The impact of plant identity and induced effects on within- and trans-generational disease resistance in the cabbage looper *Trichoplusia ni*

by

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Declaration of Committee

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Abstract

Secondary plant metabolites can have a significant impact on the susceptibility of Lepidoptera to baculovirus infection, both directly and indirectly, and thus can play a major role in determining insect fitness. Here I first examine the effects of diet complexity on the resistance of the cabbage looper (*Trichoplusia ni*) to a nucleopolyhedrovirus (TnSNPV). I then tested whether induction of plant secondary chemicals as a result of insect feeding impacts transgenerational disease resistance. I found that larvae fed on a mixed plant diet had similar resistance to virus as larvae fed on single species diets. However, larvae fed on tomato prior to virus challenge had higher resistance to virus, compared to those fed on cabbage and broccoli. Plant induction did not affect transgenerational virus resistance, although offspring mortality was lower for insects whose parents fed on broccoli compared to cabbage. This suggests that plant defensive chemistry has longer-term, indirect effects on disease resistance which could impact host dynamics.

Keywords: mixed diet; nucleopolyhedrovirus; disease resistance; transgenerational effects; immunity; plant induction

Dedication

To mom, dad, Patrick and Erika

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List of Acronyms

AcMNPV	*Autographa californica* multiple
BV	nucleopolyhedrovirus Baculovirus
GV	Granulovirus
IPM	Integrated Pest Management
OB	Occlusion body
PM	Peritrophic membrane
NPV	Nucleopolyhedrovirus
TnSNPV	*Trichoplusia ni* single nucleopolyhedrovirus
TnMNPV	*Trichoplusia ni* multiple nucleopolyhedrovirus

Chapter 1. General Introduction

Insect herbivores are inextricably linked to their host plants, resulting in a co-evolutionary arms race to overcome the development of novel defence mechanisms (Ehrlich & Raven 1964; Dawkins & Krebs 1979; Gatehouse 2002; Thompson 2009). Plants have evolved morphological structures (trichomes, hairs, spines), proteins and secondary metabolites that are toxic, repellent or anti-nutritional to their enemies to protect themselves (Rani & Jyothsna 2010; War et al. 2011a; 2011b; Mithöfer & Boland 2012). Plant secondary metabolites, in particular, are extremely diverse. They differ from other compounds produced by plants in that they do not contribute to growth and development, but instead render tissue unpalatable and toxic (Howe & Jander 2008). Much research points to the fact that ingesting these secondary metabolites decreases insect fitness (Walling 2000; Morant et al. 2008). However, insects have also developed strategies to detoxify, sequester or excrete these defensive compounds to minimize their impact (Self et al. 1964; Ivie et al. 1983; Opitz & Müller 2009; Winde & Wittstock 2011). The defensive secondary metabolites that plants produce are typically categorized as either constitutive, which are always expressed, or induced, which increase in response to insect or pathogen attack (Kessler & Baldwin 2002). Interestingly, not all constitutive chemicals are induced, as evidenced by fact that only certain categories of glucosinolates are expressed in higher concentrations as a result of herbivory (Wittstock & Gershenzon 2002; Halkier & Gershenzon 2006). However, much insect-plant research has focussed on induced defenses as they are not as metabolically costly to the plant and are usually pest specific (Agrawal & Karban 1999; Chen 2008), whereas constitutively expressed chemicals provide consistent directional selection pressure which could cause herbivores to adapt quickly (Gould 1979; Fry 1989). Induced plant chemicals have adverse effects on insect growth and fitness and have been suggested as an important factor in population regulation of forest Lepidoptera since induction is usually density-related (Haukioja & Niemelä 1977; 1979). However, this has been debated, as induction can also have neutral effects on forest insects (Niemelä et al. 1984; Myers & Williams 1987; Haukioja 1991).

What is less clear is how plant chemistry, particularly induced effects, influences an insect's natural enemies in tritrophic interactions. Most research has been focussed on how an herbivore's parasitoids are impacted by host plant chemistry. Chemicals can affect parasitoids either directly through toxic effects or indirectly through effects on host development and growth (Kennedy 2003; Gols & Harvey 2009; Kaplan et al. 2016).

While fungal entomopathogens such as *Metarhizium spp*. and *Beauveria* spp. attack by penetrating the host cuticle, viruses (baculoviruses) and bacteria (e.g., *Bacillus thuringiensis*) need to be ingested to initiate infection, and thus host plants could have both direct and indirect effects on these pathogens. The potential impact of plants on entomopathogens was recognised over 25 years ago (Duffey et al. 1995; Kouassi et al. 2001; Ali et al. 2004), and the broader impact of these interactions has been discussed in reviews (Cory & Hoover 2006; Shikano 2017). There is clear evidence that different host plant species can alter the impact or severity of disease, as well as other parameters of the host-pathogen relationship, such as speed of kill and the production of transmission stages (Hodgson et al. 2002; Raymond et al. 2002; Hodgson et al. 2006). Phytochemistry can affect some pathogens directly, and it can also cause indirect effects via changes in insect behaviour, morphology, physiology or immunity (Haviola et al. 2007; de Roode et al. 2008). For pathogens that need to be ingested to cause infection, direct chemical effects can occur within the insect gut, where chemicals bind to virus particles, create oxidative stress or, over the longer term, change the structure of the peritrophic membrane in the gut, all of which inhibit infection by negatively affecting occlusion bodies or other infectious stages (Granados & Williams 1986; Keating et al. 1988; 1989; Plymale et al. 2008). Pathogen transmission stages can also be degraded by chemical exudates on the leaf surface (Cory & Hoover 2006; Stevenson et al. 2010). Studies also indicate that host plant type and defensive chemistry negatively impact some immune components, such as haemocyte number and the encapsulation response (Bukovinszky et al. 2009; Shikano et al. 2010). However, it should be noted that these immune traits do not always correlate with their impact on the insects' ability to resist disease. For example, phenoloxidase activity does not always correlate with resistance to baculovirus infection in Lepidoptera (Saejeng et al. 2010). By affecting immune related processes such as melanisation, encapsulation, phenoloxidase activity and antibacterial activity, nutritional factors such as diet quality and quantity, and host plant chemicals can alter the outcome between insect hosts and their pathogens (Siva-Jothy & Thompson 2002; Schmid-Hempel 2005; Wilson & Cotter 2013). So secondary plant metabolites can negatively impact pathogen infection inside the gut, but the same chemicals can also cause an increase in host susceptibility through detrimental effects on insect growth and performance. Thus, there is potentially a trade-off between the effects of plant secondary chemicals on the insect and the pathogen, where the outcome is dependent on who is most affected. For example, Hunter and Schultz (1993) found that plant induction decreases baculovirus infection in gypsy moth (*Lymantria dispar*), implying that as host density increases, disease susceptibility decreases. However, disease transmission increases as population density increases in natural systems (Woods & Elkinton 1987), so this would oppose this effect. Induction thus potentially reduces variability in infection, meaning less instances of higher and lower than average risk of infection, which increases the cumulative infection rate as host density increases (Anderson & May 1991; Elderd et al. 2013).

Most studies have focussed on the interaction between a single plant, a host insect and a pathogen. However, in the wild insects can feed on multiple plant species (or multiple plant parts) which are likely to have diverse chemistries. While much of the early research carried out on grasshoppers suggest that mixtures of food plants are beneficial in terms of fitness (reviewed in Bernays & Minkenberg 1997; Hägele & Rowell-Rahier 1999; Unsicker et al. 2008), not all studies agree that diet mixing can be universally beneficial. Benefits of diet mixing on survival, growth and size of larvae have been shown in studies with Lasiocampidae, Erebidae and Arctiidae (Singer et al. 2002; Mody et al. 2007: Karban et al. 2010), but multiple larval diets had no effect or negative effects compared to single diets in studies with Geometridae and Erebidae (Stoyenoff et al. 1994; Yang et al. 2008). However, few studies have looked at the impact that mixing plant diets has on tritrophic interactions, or even factors that might contribute to it, such as immunity. The studies which have looked at immune parameters give contradictory results. For example, in Lepidoptera, Ojala et al (2005) found neutral and negative effects of mixed diets on encapsulation relative to single diets, whereas Yang et al. (2008) saw neutral and positive effects on encapsulation and phenoloxiadse activity. Thus, it is not clear if the cost of dealing with multiple diets, and therefore more complex chemistries, impacts the cost of initiating immune system defence. Moreover, there are no data on whether multiple diets impact pathogen infection in insects.

Parental diet also has the potential to affect offspring through transgenerational effects. Maternal effects (i.e. transmitted through the mother) were first identified (in Lepidoptera) by Rossiter (1991; 1996), who demonstrated that maternal host plant, and thus secondary chemistry ingested, affected offspring survival and fitness in the gypsy moth. Changes in foliage quality, food shortages, constitutive and induced secondary metabolites and other nutritional stresses, often associated with increasing population densities, can affect offspring generations in many different ways (Greenblatt & Barbosa 1980; Rossiter 1991; Bauerfeind & Fischer 2005; Myers et al 2011). In insects, data indicate that egg composition, survival, growth and development are affected by parental diet (Fox & Dingle 1994; Carisey & Bauce 2002; Rotem et al. 2003), including in Lepidoptera (Rossiter et al. 1993; Carisey & Bauce 2002; Bauerfeind & Fischer 2005). These effects, however, can be positive or negative, or can sometimes be neutral (Myers et al. 2011).

Transgenerational effects in Lepidoptera are not limited to nutrition. Factors such as the parents' thermal environment and pathogen challenge can affect offspring fitness, for example, through changes in immunity (Woestmann & Saastamoinen 2016). This work has led to the observation that immune priming can take place in the offspring of parents who have experienced stressful dietary conditions such as starvation (quantity) or nutritionally poor diets (quality) (in addition to resulting from exposure to an immune elicitor or pathogen). However, the results are variable, although several studies have shown that poor diet tends to increase disease resistance in the offspring generation. Not many studies have looked at the effects of parental diet on transgenerational disease resistance, and the few that have are not always comparable as they use organisms from very diverse taxa (Mitchell & Read 2005; Boots & Roberts 2012; Triggs & Knell 2012). For example, Mitchell and Read (2005), studying the water flea *Daphnia magna*, found increased offspring disease resistance (to a bacterium) in a poor maternal environment, but the study had the confounding variable of maternal crowding and pathogen exposure, in addition to changes in food quantity. Shikano et al. (2015) working with *Trichoplusia ni*, found that provisioning parents with poor (diluted) artificial diet resulted in increased offspring resistance to both a baculovirus and a bacterium (*Bacillus thuringiensis*), as well as both positive and negative changes in some immune measures. In another study on the western tent caterpillar (*Malacosoma pluviale californicum*), using alder *(Alnus rubra)* leaves, partial starvation had no effect on offspring disease resistance in the next generation (Myers et al. 2011). Most of these studies use changes in food quantity to examine the effect in offspring; there is less information on how (or whether) plant secondary chemicals, particularly induced chemicals, affect offspring disease resistance. Rothman (1997), studying the western tent caterpillar and alder, found no effects of plant induction on baculovirus resistance in the next generation. However, Olson (2014), looking at the same species, found that provisioning the parent generation with induced foliage resulted in offspring that were more resistant to their baculovirus. Thus, the impact of induction and plant chemistry in general, on offspring disease resistance is not clear.

1.1. Study system

1.1.1. Cabbage looper, *Trichoplusia ni*

The cabbage looper (*Trichoplusia ni)* (Hübner) is a moth in the Noctuidae family. It can complete its development on more than 150 species of plants including commercially important crops such as broccoli, cabbage, cauliflower, tomato, pepper, mustard, cucumber, potato, and cotton (Ehler 1977; Hoo et al. 1984). It prefers cruciferous crops, developing and performing better on *Brassica* plants than on other species (Li & Liu 2015). *Trichoplusia ni* is an important model organism. It is multivoltine with multiple generations per growing season (Jackson et al. 1969; Guy et al. 1985) and has the potential to be an economically impactful pest, resulting in losses of up to $2 million annually in North America (Mullan 2003). It is also an important greenhouse pest in Ontario and British Columbia (Erlandson 2013).

1.1.2. Host Plants

Larvae of *T. ni* feeding on crucifers invariably encounter glucosinolates (Wittstock et al. 2003; Li et al. 2006; Ahuja et al. 2011) that are characteristic of *Brassica* species (cabbage, mustard, broccoli, cauliflower, etc.). Glucosinolates also occur in more than 15 families in the Order Brassicales (Rodman et al. 1996; Halkier & Gershenzon 2006; Mithen et al. 2010) that are host plants of *T. ni*. Glucosinolates are stored separately in plant tissue from a catalyzing enzyme called myrosinase; when cells are disrupted by feeding or injury, they are combined resulting in a breakdown of the glucosinolates into toxic by-products such as isothiocyanates (Grubb & Abel 2006; Halkier & Gershenzon 2006; Müller et al. 2010). Glucosinolate concentrations vary between species and conspecifics (Fahey et al. 2001; Ahuja et al. 2011). Their effects differ for different herbivorous insect species, reducing their feeding or disrupting their development (Ulmer et al. 2001; Bones & Rossiter 2006; Hopkins et al. 2009; Kos et al. 2012). However, for the most part, glucosinolates negatively impact insects ingesting them (Halkier & Gershenzon 2006; Hopkins et al. 2009; Rasmann et al. 2012). *Trichoplusia ni* is negatively affected by certain glucosinolate by-products, which have adverse impacts on larval performance and weight (Kliebenstein et al. 2002; Müller et al. 2010). Some specialist herbivores, such as the diamondback moth (*Plutella xylostella*) and the cabbage white (*Pieris rapae*), deal with glucosinolates by turning them into attractant signals (Miles et al. 2005; Safraz et al. 2006; Müller et al. 2010), whereas *T. ni* detoxifies most by-products (Winde & Wittstock 2011).

T. ni will also feed on various varieties of tomato. Tomato has many different compounds that can harm and deter insects not adapted to their defensive chemicals. Glycoalkaloids such as tomatine have been known to directly impede insect survival and performance (Duffey & Stout 1996). Other defensive compounds produced by tomato, including phenolics and proteinase inhibitors, have been known to be detrimental to lepidopteran development, growth and survival, amongst other parameters (Elliger et al. 1981; Isman & Duffey 1982; Felton 2005; Gonzales-Vigil et al. 2011).

1.1.3. Baculoviruses

Baculoviruses are a family of insect-specific, double-stranded DNA viruses, that have been extensively studied in terms of their biology, ecology and molecular biology (Cory & Myers 2003; Fuxa 2004; Harrison & Hoover 2012). Baculoviruses are divided into four subgroups based on host and morphological traits: Alphabaculoviruses (Lepidopteran nucleopolyhedroviruses (NPV)), Betabaculoviruses (Lepidopteran granuloviruses (GV)), Gammabaculoviruses (Hymenopteran NPV) and Deltabaculoviruses (Dipteran NPV) (Jehle et al. 2006). They are obligate parasites which infect mostly lepidopteran hosts at the larval stage (Cory & Hoover 2006). NPVs have a unique morphology in that the infectious units (virus particles or virions) are packaged within proteinaceous occlusion bodies (OBs). This is the virus transmission stage and the protein coat allows them to persist in the environment for long periods of time, when not exposed to UV irradiation (Thompson et al. 1981; Carruthers et al. 1988). The occlusion body needs to be ingested, usually along with foliage, to initiate infection, and once inside the gut, the alkaline pH dissolves the OB, releasing the virions (Grzywacz, 2017). These will bind to gut cells, injecting their DNA and initiating replication. Infection will spread to most insect tissues, and eventually host tissue starts to liquefy, spreading millions of OBs on the plant surface, thus continuing transmission (Cory & Myers, 2003).

Trichoplusia ni can be infected by several baculoviruses in the wild; virus species that have been isolated from *T. ni* include *T. ni* granulovirus (TnGV), *T. ni* multiply enveloped nucleopolyhedrovirus (TnMNPV), and *T. ni* singly enveloped nucleopolyhedrovirus (TnSNPV) (Jaques 1970; Erlandson et al. 2007). ‘Single’ and ‘multiple’ are phenotypes of a stage of the virus which refers to the capsid number (envelope containing viral DNA) (Jehle et al. 2006; Sosa-Gómez et al. 2020).

TnSNPV and TnMNPV have been evaluated for control of *T. ni* on *Brassica* spp. (Jaques 1970; 1972; Vail et al. 1999). The latter phenotype is now recognized as a variant of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) (Theilmann et al. 2005; Harrison et al. 2012), from which a strain (FV11) has been proposed for use in the control of *T.ni* in greenhouses, due to its wider host range relative to other NPVs (PRRP 2016a; 2016b). This strain is now in two formulations: a greenhouse formulation called Loopex and a field formulation called Loopex FC (Franklin et al. 2018; Viaene 2018).

