thaliana* mutant in which accumulation of cucumber mosaic virus coat protein is delayed. *Plant Journal*, *13*(2), 211–219.

Yuan, J. S., Wang, D., & Stewart, C. N. (2008). Statistical methods for efficiency adjusted real-time PCR quantification. *Biotechnology Journal*, *3*(1), 112–123.

Zaitlin, M., & Hull, R. (1987). Plant virus-host interactions. *Annual Review of Plant Physiology*, *38*(1), 291-315.

Zhang, C., Xian, Z., Huang, W., & Li, Z. (2015). Evidence for the biological function of miR403 in tomato

development. *Scientia Horticulturae*, *197*, 619–626.

Zhang, L., Handa, K. & Palukaitis, P. (1994). Mapping local and systemic symptom determinants of cucumber mosaic cucumovirus in tobacco. *Journal of General Virology, 75*, 3185–3191.

Zhang, P. J., Zheng, S. J., Van Loon, J. J. A., Boland, W., David, A., Mumm, R., & Dicke, M. (2009). Whiteflies interfere with indirect plant defence against spider mites in Lima bean. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(50), 21202–21207.

Zhang, P., Zhu, X., Huang, F., Liu, Y., Zhang, J., Lu, Y., & Ruan, Y. (2011). Suppression of jasmonic acid-dependent defence in cotton plant by the mealybug *Phenacoccus solenopsis*. *PLoS One*, *6*(7), e22378.

Zhang, X., Yuan, Y.-R., Pei, Y., Lin, S.-S., Tuschl, T., Patel, D. J., & Chua, N. H. (2006). Cucumber mosaic virus-encoded 2b suppressor inhibits Arabidopsis Argonaute1 cleavage activity to counter plant defence. *Genes & Development*, *20*(23), 3255–3268.

Zhang, Y., Tessaro, M. J., Lassner, M., & Li, X. (2003). Knockout analysis of Arabidopsis transcription factors TGA2, TGA5, and TGA6 reveals their redundant and essential roles in systemic acquired resistance. *The Plant Cell*, *15*(11), 2647-2653.

Zhao, P., Zhang, X., Gong, Y., Wang, D., Xu, D., Wang, N., Sun, Y., Gao, L., Liu, S.S., Deng, X.W. and Kliebenstein, D.J., 2021. Red-light is an environmental effector for mutualism between begomovirus and its vector whitefly. *PLoS Pathogens*, *17*(1), e1008770.

Zhou, F., & Pichersky, E. (2020). More is better: the diversity of terpene metabolism in plants. *Current Opinion in Plant Biology*, *55*,1–10.

Zhou, J. M., Trifa, Y., Silva, H., Pontier, D., Lam, E., Shah, J., & Klessig, D. F. (2000). NPR1 differentially interacts with members of the TGA/OBF family of transcription factors that bind an element of the *PR-1* gene required for induction by salicylic acid. *Molecular Plant-Microbe Interactions*, *13*(2), 191–202.

Zhou, T., Murphy, A. M., Lewsey, M. G., Westwood, J. H., Zhang, H.-M., Gonzá Lez, I., Canto, T. S., & Carr, J. P. (2014). Domains of the cucumber mosaic virus 2b silencing suppressor protein affecting inhibition of salicylic acid-induced resistance and priming of salicylic acid accumulation during infection. *Journal of General Virology*, *95*, 1408–1413.

Zhu-Salzman, K., Bi, J. L., & Liu, T. X. (2005). Molecular strategies of plant defence and insect counter-defence. *Insect Science*, *12*(1), 3–15.

Zhu-Salzman, K., Salzman, R. A., Ahn, J. E., & Koiwa, H. (2004). Transcriptional regulation of sorghum defence determinants against a phloem-feeding aphid. *Plant Physiology*, *134*(1), 420–431.

Ziebell, H., Murphy, A. M., Groen, S. C., Tungadi, T., Westwood, J. H., Lewsey, M. G., Moulin, M., Kleczkowski, A., Smith, A. G., Stevens, M., Powell, G., & Carr, J. P. (2011). Cucumber mosaic virus and its 2b RNA silencing suppressor modify plant-aphid interactions in tobacco. *Scientific Reports*, *1*, 187.

Zitter, T. A., & Murphy, J. F. (2009). Cucumber mosaic virus. The Plant Health Instructor. *American Phytopathological Society*, DOI: 10.1094/PHI-I-2009-0518-01.