1.1.4. Research questions

The aim of my thesis is to investigate first whether mixed diets can affect *T. ni* resistance to TnSNPV. In Chapter 2, to answer this question I first investigated whether growth and development were impacted by mixing diets of cabbage, broccoli and/or tomato. I then measured the proportion of larvae dying of virus as well as the speed of death. In Chapter 3, I attempt to determine whether plant secondary defensive metabolites have a transgenerational impact on *T. ni* disease resistance. I explore whether plant induction causes sublethal effects on the parental generation, while also looking at mortality due to virus and speed of death in their offspring.

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Chapter 2. The effect of mixed plant diets on *Trichoplusia ni* growth and resistance to a nucleopolyhedrovirus (TnSNPV)

Abstract

Mixing diets has been shown to impact insect fitness and development and thus could play a role in their interactions with natural enemies. Feeding on multiple host plants, which are chemically more complex than single host plants, could be more costly to deal with and potentially trade-off with other processes such as growth or disease resistance. To test this idea, I first examined the effects of diet complexity on larval growth and development of the cabbage looper (*Trichoplusia ni*) by feeding larvae cabbage, broccoli and tomato either singly or in combinations of two or three species. Larval weight and development times were negatively impacted by increasing the complexity of diet, whereas emergence and pupal mass were not. I then measured the resistance of *T. ni* to a nucleopolyhedrovirus (TnSNPV) and found that larvae fed on a mixed plant diet had similar resistance to virus as larvae fed on single plant diets. However, larvae fed on tomato prior to virus challenge had higher resistance to virus, compared to those fed on cabbage and broccoli. Our results indicate that mixed diets in general do not alter disease resistance compared to single diets in *T. ni*, and instead suggest that plant identity is more important.

2.1. Introduction

The diet breadth of phytophagous insects is a spectrum ranging from specialist to generalist (Bernays et al. 1994). There is some debate as to describing an herbivore as a generalist, as polyphagy can mean different things. Polyphagy at the population level includes many different populations of the same herbivore species feeding on a limited range of hosts, whereas polyphagy at the individual level is a more classic definition of generalist, where a single organism feeds on a variety of plant hosts. This type of generalist can potentially benefit from feeding on multiple hosts or mixing diets, allowing them to select foods that can be complementary or that fit certain changing physiological needs (Bernays & Minkenberg 1997; Mody et al. 2007; Unsicker et al. 2008). Eating multiple host plant species can allow herbivores to attain their nutritional requirements rapidly and grow efficiently (Pulliam 1975; Simmonds et al. 1992; Bernays et al. 1994; Simpson et al. 2004; Karban et al. 2010). For example, generalist insects, such as grasshoppers (Acrididae), benefit from diet mixing by having a faster growth rate (Lewis & Bernays 1985; Lee 1990), better survival (Hodge 1933; Pfadt 1949; Barnes 1955; Unsicker et al. 2008) and improved fertility (Bernays et al. 1994; Mody et al. 2007). Herbivorous caterpillars are also able to regulate their feeding and mix food sources to acquire an optimum mix of nutrients (Walbauer et al. 1984; Telang et al. 2001; Lee et al. 2002; Lee et al. 2006; Merkx-Jacques et al. 2008). Some herbivores can benefit from diet mixing by diluting the unique set of toxic chemicals that each plant produces (Freeland & Janzen 1974; Singer et al. 2002; Marsh et al. 2006; Karban et al. 2010). Counter adaptations of insects for dealing with toxic plant chemicals include rapidly absorbing them, metabolically converting them to harmless compounds, as well as reducing the disruption of plant cells which stops the catalzying reaction from creating harmful by-products (Winde & Wittstock 2011). For generalist insects whose tactic is to detoxify chemicals, switching plants allows them to avoid encountering higher concentrations of the same chemical(s) as they feed (Bernays & Lee 1988). As plant chemistry and nutrients also vary between conspecifics and even between parts of the same plant (Zangerl & Berenbaum 1993; Hemming & Lindroth 1995; Lawler et al. 2000), the benefits of host switching are not necessarily restricted to herbivores who switch between different host species (Moreau et al. 2003; Pinaut & Quiring 2009; Kotowska et al. 2010).

Host switching is quite common in some families of Lepidoptera, such as arctiids, noctuids, lasiocampids and nymphalids (Tietz 1972; Dethier 1988; Stoyenoff et al. 1994a; Singer & Stireman 2001; Mody et al. 2007; Yang et al. 2008; Behmer 2009). Gypsy moth (*Lymantria dispar*) is a well-studied example, with larvae at high densities switching between host trees of the same and different species (Barbosa 1978; Mauffette & Lechowicz 1984; Liebhold et al. 1986). In mixed stands, many gypsy moth larvae experience multiple hosts in their diet (Stoyenoff et al. 1994b), which may reflect dietary needs at different stages in their life. Some host plants or plant stages are better for early-instar larvae, whereas others are more beneficial for late-instar larvae, improving their overall development compared to feeding on a single food source (Barbosa et al. 1986a; Johns et al. 2009). Many generalists display different forms of host switching behaviour, such as dispersal as first instar larvae (Capinera & Barbosa

1976, Lance & Barbosa 1982), repeatedly falling off their host (Bernays & Minkenberg 1997), and regularly switching to and from their host plant in the course of a day (Schultz 1983a; Dethier 1988). Even the specialist lasiocampid moth *Chrysopsyche imparilis* regularly switches between individual trees (Mody et al. 2007).

However, little is known about whether diet mixing also alters defense against an insect's natural enemies, as most studies do not examine diet mixing effects on parasitoid or pathogen fitness (Mody et al. 2007; Singer et al. 2009; Karban et al. 2010). Several studies have shown that eating certain host plants can alter resistance to parasitism (Agrawal 2000; Singer & Stireman 2003; Gols & Harvey 2009; Turlings & Erb 2018). Furthermore, host plant chemicals, such as nicotine or tomatine, ingested by herbivores can directly and indirectly harm parasitoids (Campbell & Duffey 1979; Barbosa et al. 1986b; 1991; Havill & Raffa 2000; Sime 2002). Host plants also have direct and indirect impacts on insect pathogens. The groups most frequently studied and widely used (as biological control agents) are fungi, bacteria, and baculoviruses. Different pathogen groups infect via different routes, which can alter their likelihood of plant-mediated effects. Viruses (baculoviruses) and bacteria (mainly *Bacillus thuringiensis* (*Bt*)) are usually ingested by their hosts, increasing the possibility of interactions within the insect gut, whereas entomopathogenic fungi (*Metarhizium* spp., *Beauveria* spp., etc.) infect their host through contact with the insect cuticle. However, there are still opportunities for plant impacts on entomopathogenic fungi (Cory & Ericsson 2009). Although bacteria, fungi and viruses have different infection pathways, all pathogens are susceptible to direct plant-mediated effects at the surface of the plant itself. Usually, the first encounter between the pathogen and the insect happens at the leaf surface (phylloplane). Some plants produce chemical exudates, which can directly inactivate baculoviruses (Duffey et al. 1995; Stevenson et al. 2010). Most research shows that higher levels of specific plant compounds (e.g., chlorogenic acid, rutin, tannins) can reduce viral infection by directly inactivating them in the midgut. This has been shown in many species, including, *L. dispar* moth, tomato fruitworm (*Helicoverpa zea*) and tobacco budworm (*Heliothis virescens*) (Felton et al. 1987; Keating et al. 1989; Hunter & Schultz 1993; Ali et al. 1999; Martemyanov et al. 2006). Furthermore, the stage where the pathogen proliferates through the haemolymph, is when either sequestered or ingested defensive phytochemicals can interact directly with the pathogen and potentially disrupt infection (Kouassi et al. 2001). The timing of when insects consume their diet can also impact their immunity and resistance to infection. Plant effects, pre- or post-infection, could result from indirect effects on insect growth, physiology or immunity (Ali et al. 1998; Haviola et al. 2007; Yang et al. 2008). Many studies have investigated the effect of defensive plant chemicals at the point of pathogen ingestion (e.g. Felton et al. 1987; Felton & Duffey 1990; Duffey et al. 1995) but less attention has been paid to the impact either before or after infection to see if plant chemicals have indirect effects.

2.1.1. Cost of chemicals on the immune system and pathogen infection

Little is known about how entomopathogens respond when their host consumes a mixed plant diet, and thus an increased chemical diversity, within their lifetime. Ingesting defensive chemical compounds such as iridoid glycosides, phenolics and glucosinolates can have negative effects on immune responses, such as encapsulation and melanisation in lepidopterans (Camara 1997; Haviola et al. 2007; Bukovinszky et al. 2009; Hopkins et al. 2009; Smilanich et al. 2009). However, this is not always the case. Flavonoids or alkaloids have no effect in some species (Haviola et al. 2007; Smilanich et al. 2011a) and antioxidants and iridoid glycosides can even cause positive effects on insect immune responses (Ojala et al. 2005; Laurentz et al. 2012; de Roode et al. 2019). While these studies measure traits involved in immunity, defensive chemicals can directly impede pathogen success through effects within the insect gut, such as by changes in the thickness of the peritrophic membrane, lowering gut pH, or direct inhibitory effects on the pathogens themselves (Keating et al. 1988; 1990; Hoover et al. 2000; Cory & Hoover 2006; Plymale et al. 2008). Diet mixing may be beneficial for the insect, but it comes at a cost, as strategies such as detoxifying plant defensive compounds draws on metabolic resources (Schoonhoven & Meerman 1978; Jeschke et al. 2016a; 2016b). Protection against pathogens through immunity, such as the production of immune components such as hemocytes and phenoloxidase, as well as processes like encapsulation and melanisation, are also costly (Schmid-Hempel 2005; Wilson & Cotter 2013). Therefore, there may be a trade-off between these two processes, particularly if resources are limited. McMillan et al. (2018) state that food detoxification and the immune defense share many of the same resources and occur in the same organs in insects (e.g., fat body; Chapman 2013). Therefore, if they are both activated simultaneously, there could potentially be adverse effects on both processes (Adamo 2017). It follows that the outcome in insect-pathogen interactions is dependent on who is most affected by phytochemicals. Ingesting a diverse set of chemicals may sway the outcome in favour of the pathogen.

2.1.2. System and Experiment

Using *T. ni* and the host-specific viral pathogen *T. ni* single nucleopolyhedrovirus (TnSNPV) as a study system, we examined how diet mixing can affect the resistance of an insect to a pathogen. TnSNPV belongs to the baculoviruses, a family of double stranded DNA viruses, and needs to be ingested by the larvae, usually along with foliage, to initiate infection (Cory & Myers 2003). The cabbage looper, a generalist, is a pest on a wide range of crops such as crucifers, bell pepper, tomato, cotton, soybean (Harding 1976; Hoo et al. 1984; Li et al. 2006) and has been listed as feeding on 150 species of different host plants in 36 families (reviewed in Sutherland & Greene 1984). The aim of this experiment was to determine whether *T. ni* larvae that are fed on a set of host plants with different chemical and nutrient compositions, differ in their susceptibility to TnSNPV. Larvae were fed on a diet of cabbage, broccoli and tomato either singly or in various combinations. We addressed two questions: (1) Does feeding on multiple host plants, which as a group are chemically more complex than single host plants, alter larval growth and development? (2) Does plant diversity alter resistance to TnSNPV?

2.2. Materials and Methods

To test the hypothesis that ingesting a more chemically complex diet reduces resistance to pathogens, *T. ni* larvae were fed diets from three host plants in different combinations and their performance was measured. They were then challenged with TnSNPV to assess their pathogen resistance.

Insects and Virus

Eggs of *T. ni* were received from the Great Lakes Forestry Center (Sault Ste Marie, Ontario). Hatched larvae were reared on artificial diet (provided with the eggs) in groups until the 2nd instar and then transferred to individual 96 ml cups with their designated leaf treatment. The larvae were challenged with TnSNPV (isolate FV#3433), which is specific for *T. ni* (Shikano & Cory 2016). The virus was serially diluted to reach the required doses from an initial stock of 2.65 x 10^9 occlusion bodies (OB) per ml, which were quantified using an improved Neubauer brightline hemocytometer under a phase contrast microscope (400x magnification). In order to examine the effects of the host plant (diet) treatments on larvae, untreated control larvae were sexed and weighed three days after pupation.

2.2.1. Host Plants

Cabbage loopers can feed on a wide range of plants, but we focused on species that they feed on locally including cabbage (*Brassica oleracea* var*.* Danish Ballhead), broccoli (*Brassica oleracea* var*.* Centennial) and tomato (*Lycopersicum esculentum* var. Moneymaker), all planted from seed (West Coast Seeds) in July 2019. Broccoli has a higher total concentration of glucosinolate and phenolics than cabbage (Cartea et al. 2011; Fenwick et al. 1983; Podsędek 2007; Possenti et al. 2016), and tomato was chosen due to its large difference in chemical profile and its higher concentration of glycoalkaloids compared to crucifers (Kennedy 2003). In terms of nutritional differences, tomato has approximately less protein and carbohydrate content than the crucifers (Pinela et al. 2012; Bhandari & Kwak 2015). Between *Brassica* cultivars, broccoli has slightly higher proportion of protein and carbohydrates (USDA 2018a; 2018b). Around 40 plants per treatment were grown in a mixture of peat moss and mushroom manure in a greenhouse on the Burnaby campus of Simon Fraser University for approximately 3 months until the start of the experiment (October 2019). After three weeks, the seedlings were transferred to individual pots (20 cm diam. x 30 cm).

2.2.2. Diet Experiment

There were seven diet treatments in total: three single host plants (cabbage (C), broccoli (B), tomato (T)), three two-host plant combinations (C&B, B&T, C&T) and one three-host plant combination (C&B&T). For each diet treatment, 180 second instar larvae were tested, except for treatments including tomato, where 270 larvae were used to allow for a higher rate of attrition (based on preliminary experiments). All 1,620 larvae were monitored, and larvae were fed *ad libitum* where the leaves were changed every day, depending on the treatment. Leaves for each individual larva were taken from multiple plants in a random fashion, and only one leaf was taken from a plant at a time. For the mixed treatments, only one host plant was provided each day, with each individual being given approximately equal amounts of each species over the course of the experiment. When frass began to accumulate, larval containers were switched for new 96 ml cups.

2.2.3. Virus Bioassay

When the larvae reached the 4th instar, they were weighed immediately and assigned to one of three virus challenge groups, within each of the seven diet treatments, up to a maximum of 30 randomly chosen larvae in each of the 18 treatment combinations. Larvae were challenged with TnSNPV at one of two doses: a low dose (100 OBs per larvae; 50 OBs/µl), a high dose (350 OBs per larvae; 175 OBs/µl) or left as an untreated control. Each larva was placed in a 48-well plate with a small plug of wheat-germ based artificial diet to which the viral dose or deionized water was applied in a 2 µl aliquot. Larvae were left for 24 h to consume the diet plug and were then transferred to individual 29.5 ml cups containing enough diet to maintain them through to pupation. They were kept at 25°C under a photoperiod of 16L:8D throughout the experiment. Insects were monitored daily for death, pupation and/or adult emergence. Symptoms of baculovirus death are usually very obvious, with larvae becoming pale and flaccid, and eventually rupturing to release millions of OBs in a milky white to brownish fluid. Larvae with ambiguous symptoms were smeared on a slide, stained with Giemsa quick stain and inspected under a microscope with a magnification of 1000x (with oil immersion) to check for the presence of OBs.

2.2.4. Statistical Analysis

All data were analysed using R (Version 1.1.463; package “car” (Fox & Weisberg 2019)). Where appropriate, the residuals were inspected for non-normality and transformed as needed. Models were simplified by removing non-significant terms in a hierarchical manner. Differences between treatments for ANOVAs were determined by Tukey’s HSD at $P < 0.05$. Differences between treatments for linear models were tested using the “emmeans” function in R (package “emmeans”) (Searle et al. 1980; Lenth 2021).

Fourth instar larval weights, growth rate and pupal mass were all analyzed using an ANOVA. The effect of sex on pupal mass was initially included as a factor, together with diet in a Two-Way ANOVA. Each set of data was analysed in two ways. Firstly, with diet as the independent factor (Cabbage, Tomato, Broccoli, Cabbage and Tomato, Cabbage and Broccoli, etc.) and secondly with diet complexity as an ordinal variable (of one, two or three host plant species). Larval weight, growth rate and time to pupation were analyzed a third way with complexity as an ordinal variable separated by plant type (i.e. Cabbage, Cabbage and Tomato, Cabbage and Broccoli, Cabbage and Broccoli and Tomato, etc.). Larval weights (mg) were logged (base 10) prior to analysis. Growth rate was calculated as the change in mass between pupal weight and larval weight at the 4th instar divided by time in days. The time to pupation (in days) of control larvae could not be normalized, thus a Dunn's Kruskal-Wallis post-hoc test was used to determine differences between treatments (package "FSA") (Ogle 2016; Ogle et al. 2021). Adult emergence (success or failure) of control insects which successfully pupated was analysed using a Generalized Linear Model (GLM) with a binomial distribution and a logit link function.

In the bioassay, data were censored at 15 days post infection, as larvae would have died of virus prior to this time if they had been successfully infected. Any larvae that died 1 or 2 days post-challenge were considered to be 'handling deaths' and were excluded from all analyses. Differences in mortality due to virus, diet treatment, or diet complexity were examined using a GLM (binomial distribution with a logit link function). Virus dose (ordinal) was included as a single and interacting factor. Successes (number of adjusted virus-killed larvae) and failures (larvae surviving post 15 days) were input as responses. The effect of mean larval weight, together with diet and dose on mortality, was initially included as a factor in the GLM. Virus death was adjusted for both virus and other background mortality in the untreated control insects, using the formula $Va = Vd - Td * (Vc / Tc)$, where Va is the adjusted virus mortality, Vd is the dose/treatment-specific number killed by virus, Td is the dose/treatment-specific total assayed, Vc is the number killed by virus in the untreated controls and Tc is the total number assayed in the controls. The total number assayed was also adjusted, using $Tadj = Td * (1 - ((Vc + Uc) / Tc))$, where Tadj is the adjusted dose/treatment-specific total number assayed, and Uc is the number dead by unknown causes/ background mortality for the untreated control dose. The speed of kill of the virus (time in days) was analyzed using a Linear Model and diet treatment or diet complexity and viral dose (ordinal) as interacting factors.

2.3. Results

2.3.1. Diet complexity and larval performance

Larval weight. Fourth instar larval weight differed depending on which single or mixed diet they had been fed on (Figure 2.1: *plant:* $F_{6,\ 685}$ = 39.1, $p < 0.0001$). Focusing on the single host plant diets, larvae that ate cabbage were larger than those that had fed on broccoli, which were larger than those that fed on tomato (Figure 2.1). The mixed diets clearly illustrated that any combination including tomato produced smaller larvae (as compared to broccoli and cabbage together). If we analyze the diets in terms of increasing plant species complexity, larvae that fed on three host plants had a lower weight than either single- or double-diet combinations (Figure 2.2a: *complexity*: $F_{2,\ 685}$ = 22.27, $p < 0.0001$). As for survival up to the 4th instar, larvae that fed on tomato alone had the lowest with 20%, compared to 56% on cabbage and 61% on broccoli. Survival on diets including tomato ranged from 37% to 47%.

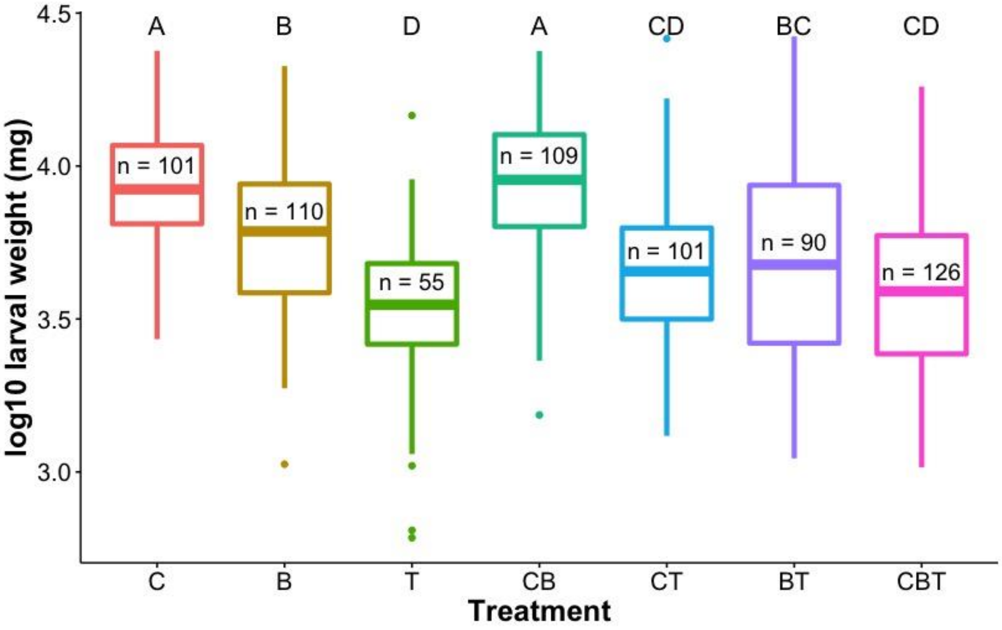

Figure 2-1 **Weights of 4th instar *T. ni* larvae fed single or mixed host plant diets [cabbage (C), broccoli (B), (T)]. Letters signify differences at P < 0.05 (Tukey's HSD). Error bars are the 95% confidence interval.**

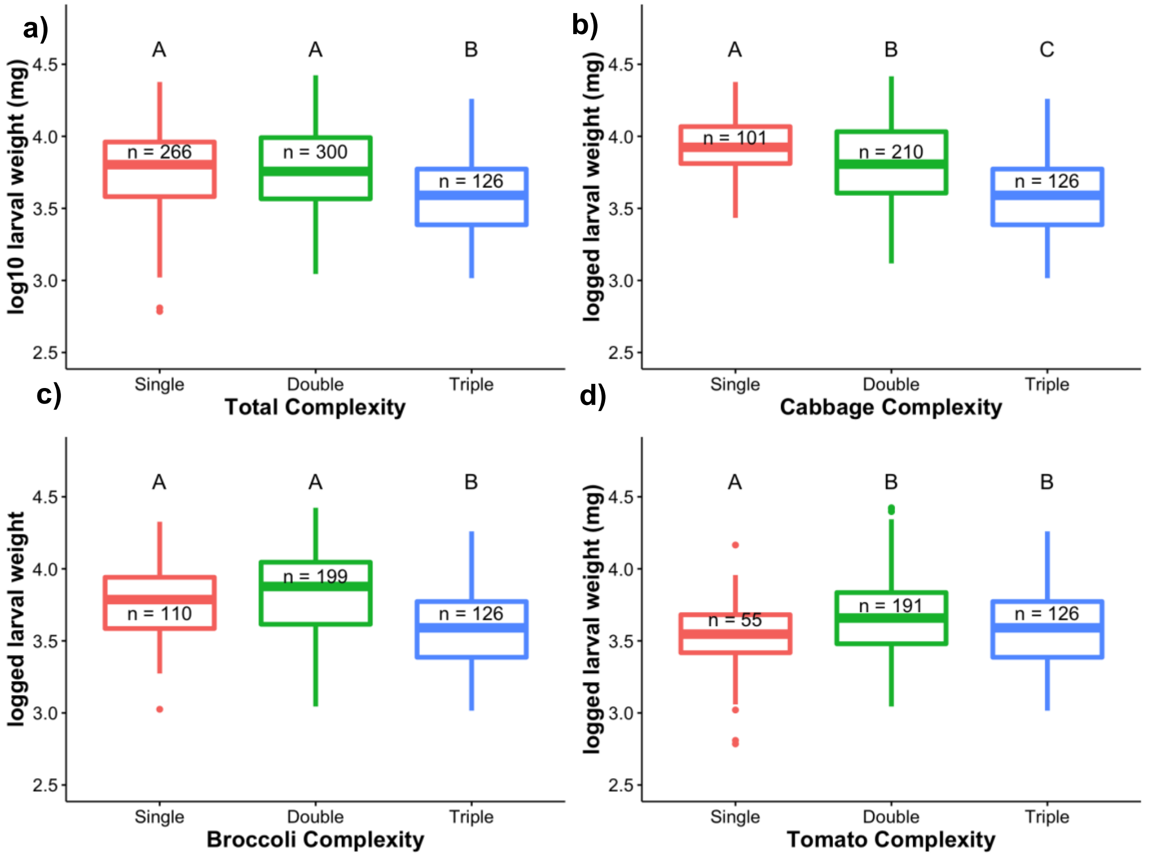

Figure 2-2 **Weights of *T. ni* larvae an increasing complexity of one, two or three host plant species of cabbage (C), broccoli (B), and tomato (T). a) Larvae fed single (C, B & T), double (CB, CT & BT) or triple (CBT) plant host combinations. b) Larvae were fed diets including cabbage with single (C), double (CB & CT) or triple (CBT) plant combinations. c) Larvae were fed diets including broccoli with single (B), double (CB & BT) or triple (CBT) combinations. d) Larvae were fed diets including tomato with single (T), double (CT & BT) or triple (CBT) combinations. Letters signify differences at P < 0.05 (Tukey's HSD). Error bars are the 95% confidence interval.**

Growth rate. The growth rate of control larvae, measured from 4th instar to pupation, was significantly affected by plant diet (Figure 2.3: *plant*: $F_{6, 187} = 3.8$, $p = 0.0013$), but there were no clear trends, with all the single host plants producing a similar growth rate and the only difference in the mixed treatments suggesting that the presence of cabbage reduced the feeding rate. When focusing on increasing diet complexity, larval growth rate on the double diets was 10% faster than on the triple diet (Figure 2.4a: *complexity*: $F_{2, 187} = 3.51$, $p = 0.032$).

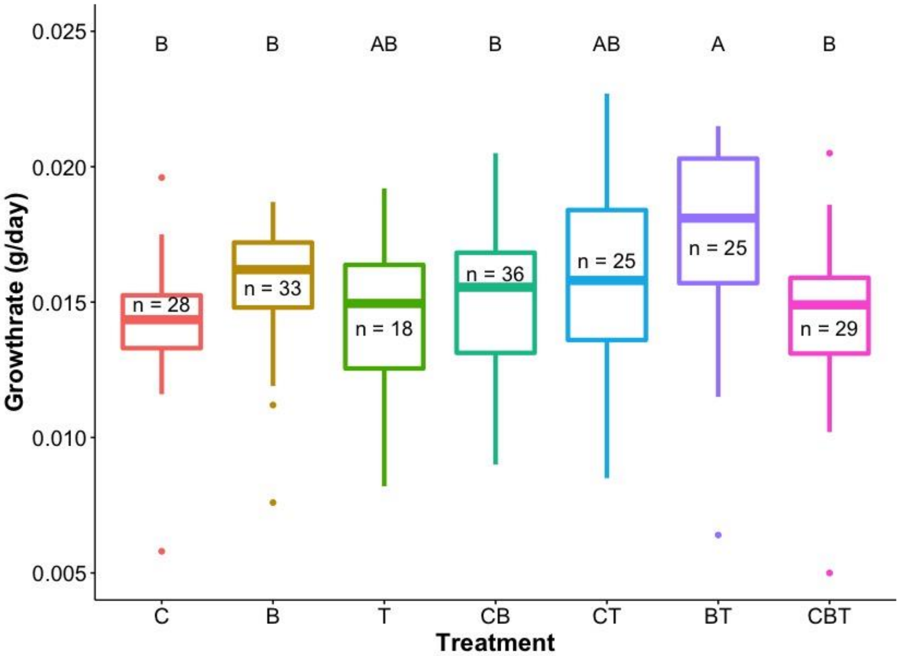

Figure 2-3 Growth rate (g/day) of control *T. ni* larvae between 4th instar and pupation, fed combinations of cabbage (C), broccoli (B), and/or tomato (T). Letters signify differences at $P < 0.05$ (Tukey's HSD). Error bars are the 95% confidence interval.

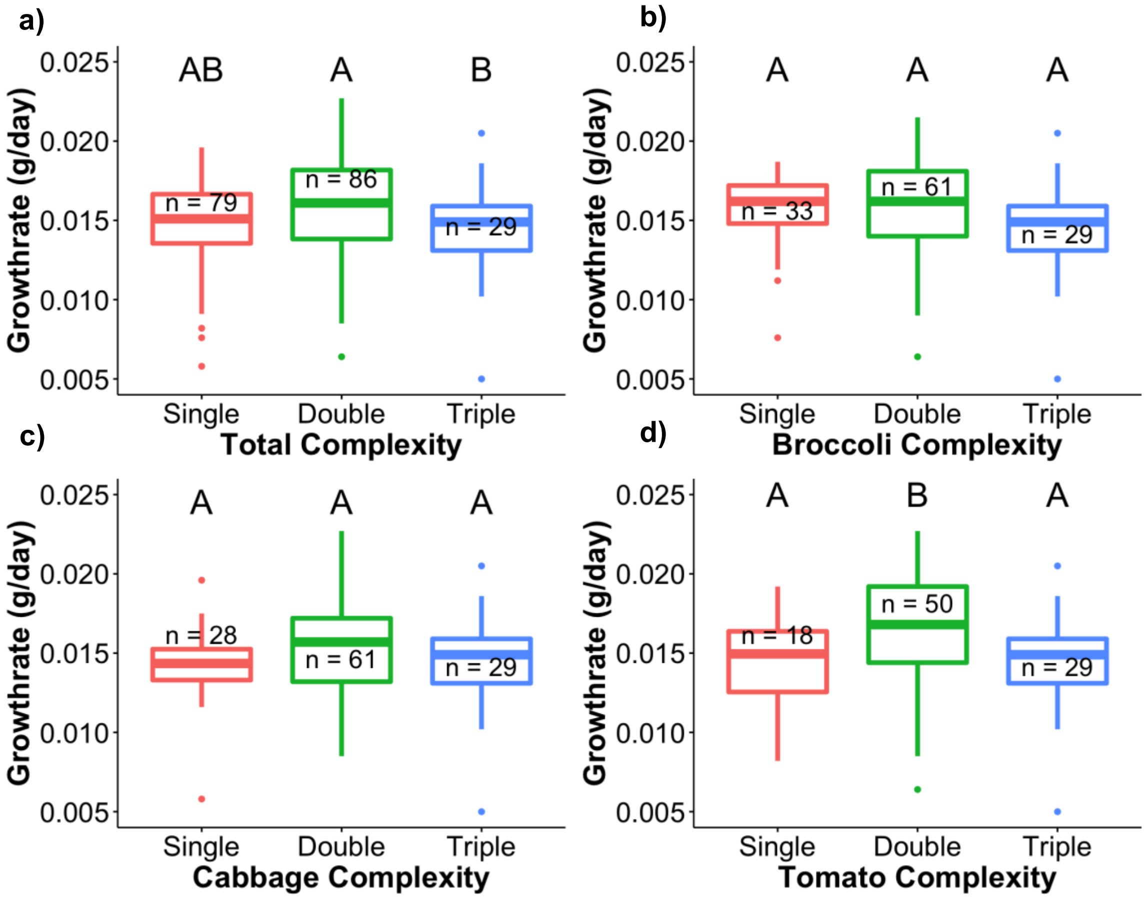

Figure 2-4 **Growth rate (g/day) of control *T. ni* larvae fed an increasing complexity of one, two or three host plant species of cabbage (C), broccoli (B), and tomato (T). a) Larvae fed single (C, B & T), double (CB, CT & BT) or triple (CBT) plant host combinations. b) Larvae were fed diets including broccoli with single (B), double (CB & BT) or triple (CBT) plant combinations. c) Larvae were fed diets including cabbage with single (C), double (CB & CT) or triple (CBT) combinations. d) Larvae were fed diets including tomato with single (T), double (CT & BT) or triple (CBT) combinations. Letters signify differences at P < 0.05 (Tukey's HSD). Error bars are the 95% confidence interval.**

Time to pupation. The time to pupation was significantly affected by diet treatment (Figure 2.5: *plant*: $X^2_6 = 140.28$, $p < 0.0001$). On the single species diets, tomato-feeding larvae took 63% and 60% longer to pupate than larvae fed on cabbage or broccoli. With increasing diet complexity, there was an increase in time to pupation (Figure 2.6a: *complexity*: $X^2_2 = 21.81$, $p < 0.0001$), with larvae fed single and double diet combinations pupating ~3.5 days (18%) and ~3 days (14%), respectively, sooner than larvae fed a combination of three plants. For cabbage diets, as complexity increased, there was a significant increase in the time it took to pupate (Figure 2.6b: *cabbage diet* *complexity*: $X^2_2 = 38.65$, $p < 0.0001$), with larvae fed three plants taking 20% (~4 days) longer to pupate than those fed two diets, and insects fed two plants taking 14% (~2 days) longer to pupate than larvae on single diets. Similarly, for broccoli complexity, larvae fed the three-combination diet took on average four (19.5%) more days to pupate than larvae eating double diets, and took ~5.5 more days (31%) to pupate than larvae eating single diets (Figure 2.6c: *broccoli diet complexity*: $X^2_2 = 31.38$, $p < 0.0001$). Lastly, when increasing tomato diet complexity, larvae feeding on tomato alone took an average of ~5 days longer to pupate than larvae fed two diets and ~4 more days than larvae fed the three-combination diet (Figure 2.6d: *tomato diet complexity*: $X^2_2 = 12.21$, $p = 0.002$).

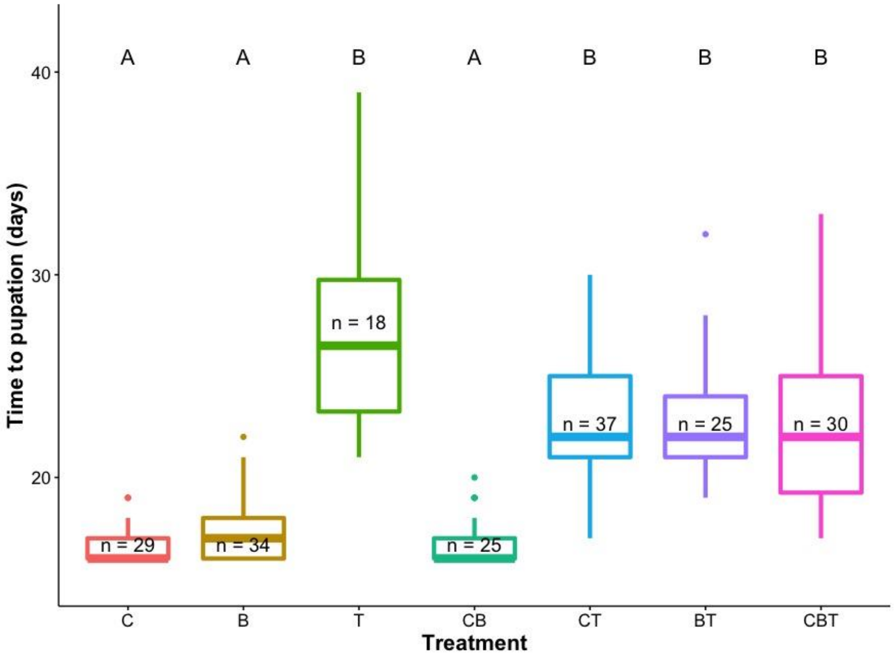

Figure 2-5 **The time to pupation of *T. ni* larvae fed combinations of cabbage (C), broccoli (B), and/or tomato (T) plant diets. Letters signify differences at P < 0.05 (Dunn's Kruskal-Wallis test). Error bars are the 95% confidence interval.**

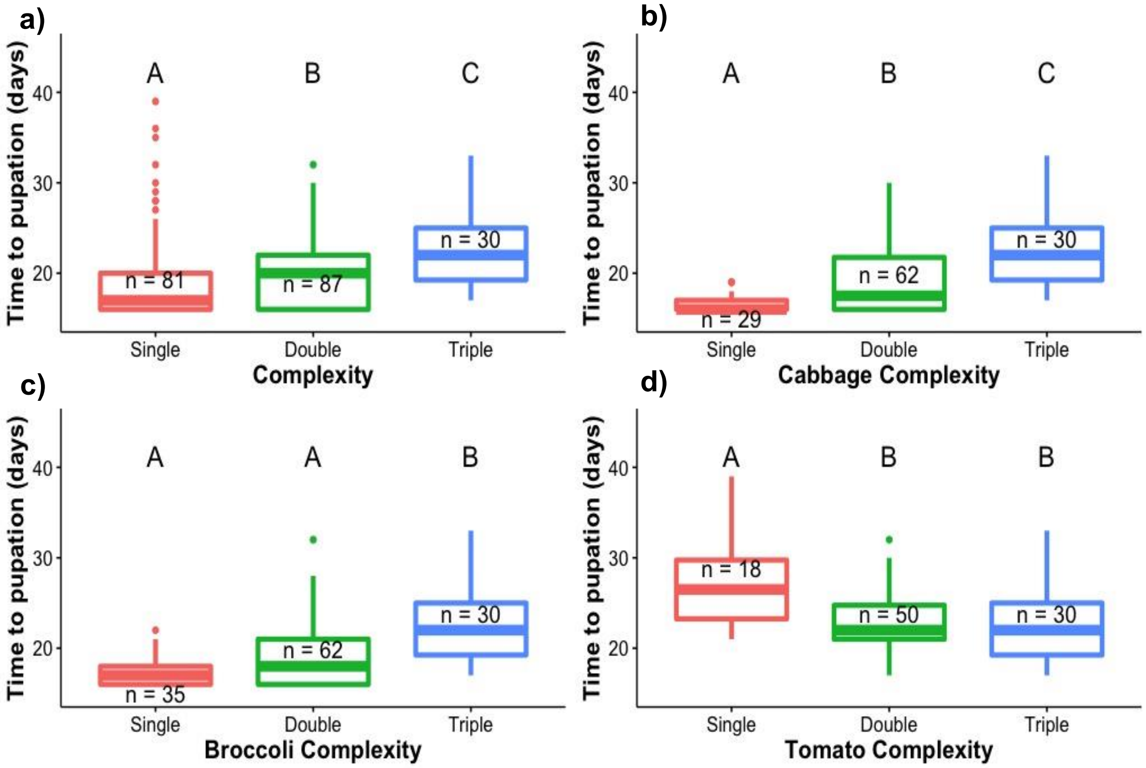

Figure 2-6 **The time to pupation of *T. ni* larvae fed on an increasing complexity of one, two or three host plant species of cabbage (C), broccoli (B), and tomato (T). a) Larvae fed single (C, B & T), double (CB, CT & BT) or triple (CBT) plant host combinations. b) Larvae were fed diets including cabbage with single (C), double (CB & CT) or triple (CBT) plant combinations. c) Larvae were fed diets including broccoli with single (B), double (CB & BT) or triple (CBT) combinations. d) Larvae were fed diets including tomato with single (T), double (CT & BT) or triple (CBT) combinations. Letters signify differences at P < 0.05 (Dunn's Kruskal-Wallis test). Error bars are the 95% confidence interval.**

Pupal mass. Pupal weights were significantly affected by plant diet; tomato-feeding larvae produced larger pupae than cabbage feeders (~15% larger), but larvae that fed on broccoli had similar pupal weights to both (Figure 2.7: *plant*: $F_{6,\,187} = 5.7$, $p < 0.0001$). Insects fed on a combination of cabbage and broccoli had lighter pupae than either plant fed on in combination with tomato. Diet did not affect each sex differently; however, males were 11% larger than females (*sex*: $F_{1,\,187} = 51.56$, $p < 0.0001$; sex *by* plant: $F_{6,\,187} = 1.51$, $p = 0.176$). Overall diet complexity did not affect pupal weight (*diet complexity*: $F_{2,\,187} = 1.34$; $p = 0.265$).

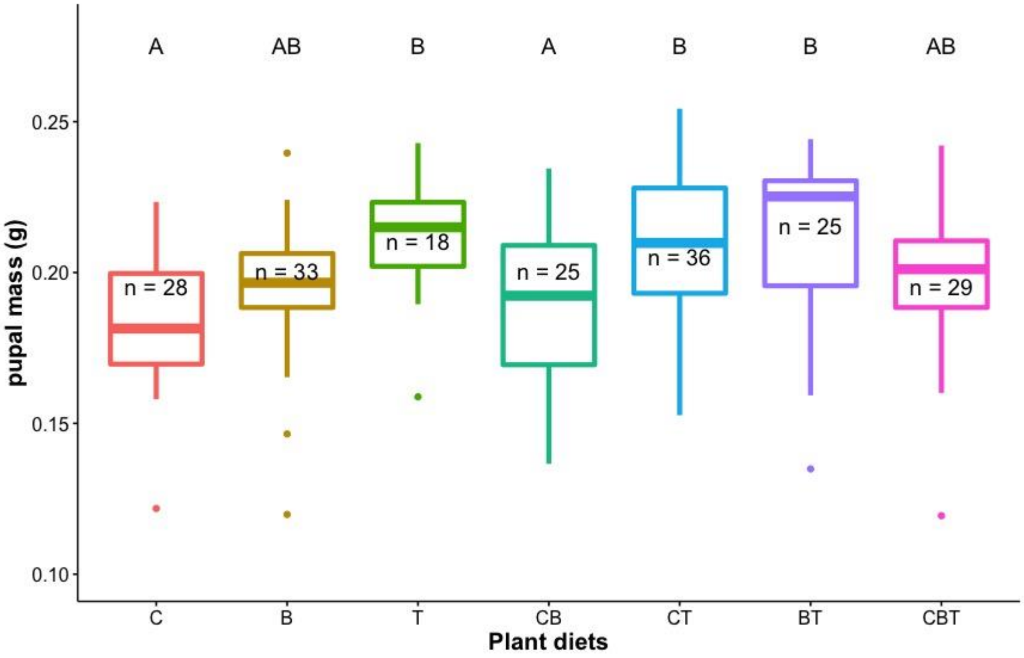

Figure 2-7 **Pupal mass of *T. ni* larvae fed combinations of cabbage (C), broccoli (B), and/or tomato (T). Letters signify differences at P < 0.05 (Tukey's HSD). Error bars are the 95% confidence interval.**

Adult Emergence. Adult emergence was not affected by plant diet (*plant*: X^2_6 = 10.15, *p* = 0.119). However, larvae fed on single cabbage and broccoli had 19% and 21% higher level of emergence of live adults than tomato fed insects. Increasing diet complexity had no effect on adult emergence (*diet complexity*: X^2_2 = 0.343, *p* = 0.842).

2.3.2. Viral Bioassay

Mortality due to virus. Host plant diet prior to virus challenge altered the virus mortality of the larvae (Figure 2.8: *plant diets:* X^2_6 = 18.61, *p* = 0.0048). Mortality of larvae which had fed on the broccoli and tomato single diets were around twice and three times, respectively, lower than that of larvae that had fed on cabbage. However, mortality of larvae that fed on the mixed diets did not differ. Mortality increased with virus dose as expected, and this was not affected by diet (*virus dose*: X^2_1 = 20.76, *p* = <0.0001; *virus dose by plant diet*: X^2_6 = 11.918, *p* = 0.064). Average larval weight was initially included as a factor but did not influence virus mortality (*average larval weight*: X^2_1 = 0.003, *p* = 0.95). Increasing diet complexity had no effect on virus-induced mortality (diet *complexity*: X^2_2 = 0.17, *p* = 0.977; *virus dose*: X^2_1 = 20.25, *p* = 0.017; *virus dose by diet complexity*: X^2_2 = 0.30, *p* = 0.96).

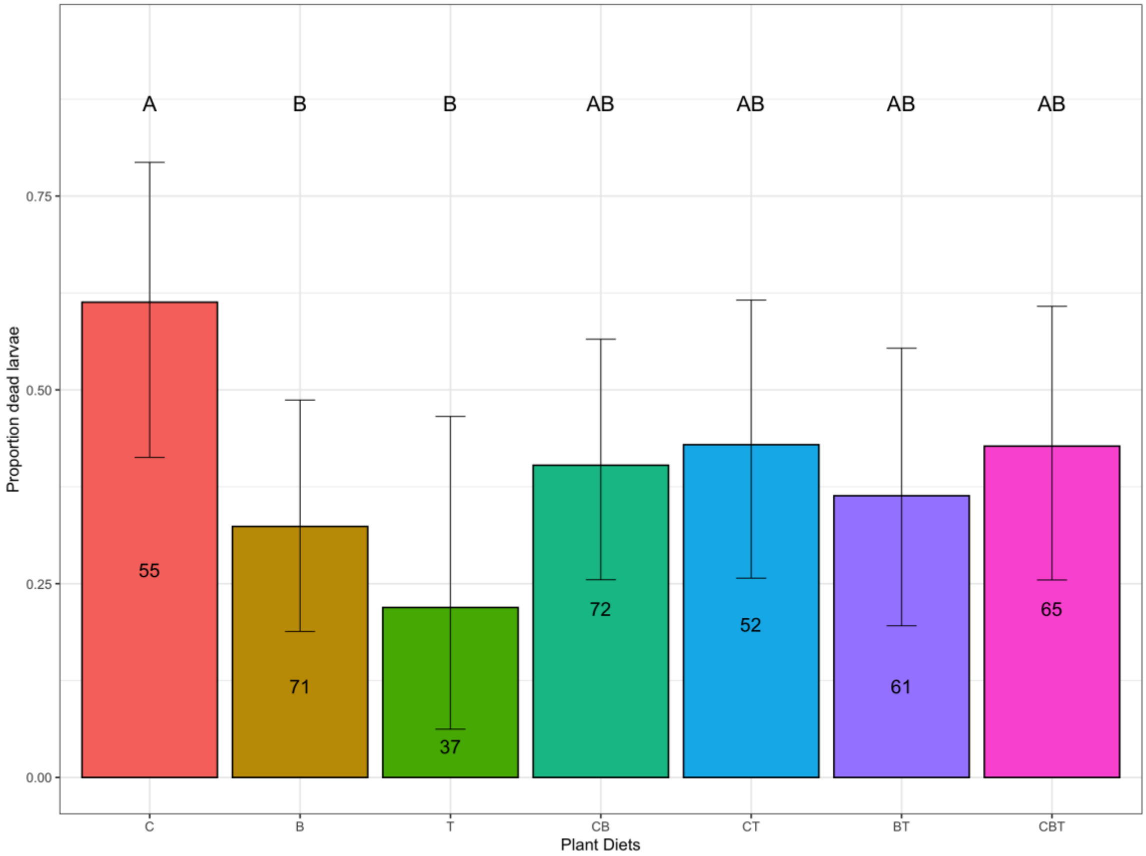

Figure 2-8 Proportion of *T. ni* larvae which died of TnSNPV infection after feeding on combinations of cabbage (C), tomato (T) and/or broccoli (B) plant diets. Viral dose was removed from figure (as there was no significance) to make differences between diets clearer. N are adjusted total values. Letters signify differences at P < 0.05 (R package "emmeans"). Error bars are binomial 95% confidence intervals.

Speed of kill. The diet that the larvae fed on before virus challenge had no effect on time to death (*plant diet*: $F_{6,\ 693}$ = 1.08, p = 0.37), nor did virus dose (*virus dose*: $F_{1,\ 693}$ = 1.08, p = 0.30; *virus dose by plant diet*: $F_{6,\ 693}$ = 2.00, p = 0.068).

2.4. Discussion

We set out to test whether plant species and increasing diet complexity affected *T. ni* growth and development and whether this impacted their resistance to NPV infection. We expected that a diet of increasing chemical complexity would negatively impact *T. ni* life history traits, and these negative effects would translate into a cost to the insect's response to disease in the form of higher mortality and faster death due to virus.

Our results show that individual plant species and diet complexity do alter specific insect life history parameters, and subsequently their resistance to viral infection. However, differences in viral resistance was only seen between single plant species, with a lower mortality in tomato and broccoli fed larvae compared to cabbage. We found that feeding on tomato negatively impacted larval weight and development time, but that did not translate into increased viral susceptibility. Larvae reared on tomato alone experienced high levels of mortality, where around 20% survived to the 4th instar. This may have led to selection playing a role, where larger larvae were the ones surviving, resulting in increased viral resistance. However, when average laval weight in the analysis was included, it did not affect resistance. Increasing complexity did not always correlate with a negative effect on growth and development traits. This did not translate to lower viral resistance as all the mixed plant diets resulted in a level of susceptibility that was intermediate and not different from the single plant diets.

2.4.1. The effect of plant identity and increasing diet complexity on insect fitness-related traits

Larvae fed tomato were smaller and had longer development times as compared to cabbage- and broccoli-fed larvae. Similarly, larvae that were fed on mixed diets which included tomato had lower larval weights and longer development times. However, the opposite effect was seen in terms of pupal mass, where tomato feeders were heavier, for all diets (both single and mixed) that included tomato. This is partly a result of development time, where time to pupation was longer on tomato containing diets, than on those which included cabbage and broccoli. This suggests that insects need to reach a minimum weight before they can pupate, although the higher weight of tomato feeders implies that there was some type of over compensation, or that tomato selected for larger larvae. Moreover, larvae eating the three-diet combination were trending towards being intermediate in larval weight, pupal mass and growth rate between single-diet and two-diet feeding larvae. Tomato is clearly worse for some traits, therefore when mixing with other diets, the measures such as larval weight and development time often improve. Our data suggests that mixed diets including tomato cause larvae to compensate on other host plants and avoid eating tomato. It isn’t clear if the larvae feeding on tomato were consuming an equal amount of tomato relative to other host plants as we did not calculate how much was eaten. We did not measure plant chemical profiles, but assume that the cost of consuming broccoli and tomato, which have relatively more defensive compounds than cabbage, can account for the differences in *T. ni* larval weight, pupal mass and development times we observed between them. We speculate that the cost of dealing with specific defensive plant compounds may come at the expense of *T. ni* growth and fitness traits. Although cabbage and broccoli are both *Brassicas*, there are differences between them, with broccoli containing approximately double the glucosinolate concentration (Fenwick et al. 1983; Possenti et al. 2016), and a higher total phenolic concentration as compared to cabbage (Podsędek 2007; Cartea et al. 2011). Broccoli also has a higher concentration of certain glucosinolates than cabbage, such as glucoraphanin and glucobrassicin (Rochfort & Jones 2011; Brown et al. 2015; Palani et al. 2016). Isothiocyanates formed under glucosinolate hydrolysis are detoxified by *T. ni* larvae via a glutathione-S-transferase (GST) enzyme (Wadleigh & Yu 1988; Winde & Wittstock 2011; Jeschke et al. 2017), which has been known to have metabolic costs in generalist lepidopteran species (Jeschke et al. 2016a; 2016b). Thus, the costs of detoxifying specific or higher concentrations of chemicals in broccoli could have accounted for the differences in larval weight as compared to cabbage. However, the chemical difference between crucifers and tomato is much larger, which could account for the smaller larval weight of the tomato-fed larvae. Although the specific costs of detoxifying tomato's defensive chemicals on larval growth have not been measured to my knowledge, many of these chemicals (mainly proteinase inhibitors) are known to degrade essential amino acids in the gut of *T. ni*, which impacts nutrient uptake and survival (Gonzales-Vigil et al. 2011). Previous work shows that *T. ni* detoxifies the glycoalkaloids contained in tomato, among other plants, through the production of enzymes and ABC transporters in fat bodies (Yang et al. 2007; Herde & Howe 2014; Adamski et al. 2016), so the cost of producing these may also impede growth and development. Furthermore, tomato is known to impact *T. ni* more than other host plants through direct negative effects on development and survival (Meneses-Arias et al. 2000; Shikano et al. 2010). The glycoalkaloids, phenolics and proteinase inhibitors in tomato negatively affect growth, development and survival in other lepidopterans (Elliger et al. 1981; Isman & Duffey 1982a; 1982b; Duffey & Stout 1996; Felton 2005; Janmaat & Myers 2005). Similarly, physical structures such as the trichomes on tomatro leaves can impact herbivorous insects as well. Trichomes contain chemical compounds that impede insect feeding (Kennedy 2003). Therefore, the costs of detoxifying these compounds, as well as the directly toxic effects, are likely to have had a greater negative impact on the growth of tomato feeding larvae, as compared to the *Brassica*s. Differences between larval weight may also be attributed to differences in the nutritional components of the plants; however, less is known about the nutritional composition of the plant species that we tested, as most studies focus on the quality of the fruit/floret for human consumption (Kurilich & Juvik 1999; Guil-Guerrero & Rebolloso-Fuentes 2009; Pinela et al. 2012; Liu et al. 2018). According to literature, tomato has slightly lower estimated levels of protein and carbohydrates than the *Brassicas* (Pinela et al. 2012; Bhandari & Kwak 2015). Comparing between cruciferous cultivars, broccoli has slightly higher proportion of protein and carbohydrates than cabbage (USDA 2019a; 2019b). Although broccoli seems to be better nutritionally (higher protein: carbohydrates ratio), our results do not always reflect this, as the larval weight is at an intermediate between cabbage and tomato. This indicates that perhaps there is a trade-off between nutritional compounds and defensive chemistry, since broccoli has the highest nutritional value compared to cabbage and tomato but is intermediate in terms of defensive chemistry.

In our experiment, increasing the complexity of diet affected some, but not all, life history parameters measured. Larval weight and development times were negatively impacted, whereas emergence and pupal mass were not. Focusing on tomato, the weight of larvae fed only tomato did not differ from that of larvae which ate mixed diets with tomato as a component. Furthermore, there could be a trade-off, where the gains from a more suitable diet such as cabbage or broccoli (compared to tomato) is negated by increased diet complexity no matter how many more suitable diets are added. Increasingly more complex diets may add more unique chemical compositions for the insect to deal with, or they dilute beneficial nutrients, which could be hampering larval growth compared to just one-plant or two-plant diets (Ojala et al. 2005). Multiple diets can also dilute a particularly toxic plant, such as tomato in our case. Research on diet mixing versus single foods indicates that it generally improves a variety of fitness traits, although much of this research has focussed on grasshoppers (Acrididae) and a limited number of lepidopteran species (Bernays & Minkenberg 1997; Miura & Ohsaki 2004; Mody et al. 2007; Karban et al. 2010). However, in many other polyphagous herbivores in the Lepidoptera, Hemiptera, and Diptera, mixtures of food plants rarely improved performance (Bernays & Minkenberg 1997; Hägele & Rowell-Rahier 1999). Several studies debate the benefits of diet mixing. For example, Stoyenoff et al. (1994b) who studied *L. dispar larvae* which are longer-lived and switch host plants more often than *T. ni* (Lance & Barbosa 1982; Liebhold et al. 1986), suggest that early feeding (first two weeks) on red oak (*Quercus rubra*) resulted in better weight, relative growth rate and larval development, regardless of the diet larvae were fed afterwards. In contrast, *L. dispar* larvae feeding for the first two weeks on bigtooth aspen (*Populus grandidentata*) had decreased growth and development, regardless of what other plant was fed afterwards. Stoyenoff et al. (1994b) suggested that the performance of *L. dispar* larvae was affected by the type of host plant and the order in which they are fed in. Similarly, Li & Liu (2015) showed that *T. ni* larvae that fed on cotton (*Gossypium hirsutum*), which has high levels of toxic terpenoids (Stipanovic et al. 1988; Liu et al. 1999; Bezemer et al. 2004), after they fed on cabbage, had longer development times and lower survival than those fed continually on cabbage. This suggests that it may be the order in which diets are fed that most strongly affect life history parameters (Barbosa et al. 1986). Further experiments would need to be carried out using our system to see if the order in which the plants were fed affect *T. ni* growth parameters. Evidence points to the fact that, in many species of Lepidoptera, individuals develop a preference for whichever plant they fed on first, resulting in poor performance if fed non-preferred plants (Jermy 1987; Soler et al. 2012). In the case of *T. ni,* larvae, they are not particularly mobile, and individuals seem to strongly prefer their rearing plants over other acceptable hosts (Lee 1990; Shikano et al. 2010). This could mean that the order in which we fed our plants could be the reason why we see no effect of mixed diets on growth rate, pupal mass etc. However, if mixed-plant diets are better than single-plant diets only when mixed-plant diets contain complementary food nutrients or have lower toxin content (Hägele & Rowell-Rahier 1999), then the plant combinations we tested in our experiments did not seem to have any of these benefits.

2.4.2. The effect of plant identity and increasing diet complexity on disease resistance

We then examined whether being fed different plant species prior to virus challenge affected insect viral resistance and whether increasing diet complexity decreased host resistance. In terms of plant species, the expectation from the first half of the study was that since cabbage fed larvae were heavier than those fed on tomato and broccoli only, they should be more resistant to viral infection as weight often influences susceptibility (Pourmiza 2000; Spoleder et al. 2007). We therefore included the average larval weight into the analysis of the assay to account for this, but it did not influence the results. Our hypothesis that diet complexity would negatively impact mortality due to virus and speed of kill was not supported, as increasing diet complexity did not alter disease susceptibility. There is some suggestion that mixing diets can offset some of the costs of plant secondary metabolites on insect growth (Waldbauer & Friedman 1991; Bernays et al. 1994; Hägele & Rowell-Rahier 1999; Unsicker et al. 2008; Karban et al. 2010) and thus their immune function; however, encountering increased diversity of host plant chemistry did not benefit *T. ni* larvae over single diets in terms of growth and development. The variable effects on growth and fitness traits, as well as the inconclusive effects of mixed diets on disease resistance, point to the fact that mixed diets may not have been detrimental to the immune function of *T. ni* larvae. A study by Ojala et al. (2005) also found that mixed diets fed to *Parasemia plantaginis* (Lepidoptera: Arctiidae) did not improve larval immune response, however in their case mixing reduced it compared to some single diets. They proposed that it is not necessarily the plant chemistry that is important, but the fact that one species (lettuce, *Lactua sativa*) in the mixed diet interferred with the encapsulation immune response by the larvae. They further suggest that since lettuce has lower levels of secondary metabolites it implies that something else is responsible for this poor encapsulation, such as their relatively higher levels of nitrogen. It is noteworthy, however, that the *P. plantaginis* study differs from ours in that encapsulation was studied as an immune response parameter, and that *P. plantaginis* is slightly longer lived than the *T. ni* as well as feeds on both herbaceous and arborescent plants. The *P. plantaginis* study suggests that mixed diets may affect insect immunity to a lesser degree than generally thought, as one diet component may dilute the compounds that otherwise would have promoted growth and immunity. In our case, we know that host plant identity, probably through its chemistry, does play a role as susceptibility to virus was reduced in larvae fed single tomato, but this dilution effect may still be taking place in the mixed diet treatments with tomato. The effect of the more resistant tomato-fed larvae could have been diluted by the other two "better" diets in the two or three mixed plant combinations.

Between single diets, tomato-fed larvae had the lowest mortality due to virus, whereas cabbage caused the highest mortality. However, since survival up to the 4th instar was poor in tomato, it could simply be a matter of selecting the fitter insects. However, tomato fed larvae were the smallest compared to the *Brassica*s, indicating that there must be another factor affecting disease resistance with tomato, particularly since average larval weight had no effect on disease in our analysis. However, it is not clear whether diet indirectly affected resistance through changes in insect physical defense mechanisms or its immune response. Although we did not measure these mechanisms, when looking at the effects of plant defensive compounds on disease resistance prior to infection there are two possible avenues in which the virus can be impacted; either through the impacts of chemistry on insect morphology, or by indirectly impacting traits in the immune response in insects which break down or excrete the chemicals. In terms of morphological features, research shows that plant type can affect the thickness of the peritrophic membrane (PM) in the gut at the expense of insect growth (Pechan et al. 2002), which can stop microbes from entering the midgut (Lehane 1997), protecting against virus infection (Cory & Hoover 2006; Plymale et al. 2008; Chen et al. 2018). A study on *T. ni* found that consuming cabbage versus potato, resulted in higher levels of chitinase in their midgut, thinning the PM (Chen et al. 2018). This was attributed to potato creating a more alkaline gut environment as compared to cabbage. Because tomato and potato are in the same genus (*Solanum*) and share glycoalkaloids (Schwarz et al. 1995), a similar situation may have occurred in our experiment, where tomato-unlike cabbage-derived diet constituents may have caused PM thickening in experimental larvae, thus protecting them from viral infection. Moreover, the glycoalkaloid tomatine is basic (Duffey & Stout 1996), which may have created a more alkaline gut and thickened the PM. However, studies on pH changes in the gut of *T.ni* larvae feeding on tomato are lacking. Ingestion of tomatine has also been known to cause oxidative stress to the insect midgut (Duffey & Stout 1996), which can result in gut cells sloughing off before the virus is able to penetrate and replicate (Hoover et al. 2000).

Although we did not measure the effect of specific chemicals on immune parameters, many studies show that defensive compounds in plants have very variable effects on the immune response of different lepidopteran species. Detoxifying plant defensive chemicals is costly and there can be a trade-off with immune defense, since both share the same resources (Adamo 2017). Defensive compounds such as iridoid glycosides and hydrolyzable tannins reduce encapsulation as well as melanisation in species such as the autumnal moth (*Epirrita autumnata*) and the common buckeye (*Junonia coenia*) (Haviola et al. 2007; Smilanich et al. 2009). However, other chemicals such as carotenoids enhance melanisation in the monarch butterfly, *Danaus plexippus* (de Roode et al. 2008), and some (iridoid glycosides and pyrrolizidine alklaloids) have no effect on melanisation in the arctiine moth *Grammia incorreupta* (Smilanich et al. 2011a; 2011b). Therefore, it is not clear whether the effects in these studies are unique to their respective systems, and more work would need to be done to establish if the specific defensive chemicals in our host plants affect traits involved in immune responses of *T. ni*. Furthermore, it would need to be established whether these parameters even correlate with susceptibility to disease in our system.

Our results showed that viral infection was not changed by mixed diets because larval mortality was similar to tomato-fed larvae (lower mortality) and in cabbage-fed larvae (higher mortality). However, the increase in diet complexity in the two or three diet combinations could have diluted the effect on viral infection we would have seen if larvae had just been feeding on tomato alone. To address these issues, it would be beneficial to repeat this experiment with other combinations of plants to separate the effects of diversity with those of tomato (individual plant identity).

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Chapter 3. The transgenerational effects of host plant and plant induction on *Trichoplusia ni* fitness and virus resistance

Abstract

Defensive secondary plant metabolites significantly impact susceptibility to baculovirus infection in Lepidoptera both directly and indirectly. However, it is not clear whether induced defensive chemistry has longer term transgenerational impacts on disease resistance. I examined if plant chemical induction, as a result of insect feeding, has transgenerational effects on the disease resistance of the cabbage looper (*Trichoplusia ni*), and if these effects were altered by host plant species. I determined that plant induction did not affect transgenerational virus resistance or egg size, although it did have negative sublethal effects on the pupal mass and fecundity of parents fed broccoli relative to cabbage. However, offspring mortality due to virus was lower for insects whose parents fed on broccoli compared to cabbage. This suggests that plant identity is important and has the potential to have longer term impacts on host-pathogen dynamics. But further work is needed to examine the mechanisms behind this.

3.1. Introduction

Inter- and intra-specific variation in host plant species can have significant impacts on insect growth, development and fitness (Hodge 1933; Seamans & McMillan 1935; Snyder 1954; Smith 1959; Awmack & Leather 2002; Shikano et al. 2010). Plants defend themselves from herbivore attack in numerous ways, ranging from trichomes to waxy leaf surfaces, but some of the most effective defenses are through the production of secondary chemicals. Constitutive chemicals will always be expressed and do not substantially increase in concentration under stress, whereas induced defenses increase by significant amounts within the host plant under insect or pathogen attack (Karban & Baldwin 1997; Bostock 2005; Hanley et al. 2007; Chen 2008; Howe & Jander 2008; Karban 2011; War et al. 2012). Plant chemicals induced as a result of herbivore feeding, either rapidly or over longer time periods (Rossiter et al. 1988; Giamoustaris et al. 1995; Borek et al. 1998; Widstrom et al. 1998) have repellent, anti-nutritive, anti-digestive and sometimes toxic qualities (Fraenkel 1959; Ehrlich & Raven 1964), affecting insect performance (Felton et al. 1992; Duffey & Stout 1996; Brian Traw & Dawson 2002).

In addition to their direct effect on insects, plant secondary chemicals can also impact natural enemies. Indirect effects on parasitoids have been well studied and demonstrate the negative effect of defensive compounds through reductions in insect host growth and survival (Bloem & Duffey 1990; Bourchier 1991; Gols et al. 2008). There can also be direct effects, including toxic effects of plant secondary metabolites that impede parasitoids' performance (Campbell & Duffey 1981; Singer & Stireman 2001; Gols & Harvey 2009). However, there are some cases of the parasitoid benefitting from the weakening of the host immune responses, resulting in higher parasitism success (Karimzadeh & Wright 2008; Kos et al. 2012). Host plant defensive chemicals also impact insect pathogens through various mechanisms: directly on plant surfaces or within an insect's gut (Felton & Duffey 1990; Forschler et al. 1992; Young et al. 1995; Hoover et al. 2000), and indirectly through changes in insect behavior and morphology as well as negative effects on insect immunity (reviewed in Cory & Hoover 2006). Furthermore, induced plant chemicals can sometimes protect insects from disease (Hoover et al. 1998a; Hoover et al. 1998b; Smilanich et al. 2018). Several studies have explored the different immune traits impacted by host plants and their chemicals (Ojala et al. 2005; Haviola et al. 2007; Smilanich et al. 2009; Shikano et al. 2018). However, there is not a consensus as to whether induced plant chemicals always negatively impact insect herbivores.

While we are gaining an understanding of within-generation impacts of plant defensive chemistry at multiple trophic levels, whether there are transgenerational consequences of exposure to them is less clear. Rossiter (1991; 1992) was one of the first to discuss maternal effects and argued that they influence population dynamics through time delayed effects, which can depend on the maternal nutritional environment. These effects can be adaptive as maternal dietary conditions are an indication of future environmental conditions and resource availability for their offspring (Rossiter 1996; Mousseau & Fox 1998). It is thus beneficial for females to produce offspring that are acclimatized to the mother's current condition (Fischer et al. 2003; Ladner & Altizer 2005; van Asch et al. 2010; Cahenzli et al. 2015). However, recently it has been recognized that both paternal and maternal nutrition can have transgenerational effects. Many early studies on the transgenerational effects of nutrition manipulated the content of artificial diets, often investigating the impact of specific components, such as proteins, carbohydrates or other macro- and micro-nutrients (Keena et al. 1995; Rotem et al. 2003; Littlefair & Knell 2016). These studies give us an indication that parental diet, without considering plant secondary metabolites, plays a role in offspring quality and condition. However, artificial diet is not realistic in terms of what insects eat in their natural environment. Both reduced diet quality and quantity in the parental generation have been shown to have positive, negative and neutral transgenerational impacts on offspring success and condition (Rossiter et al. 1993; Ots et al. 2005; Triggs & Knell 2012). Offspring traits affected by parental diet in insects include changes in egg size and composition (Bauerfeind & Fischer 2005; Bauerfeind et al. 2007; Karl et al. 2007), larval development (Greenblatt & Barbosa 1980; Rossiter 1991; Fox & Dingle 1994; Cahenzli & Erhardt 2012) and larval survival (Morris 1967; Carisey & Bauce 2002).

What is less clear are the effects parent dietary condition will have on offspring disease resistance. Broadly, transgenerational effects often take the form of enhanced immunity and increased resistance to disease, in offspring whose parents had undergone some sort of stress and/or immune challenge (Moret 2006; Roth et al. 2010). These effects are also known as “anticipatory parental effects” or “adaptive transgenerational plasticity” (Mousseau & Fox 1998; Marshall & Uller 2007; Uller et al. 2013; Kangassalo et al. 2020). Focusing on the impact of parental diet, there is no clear pattern as to whether poor parental diet has positive or negative effects on offspring disease susceptibility or immune parameters. Research on the effects of diet and host plant on transgenerational disease resistance do so within the framework of the effects of food stress, defined as starvation (withholding food) and food limitation (reduced quantity of food through dilution) or varying diet quality. Mitchell and Read (2005) studying the water flea *Daphnia magna* showed an increase in disease resistance when they were fed limited food quantities, whereas Triggs and Knell (2012) recorded a decreased immune response with the Indian meal moth *Plodia interpunctella* fed a diluted diet. Even though these studies are on two very different organisms, one a freshwater water flea (*D. magna*) and the other a common stored product pest (*P. interpunctella*), they do show that poor (or at least more dilute) parental diet does not necessarily result in offspring that have greater immunocompetance. Poor diet conditions, as can happen with higher insect densities, can potentially signal an increasing threat of infection, leading to the increased investment in disease resistance of future generations (Ben-Ami et al. 2010; Frost et al. 2010). Another study involving *Daphnia* showed that food-stressed parents produced offspring that were more resistant to the bacterium *Pasteruria ramosa* (Stjernman & Little 2011). Also, lepidopteran larvae that fed on a lower quality diet (diluted nutrients) produced offspring which were more resistant to bacteria (*Bacillus thuringiensis*), entomopathogenic fungus (*Beauvaria bassiana*), and baculovirus infection (Boots & Roberts 2012; Kangassalo et al. 2015; Shikano et al. 2015). However, poor parental diet quality does not always result in more resistant offspring. Food-stressed (intermittently starved) western tent caterpillar (*Malacosoma pluviale californicum*) parents, and cabbage looper (*Trichoplusia ni*) parents fed diets of various protein to carbohydrate ratios produced offspring whose resistance to a nucleopolyhedrovirus (NPV) was not altered (Myers et al. 2011; Shikano et al. 2016). Chronic malnutrition (using diluted artificial diet) of vinegar fly *Drosophila melanogaster* parents increased susceptibility to infection in offspring generations (Vijendravarma et al. 2015).

While the transgenerational effect of diet has received some attention in a limited number of systems, little is known about the impact of induced effects, as it is hard to separate these from baseline chemistry. Although induction can be triggered rapidly (within the same generation of the insect), delayed induced responses do occur, with some debate as to whether they can influence population dynamics in forest insects (Haukioja & Neuvonen 1987; Rossiter 1988; Karban & Myers 1989; Haukioja 1990). Studies which have found transgenerational effects of host plant chemistry looked at the effect on offspring life history traits and fitness (Gould 1988; Rossiter 1991), but did not test whether this translates to an impact on disease susceptibility. There is one study which suggests that plant induction results in increased disease resistance in the next generation of western tent caterpillars (Olson 2014). Offspring whose parents fed on induced red alder leaves were more resistant to a baculovirus (*Malacosoma californicum pluviale* NPV). This effect on parents was sublethal and thus did not appear to be the result of selection for larger and/or more resistant insects. Looking at the same system, Rothman (1997) found that high larval density as a proxy for plant induction resulted in no transgenerational effects. Thus, it is unclear how widespread this effect is, as *M. c pluviale* feed on longer lived tree species and plant induction can be quite marked (Myers 2000; Sarfraz et al. 2013). It would be interesting to establish if a similar transgenerational response occurs in lepidopterans from agricultural systems, such as *T. ni,* which feeds on a very different set of host plants.

My aim is to examine whether induced plant defensive chemicals alter transgenerational disease resistance in cabbage looper *T. ni*. Larvae of *T. ni* are voracious pests on a wide range of plants grown in greenhouses and agricultural fields, such as crucifers, tomatos, and peppers (Hoo et al. 1984; Wittstock et al. 2003; Li et al. 2006; Ahuja et al. 2011). In the field, *T. ni* larvae are commonly infected by naturally occurring baculoviruses (Jaques 1962; Elmore & Howland 1964; Jaques 1970; Capinera 2008). Baculoviruses are a family of insect-specific DNA viruses, containing two main groups, the nucleopolyhedroviruses (NPV) and the granuloviruses (GV). NPVs need to be ingested by the larvae, usually along with foliage, to begin infection (Cory & Myers 2003; Grzywacz 2017). To examine whether host plants affect transgenerational disease resistance, I used *T. ni*, a nucleopolyhedrovirus specific to *T. ni (Trichoplusia ni* single NPV) and three common *T. ni* host plants (cabbage, broccoli and tomato) as a study system. Larvae were fed induced or non-induced foliage from tomato, broccoli and cabbage, and their offspring were exposed to the NPV, measuring differences in infection. I ask two questions: (1) Do host plant species have transgenerational effects on disease resistance? (2) Are transgenerational effects altered by chemical induction?

3.2. Materials and Methods

3.2.1. Insects

Larvae of *T. ni* were obtained from a colony that was initiated by mixing a long-term colony (originally collected from a commercial tomato greenhouse in British Columbia, BC (Janmaat & Myers 2003)), with *T. ni* progeny recently collected from a field in Delta, BC, and then reared for several generations. Insects were kept at 25°C under a photoperiod of 16L:8D on a semi-artificial, wheat germ-based diet of approximately 1:1.1 protein to carbohydrate ratio (Bio-Serv, Frenchtown, NJ, USA; Shikano & Cory 2016). Pupae were surface-sterilized with a 0.5% bleach solution to reduce pathogen contamination, before being placed in groups of 30-40 in cages (~15 cm radius x 30 cm height). Eggs were surface-sterilized with a 0.5% bleach solution and rinsed with distilled water. First instar larvae were moved to individual 29-ml containers of diet, and 2nd instar larvae were used in experiments.

3.2.2. Plants

We chose three local host plant species of *T. ni*: cabbage (*Brassica oleracea* var. *capitata* cv. Danish Ballhead), broccoli (*Brassica oleracea* var. *italica* cv. Centennial) (both Cruciferae)) and tomato (*Lycopersicum esculentum* cv. Amish Paste) (Solanaceae). Broccoli has a higher total concentration of glucosinolate and phenolics than cabbage (Cartea et al. 2011; Fenwick et al. 1983; Podsędek 2007; Possenti et al. 2016), and tomato has a distinct chemical profile which substantially differs from both cruciferous plants (Kennedy 2003). Plants were seeded in mid-May 2018 and grown in pots in a greenhouse on the Burnaby campus of Simon Fraser University. Mushroom manure and peat moss were mixed to form potting soil (GardenWorks, Burnaby, BC). Single cabbage and broccoli plants were grown per pot (~20 cm width x 30 cm height), whereas tomatoes were grown in sets of three in larger containers (~40 cm width x 45 cm height) due to logistical constraints. Plants were watered every one or two days depending on temperature. Plants were transferred to larger pots in early June and foliage used for the experiment was taken in late July.

Eighty plants each of cabbage, broccoli and tomato were assigned to the induced plant group and the non-induced plant group. In order to produce induced leaves, five 3rd or 4th instar *T. ni* larvae were placed on each plant; one larva was removed when defoliation became more marked to ensure enough leaf material remained to feed to the experimental larvae. The first harvest of foliage for the experiment began 48 hrs after inducing larvae were placed on plants, and these larvae were left on the plants for the remainder of the experiment (July 25th to August 27th). Plants were wrapped with fine netting to prevent insects from escaping. All leaves from the plant, with or without feeding damage, were used in the experiment. Leaves from multiple plants were used throughout the experiment in a random fashion, with only one leaf being taken from a plant at a time.

3.2.3. Experimental Design

Parental Generation

Eighty 2nd instar larvae per plant per induction treatment (3 plant species x 2 induction treatments) (480 in total) were transferred to 96 ml cups containing their respective foliage treatment. The larvae were given fresh foliage every other day until they reached 3rd instar, and were then fed fresh leaves every day until they pupated (~10 -14 days). Leaves of cabbage and broccoli typically measured ~10-15 cm^2, whereas a whole leaflet of tomato was offered per day. Each larva was weighed at the start of the 4th and 5th instars and surviving insects were weighed 3 days after pupation.

Mating and egg collection

Each pupa was sexed and single male/female pairs were placed in 591 ml paper cups with a 10% sugar solution to feed the adults once they emerged. A piece of black construction paper matching the circumference of the cup was placed in each container for the insects to lay eggs on. Egg sheets from each pair were collected 3 days after egg laying began (this usually coincides with peak egg production). To count the number of eggs and to estimate egg size, egg sheets were scanned (CanoScan LiDE210 Color Image Scanner; Canon, Tokyo, Japan), and scans were edited in Photoshop (Adobe, 345 Park Av, San Jose, California) to remove blemishes before exporting scans into ImageJ (Rasband 1997- 2018). Eggs were then left to hatch on diet. Insufficient numbers of larvae survived to pupation and adults in the tomato treatments to produce eggs.

Virus bioassay

When offspring larvae reached the 3rd instar, they were placed individually in 48-well plates with diet plugs treated with virus or distilled water. The larvae were challenged with two doses of *Trichoplusia ni* single nucleopolyhedrovirus (TnSNPV FV#3433), which is specific for *T. ni.* (Shikano & Cory 2016): a high dose (350 OBs per larva; 175 OBs/µl) and a low dose (100 OBs per larva; 50 OBs/µl). Controls were given 2 µl of distilled water. Twenty larvae were challenged with each dose (20 larvae x 3 doses x 2 host plants x 2 induction treatments). Larvae were left 24 h at 25°C to consume the dose and were then transferred to individual 29.5 ml cups containing artificial diet. Larvae were monitored daily for death or pupation. Baculovirus-induced death usually results in very obvious symptoms; the larvae become pale and flaccid and rupture releasing millions of OBs. Larvae with ambiguous death symptoms were smeared on a slide with quick stain Giemsa and inspected under a microscope (magnification 1000x with oil immersion) to investigate the cause of death.

Statistical analysis

All data were analyzed using R (Version 1.1.463). Data were inspected for non-normality and transformed as necessary. The maximal model was first fitted and then simplified by removing non-significant interaction terms. Differences between treatments for ANOVA analyses were examined by Tukey's HSD at $P < 0.05$. Differences between treatments for linear models were tested using the "lsmeans" function in R (package "emmeans") (Lenth; Searle et al. 1980).

Larval weights and pupal mass. Fourth instar larval weights (mg) were logged (base 10) due to non-normality, whereas fifth instar weights were not, and both were analyzed separately with a two-way ANOVA with plant type (Cabbage/Broccoli/Tomato) and foliage induction (Normal/Induced) as interacting factors.

Fecundity and egg size. Egg count was analyzed using a generalized linear model with a quasipoisson distribution due to overdispersion of data. The effect of plant type, induction and their interaction on logged (base 10) egg area (cm^2) was analyzed using a linear mixed model with insect family as a random effect.

Virus bioassay. Data were censored at 15 days post infection, as larvae would have died of virus prior to this time if they had been successfully infected. All larvae that died 1 or 2 days post-infection were considered to be 'handling deaths' and excluded from all analyses. Virus mortality was analyzed using a Generalized Linear Model with a quasibinomial distribution and a logit link function as the data were overdispersed. Successes (number of adjusted virus-killed larvae) and failures (surviving larvae) were input as responses. Plant type, induction and viral dose (ordinal) were included as interacting factors. A similar analysis was done with insect family as a factor, to determine differences in mortality due to virus, between them. A quasibinomial distribution with logit link function was also used.

Any background mortality was adjusted for using the following formulae. In untreated controls, virus death ranged from 5-26%, with most treatments having no contamination. Virus mortality was adjusted for virus death in the untreated control insects, using the formula $Va = Vd - Td * (Vc / Tc)$, where Va is the adjusted virus mortality, Vd is the dose/treatment-specific number killed by virus, Td is the dose/treatment-specific total assayed, Vc is the number killed by virus in the untreated controls, and Tc is the total number assayed in the controls. The total number assayed was adjusted using Tadj = Td * (1 – ((Vc + Uc) / Tc)), where Tadj is the adjusted dose/treatment-specific total number assayed, and Uc is the number dead by unknown causes/ background mortality for the untreated control dose. Unknown and background mortality ranged from 5-56%, with most families having less than 12%.

Time to death was analyzed using a linear mixed model (package "lme4") with plant identity, induction and viral dose as the explanatory variables. Insect family was input as a random effect.

3.3. Results

3.3.1. Performance of the parent generation

Larval weight. Fourth instar larval weight was significantly affected by host plant species, but not by induction (*plant:* $F_{1,443} = 33.45$, $p < 0.0001$; *induction by plant:* $F_{3,441} = 0.29$, $p = 0.748$), with insects fed on tomato being smaller than those fed broccoli or cabbage (Figure 3.1a). Larvae reared on induced foliage were marginally smaller than those reared on uninduced plants (*induction:* $F_{1,443} = 3.50$, $p = 0.062$). When the larvae were measured again at the 5th instar, the differences among the treatments were more marked (*plant:* $F_{1,413} = 47.45$, $p < 0.0001$; *induction:* $F_{1,413} = 47.86$, $p < 0.0001$; *induction by plant:* $F_{3,411} = 32.93$, $p < 0.001$). Larvae that were fed on induced tomato, were ~94% smaller than any of the other larvae (Figure 3.1b). In addition, larvae that were fed normal cabbage were also significantly larger than those fed on induced broccoli and normal tomato (Figure 3.1b).

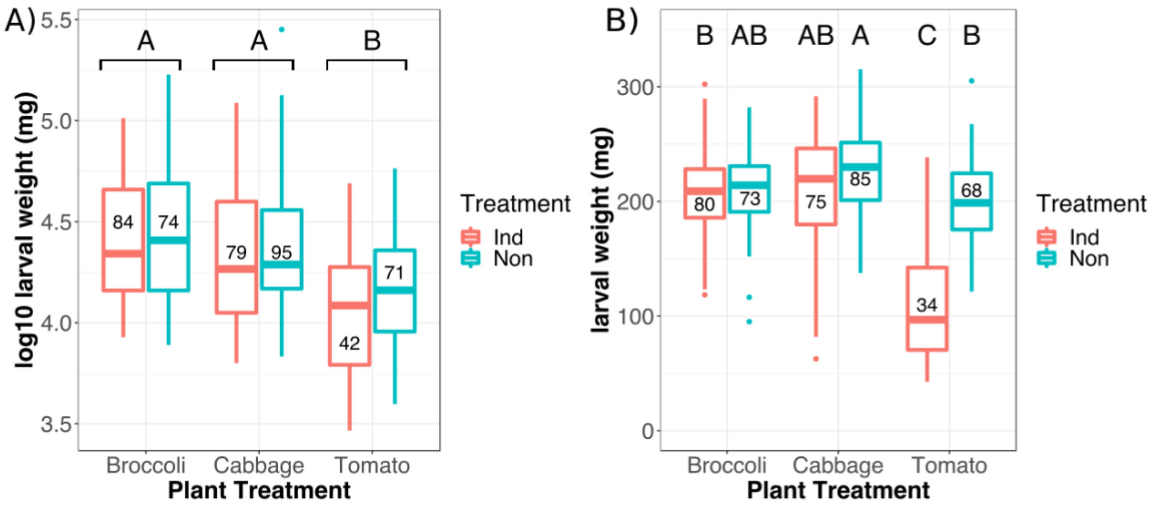

Figure 3-1 **A) Weight of 4th instar *T. ni* larvae after feeding on induced or non-induced tomato, broccoli and cabbage leaves. B) Weight of 5th instar *T. ni* larvae after feeding on induced or non-induced tomato, broccoli and cabbage leaves. The letters indicate significant differences at P < 0.05 (Tukey HSD test). Error bars are the 95% confidence interval.**

Pupal mass. Pupae which resulted from larvae that fed on induced broccoli were ~14% smaller than the others (Figure 3.2; *induction by plant:* $F_{3,\,185} = 5.21$, $p = 0.023$ *plant:* $F_{1,\,187} = 7.43$, $p = 0.007$; *induction:* $F_{1,\,187} = 4.88$, $p = 0.028$). Insufficient numbers of insects survived to the pupal stage in the tomato treatments to measure pupal weights. Survival to pupation on tomato was lower than the other treatments, with 13% survival on normal tomato and none surviving in induced feeding insects.

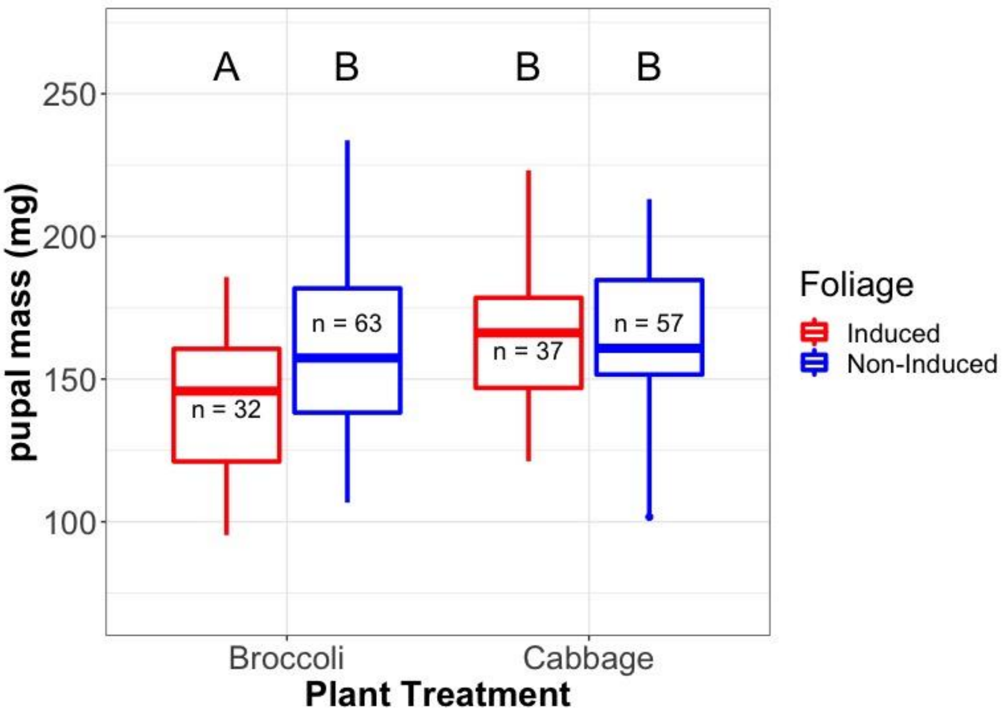

Figure 3-2 **Pupal mass of *T.ni* larvae reared on induced and non-induced cabbage and broccoli. Letters signify differences at P < 0.05. Error bars are the 95% confidence interval.**

Fecundity & egg size. Fewer eggs were laid by parents that fed on induced broccoli than by parents that fed on induced cabbage. Parents that fed on induced broccoli produced marginally fewer eggs than non-induced broccoli feeders (Figure 3.3) (lsmeans contrast: p = 0.071, df = 3; *induction by plant*: $F_{3, 66}$ = 4.79, p = 0.032; *plant:* $F_{1, 68}$ = 0.47, p = 0.49; *induction*: $F_{1, 68}$ = 0.22, p = 0.64). Egg size did not differ among treatments (*induction by plant:* $F_{3, 66}$ = 1.21, p = 0.27; *induction:* $F_{1, 68}$ = 0.03, p = 0.86; *plant:* $F_{1, 68}$ = 0.27, p = 0.61).

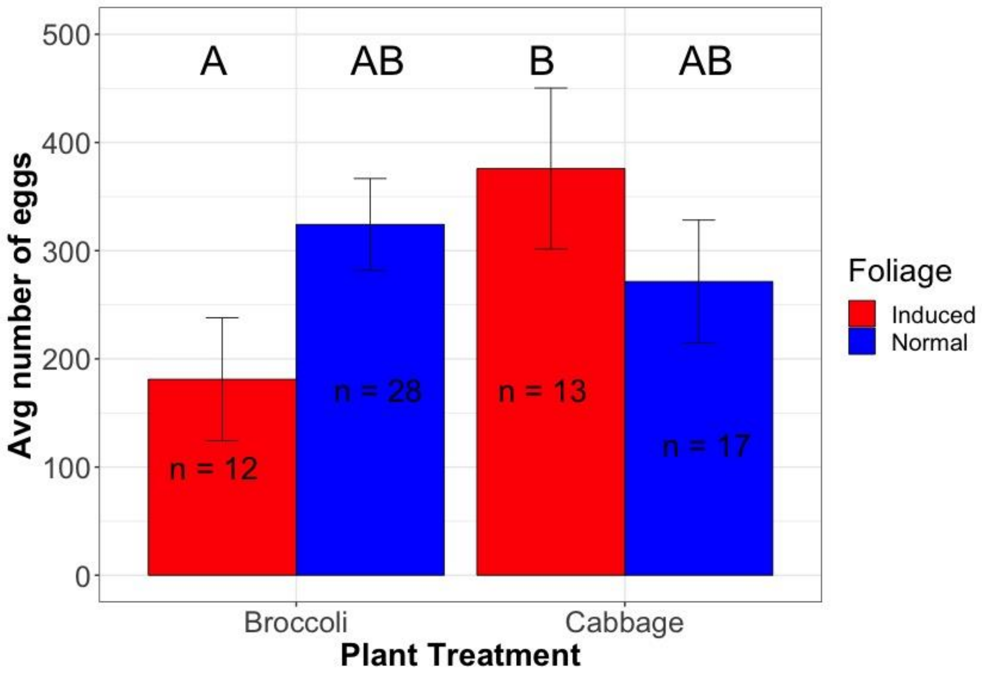

Figure 3-3 **Mean (±SE) fecundity of adult *T. ni* pairs fed induced or non-induced broccoli and cabbage foliage. The different letters designate significant differences according to the lsmeans function in R.**

3.3.2. Effects of plant induction and plant species on the offspring resistance to nucleopolyhedrovirus (TnSNPV)

Mortality due to virus. Plant identity in the parental generation affected the susceptibility of the offspring to virus (Table 3.1), with higher mortality in insects whose parents were reared on cabbage, compared to broccoli (Figure 3.4). Induction had no effect. As expected, mortality was greater at the higher virus dose. On a family basis, there was a large degree of variation in viral mortality among cabbage families ($F_{18, 742}$ = 3.97, p = 0.0022), with mortality ranging from 31.6% to 100%, but less in the broccoli families, with mortality ranging from 30% to 72.5% ($F_{17, 743}$ = 0.53, p = 0.9) (Figures 3.5a – d).

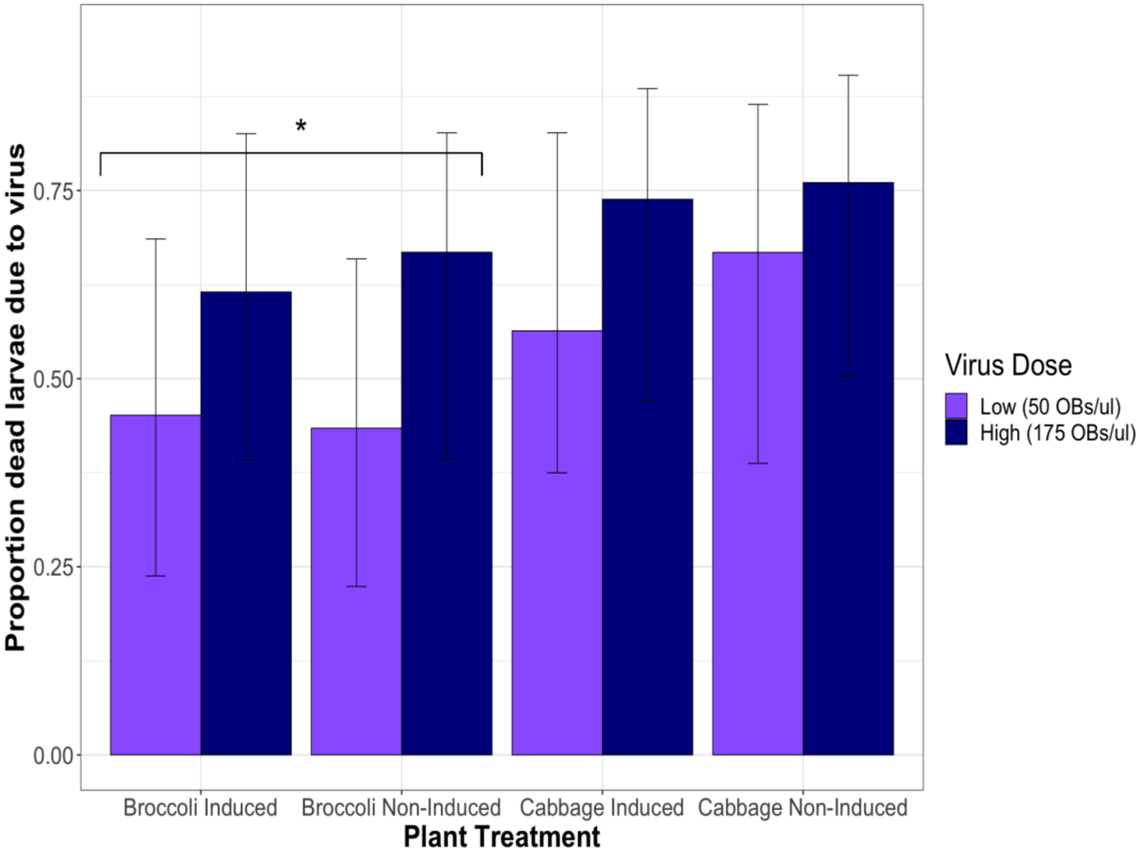

Figure 3-4 Proportion of viral mortality of 4th instar *T. ni* larvae, which were the offspring of parents who ate induced or non-induced cabbage or broccoli. (N = Broccoli Induced = 211, Broccoli Non-Induced = 457, Cabbage Induced = 371, Cabbage Non-Induced = 353). The asterisk designates a significant difference at P <0.05 for plant identity. Error bars are binomial 95% confidence intervals.

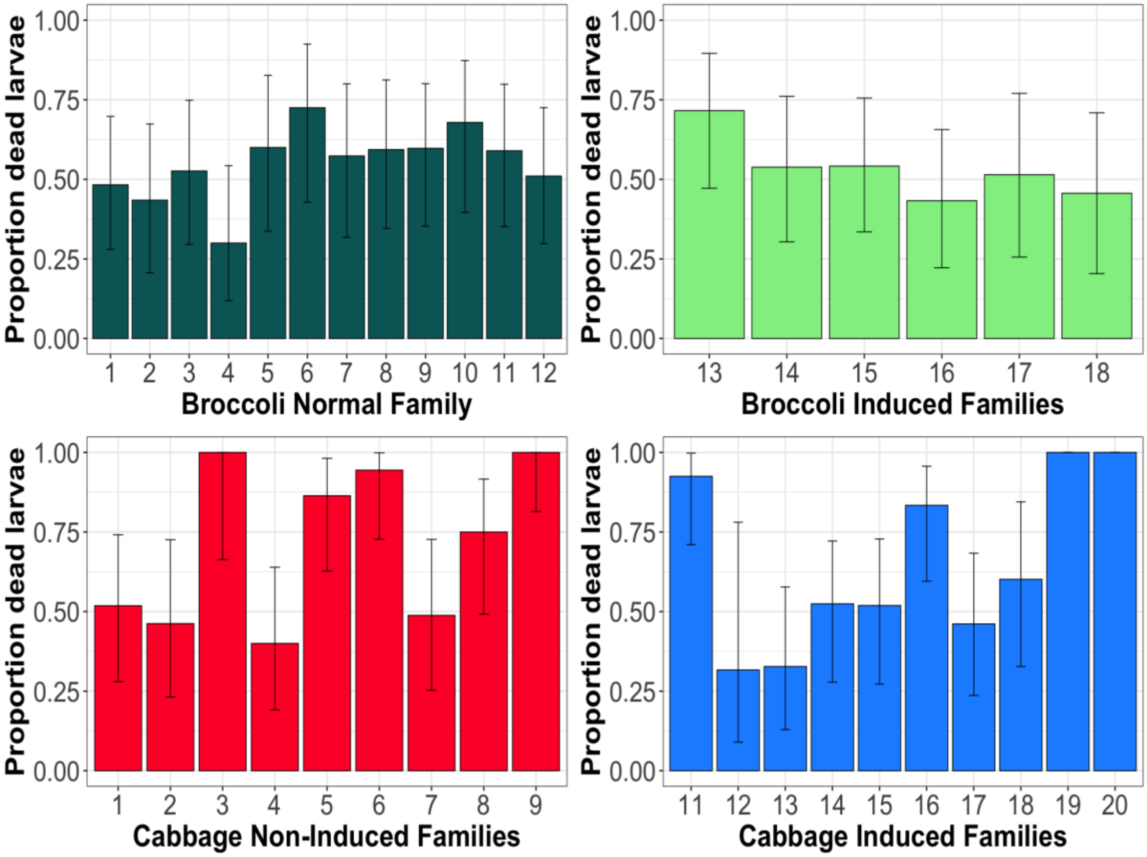

Figure 3-5 A – D) Proportion (±SE) of *T. ni* larval offspring dead from virus infection whose parents have eaten Cabbage or Broccoli with foliage treatments (Non-induced or Induced), separated out by family. Cabbage normal family 10 removed due to contamination. Error bars are binomial 95% confidence intervals.

Table 3.1 Results from a generalized linear model for the effect of plant identity, induction, viral dose and their interaction on mortality due to virus of *Trichoplusia ni* larvae infected with *Tn*SNPV. Values where P < 0.05 are given in bold. Stepwise backward elimination method was used to remove non-significant interaction terms (P ≥ 0.05) from the final mode. Asterisks indicate the factors used in final model.

	DF	F value	P value
Plant *	1	**5.918**	**0.013**
Induction	1	0.241	0.757
Dose *	1	**38.738**	**0.002**
Plant x Induction	3	0.14	0.689
Plant x Dose	3	0.535	0.542
Induction x Dose	3	0.003	0.992
Plant x Induction x Dose	7	0.611	0.728

Speed of kill of virus. Time to death was longer in offspring whose parents had eaten broccoli (*plant:* $F_{1,\ 759}$ = 31.57, p <0.0001) (Figure 3.6). Induction in the parental generation had no effect on offspring time to death (*induction:* $F_{1,\ 759}$ = 0.021, p = 0.88). Larvae died more rapidly at the higher virus dose (*viral dose:* $F_{1,\ 759}$ = 7, p = 0.008). The interactions between factors were all non-significant (*induction by plant:* $F_{3,\ 757}$ = 1.47, p = 0.23; *viral dose by plant:* $F_{3,\ 757}$ = 0.82, p = 0.36; *viral dose by induction:* $F_{3,\ 757}$ = 0.17, p = 0.68; *viral dose by induction by plant:* $F_{7,\ 753}$ = 0.4, p = 0.53).

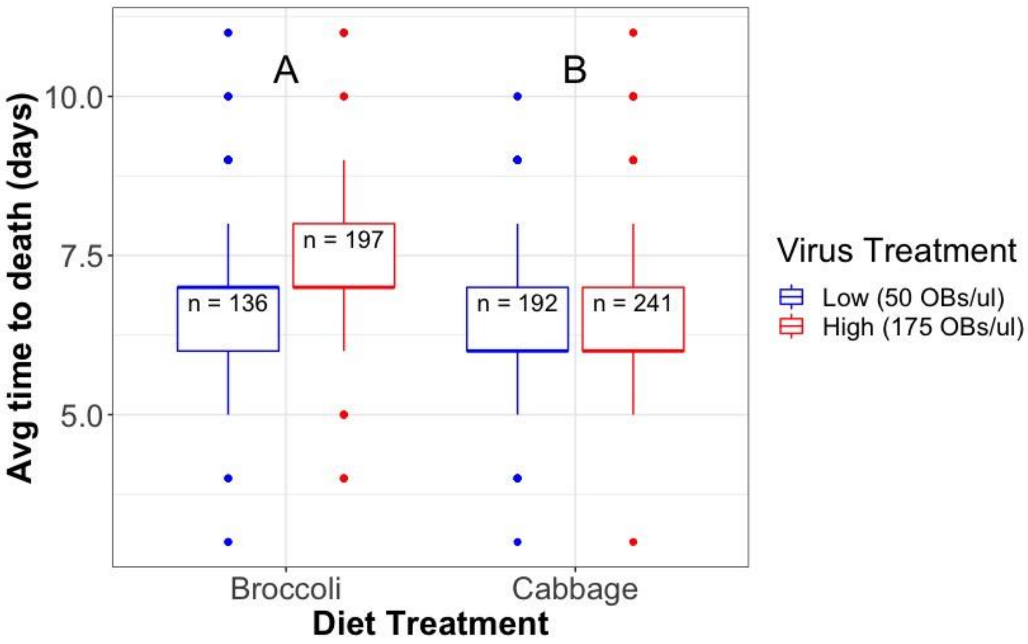

Figure 3-6 **Mean number of days until death from virus for *T. ni* offspring whose parents have fed on cabbage or broccoli. The different letters designate significant differences according to a Tukey's test. Error bars are the 95% confidence interval.**

3.4. Discussion

This experiment addressed whether life history traits of *T. ni* were affected by induced secondary plant metabolites in different plant species, and whether these effects resulted in changes in disease resistance in their offspring. Although feeding on induced foliage caused changes in larval growth and development in the parents, it did not alter viral resistance in offspring. However, there were strong effects of plant identity with the offspring of parents that had fed on broccoli being more resistant to virus.

Table 3.2 Sublethal effects of plant induction and plant identity

	Induction	Plant identity		Interaction
Larval weight (4th)	Marginal negative effect	broccoli & cabbage no difference	Tomato smaller	No effect
Larval weight (5th)	Interaction effect	Interaction effect	Interaction effect	Lower on tomato induced
Pupal mass	Interaction effect	Interaction effect	Tomato NA	Lower on broccoli induced
Fecundity	Interaction effect	No difference	Tomato NA	Induced broccoli less eggs than induced cabbage
Egg size	No effect	No difference	Tomato NA	No effect

3.4.1. Effect of host plant and induction on parents

The effects of both induction and plant identity were seen in the parental generation. Plant identity had a strong effect on insect growth, with insects feeding on tomato being lighter and slower to develop than those on the two brassicas, resulting in poor survival to the pupal stage. Plant induction also had a marked effect in tomato-feeding insects with fifth instar larvae being much lighter than in any of the other treatments (58.8% difference). Tomato contains a myriad of defensive chemicals, including tomatine, phenol oxidases, catecholic phenolics and other molecules such as proteinase inhibitors and lipoxygenases [some of which inducible by herbivory (Elliger et al. 1981; Duffey & Stout 1996; Felton 2005)] that are directly toxic to larvae, impeding feeding, nutrient uptake, larval weight and development times (Gallardo et al. 1990;

Duffey & Stout 1996; Safraz et al. 2011; Coapio et al. 2018). These defensive chemicals of tomato could have impacted larval weight more strongly than those of crucifers. In other studies, tomato was found to have more negative effects on *T. ni* growth and development than cabbage, pak choi, and lettuce (Hoo et al. 1984; Shikano et al. 2010). In addition, the trichomes on the surface of tomato foliage as well as the chemicals cause mortality in neonate *T. ni* larvae (Meneses-Arias et al. 2000). Some of these compounds are especially disruptive as they can trigger the remodeling of *T. ni* digestive enzymes, and due to the metabolic costs associated with this process, hinder the ability of larvae to detoxify defensive chemicals of tomato plants (Herde & Howe 2014). Unlike cabbage and broccoli, tomato is a relatively new host plant for *T. ni* larvae (Meneses-Arias et al. 2000), thus it may still affect growth and development of *T. ni* larvae that have not yet adapted to this new food source. Tomato as a new food source could also have accounted for the high level of larval mortality experienced on tomato diets where selection may have played a role. The larger more robust larvae feeding on tomato survived, but even among those not many reached the pupal stage.

There was less of a difference in growth on the crucifers, as would be predicted. However, the negative effects of induction appeared to be stronger on broccoli than on cabbage. The pupal weights of larvae feeding on induced broccoli were smaller compared to the larvae feeding on non-induced broccoli. Also, parents who fed on induced cabbage had a higher fecundity than parents who fed on induced broccoli. In foliage feeding lepidopterans pupal mass size is indicative of fecundity (Haukioja & Neuvonen 1985; Slansky & Scriber 1985; Rossiter et al. 1988; Awmack & Leather 2002). However, there was no trade-off between fecundity and egg size, indicating that induction did not trigger increased investment in offspring, at least in terms of increased provisioning. Other studies which exposed parental generations to diet stress resulted in a trade-off between fecundity and egg size, but the stressors in these studies were not induction but reduced dietary quality and variation in macronutrient content (Torres-Vila & Rodriguez-Molina 2002; Rotem et al. 2003; Shikano & Cory 2015). There was also no trade-off between fecundity and egg size in western tent caterpillars (*Malascome californicum pluviale*), but females that were fed induced foliage did produce smaller eggs (Olson et al. 2017). Although we do not know what the specific differences are between induced chemicals in our *Brassica* cultivars, on average broccoli has higher concentrations of glucosinolates and phenolics than cabbage (Mullin & Sahas-Rabudhe

1977; Fenwick et al. 1983; Podsędek 2007; Cartea et al. 2011; Possenti et al. 2016), which may explain the differences in the effects of induction. Moreover, broccoli usually has higher concentrations of glucoraphanin and progoitrin, and unlike cabbage contains gluconapoleiferin (Carlson et al. 1987; Kushad et al. 1999; Cartea & Velasco 2008). Cabbage, on the other hand, contains large amounts of sinigrin, glucobrassicin, glucoerucin and glucoiberin compared to broccoli (Kushad et al. 1999; Cartea & Velasco 2008; Cartea et al. 2008; Traka & Mithen 2009), and unlike broccoli contains glucoiberverin (Cartea et al. 2008). Because cabbage has a lower concentration of total defensive compounds as well as different types of glucosinolates, *T. ni* larvae could have detoxified the induced chemicals produced by cabbage with no or minimal effect to their development. The smaller induction effect on *T. ni* larvae caused by cabbage may be attributed to their ability to detoxify the glucosinolate sinigrin, which is found in higher concentrations in cabbage (Kushad et al.1999; Cartea & Velasco 2008; Cartea et al. 2008), more so than other lepidopteran species such as *Anticarsia gemmatalis* (a crucifer specialist) and a generalist *Spodoptera frugiperda* when feeding on this chemical incorporated in artificial diet (Wadleigh & Yu 1988a; 1988b; Winde & Wittstock 2011). Furthermore, differences in glucosinolate profiles of cabbage cultivars did not affect larval performance of the host generalist cabbage moth (*Mamestra brassicae*) compared to the host specialist diamondback moth (*Plutella xylostella*) (Poelman et al. 2008). Larval performance of these herbivores did not correlate with the total concentration of glucosinolates or any single glucosinolate of the eight cabbage cultivars that were tested, implying that glucosinolates in cabbage may not strongly affect insect herbivores.

3.4.2. Effect of parent host plant and induction on disease

Contrary to our prediction, both offspring disease resistance and virus speed of kill were unaffected by induction in the parents' diet. If, as predicted, plant induction had reduced diet quality and acted as a stressor (i.e., cost) to parents, there should have been a trade-off between growth and traits involved in provisioning of offspring in preparation for a future more hostile environment. We found no evidence of this type of trade-off, as the lower pupal mass in parents fed induced broccoli did not result in larger eggs or more resistant offspring. We conclude that plant induction as a stressor to *T. ni* parents does not affect the disease resistance of offspring. This result is supported by other studies which have looked at immunity traits. Although Shikano et al. (2016) did not specifically study the effects of induction, they found that low-quality parental nutrition or changes in parental nutrition do not impact the cellular immunity (haemocyte number) of *T. ni* offspring. Furthermore, Olson et al. (2017) found that immune functions such as antibacterial activity, and protein and lipid concentrations in the eggs of *M. c. pluviale*, did not differ between parent food treatments, even though egg size was negatively affected by feeding on induced foliage. However, our results contrast with the findings in the two studies that investigated the effect of plant chemistry on disease resistance. Working with *M. c. pluviale,* Olson (2014) showed a positive effect of plant induction on offspring NPV resistance. Similarly, monarch butterfly (*Danaus plexippus*) parents that consumed host plants rich in cardenolides (defensive compounds) produced offspring more resistant to a protozoan parasite (Sternberg et al. 2015). However, both the design and response criteria differed between the Olson (2014) study and our study. Olson (2014) recorded higher phenoloxidase activity and encapsulation in offspring generations but did not find any increases in haemocyte density, indicating some effect of plant induction on offspring immunocompetence. The underlying mechanisms as to how induced plant chemicals have an adverse impact on pupal mass, growth rate and fecundity in western tent caterpillars are not clear (Rothman 1997; Myers et al. 2011; Sarfraz et al. 2013), especially because both beneficial effects, or no effects, of plant induction have also been reported (Myers & Williams 1984; 1987; Rothman 1997). It is possible that induction is greater in the longer-lived host plant species fed on by the western tent caterpillar, resulting in stronger effects and higher parental investment. The tent caterpillar is also a capital breeder, so the adults do not feed and are thus unable to offset any lack of nutrients in the larval stage, whereas *T. ni* larvae feed on shorter lived plants and the adults feed. Monarch butterfly larvae sequester cardenolides in their tissue as a defense against predators (Malcolm & Brower 1989; Malcolm et al. 1989), and adult female butterflies may oviposit cardenolide-laden eggs, thereby potentially contributing to disease resistance of their offspring (Sternberg et al. 2015). In both western tent caterpillars and monarch butterflies, the mechanisms underlying transgenerational resistance to disease remain unknown.

Although we did not find a link between plant induction and disease resistance, larval offspring whose parents had fed on broccoli had a lower mortality due to virus than those whose parents had fed on cabbage. The increase in defensive compounds in broccoli may have been an indication of a hazardous environment for offspring, triggering increased investment in disease resistance (Ben-Ami et al. 2010; Stjernman & Little 2011). However, this increase in disease resistance is not linked with egg size, although according to the literature, egg size in insects is thought to be related with increased disease resistance because large eggs may contain more components beneficial to offspring immunity (Rothman & Myers 1996; Sadd & Schmid-Hempel 2007; Moreau et al. 2012; Zanchi et al. 2012; Shikano et al. 2015). Studies on poor parental nutrition or poor diet quality have shown no effect, or a negative effect, on offspring disease resistance (Myers et al. 2011; Triggs & Knell 2012; Shikano et al. 2016), but there are instances of offspring with improved responses to disease (Mitchell & Read 2005; Boots & Roberts 2012; Shikano et al. 2015). These studies did not entail feeding parent insects actual host plants but instead investigated the effects of parent diet quality and quantity, either through manipulation of nutrients in artificial diets or by diluting nutrients. For example, when *T. ni* parents were fed a standard diet diluted with cellulose, they produced offspring that were more resistant to *Bacillus thuringiensis* (*Bt*) and *T.ni* SNPV (Shikano et al. 2015). Similarly, Indian mealmoth (*Plodia interpunctella*) that was fed a maternal diet diluted with cellulose, produced offspring which were more resistant to a granulovirus (Boots & Roberts 2012). Comparable results are reported in a study with *Daphnia magna* (Mitchell & Read 2005), but here it is not clear whether offspring became more resistant due to stress caused by a nutritionally inadequate maternal environment or due to the numerical density of individuals. Insufficient quality or quantity of diet does not always result in changes to offspring disease resistance. Reducing the amount of Brewer's yeast in the diet of *Drosohpila melanogaster* parents had no effect on their offspring's resistance to a bacterium (Valtonen et al. 2012). Moreover, depriving western tent caterpillars of foliage every other day had no effect on offspring resistance to NPV, even though it had negative sublethal effects on the parents such as lower body mass and fecundity (Myers et al. 2011). The data from all these studies, including the current one, suggest that there is no universal rule as to how resistant offspring will be in relation to dietary stress experienced by their parents. In further studies, it might be helpful to specifically investigate the effects of plant metabolites or toxicants and separate them from the effects of food quantity, food quality, and intact plant foliage on life history traits of parent insects and disease resistance of their offspring.

In summary, although it is unclear why offspring whose parents consumed induced foliage and those who ate broccoli (as compared to cabbage) differed in their ability to resist disease, our results imply that plant identity may have longer term effects on host-pathogen dyanmics. However, futher studies should be conducted in order to identify if the transgenerational effects of broccoli carry over to other systems with different pathogen groups. The type of chemicals or the level of chemical of the same type present in broccoli as opposed to cabbage may have more strongly affected the larvae than the effects of induction, resulting in transgenerational disease resistance. The effect of plant identity on pupal mass could be due to a different compliment of defensive chemicals in cabbage and broccoli, such as glucosinolates being present in cabbage. Further studies should separate the effects of defensive chemistry and nutritional composition between host plant varieties on disease resistance.

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Chapter 4. General Conclusion

In Chapter 2 of my thesis, we tested whether feeding *Trichoplusia ni* multiple host plants alter larval growth and development more than a single host plant, and whether mixed diets alter resistance to a viral pathogen. Our hypothesis was that multiple host plants would be more chemically compelx and negatively impact resistance to TnSNPV. I demonstrated that mixed diets have no effect on disease resistance compared to single diets fed to the cabbage looper. As one of the few experiments looking at mixing diets and its effects on entomopathogen resistance, much more work should be done to push this field further. Past research has shown that mixing diets have conflicting results on whether they benefit insect fitness as opposed to single host plant species diets. Our experiment adds to this research and suggests that there is no universal outcome to mixing diets, which might suggest that plant identity is more important. It highlights the need for more research using different combinations of plants, as other systems may not have the same pattern. Furthermore, repeating the experiment with a different set of host plants may help distinguish between the effects of plant identity and chemical diversity. This can be of particular importance for other economically important pests that feed on multiple plant hosts throughout their development. It might also be interesting to look at the impact of feeding on mixtures of plant varieties. This could affect growers, as agricultural fields are most often large stretches of one type of crop, although there are many exceptions. Mody et al. (2007) is the only study to my knowledge looking at intraspecific diet-mixing, using the specialist *Chrysopsyche imparilis* (Lepidoptera: Lasiocampidae), and it would be useful to contrast this study with a generalist such as *T. ni*. It may be worth pursuing in more depth whether specific immune parameters or changes to the gut occur when insects feed on mixed diets.

In my second results chapter, I measured the effect of plant induction and plant identity on transgenerational disease resistance. Our prediction was that plant induction would act as a stressor in the parent generation, which would result in increased provisioning in their offspring, increasing their disease resistance. I established that, although plant induction in the parent generation does not result in more resistant offspring to nucleopolyhedrovirus, broccoli does as compared to cabbage. The results for induction contradict previous research on the transgenerational disease resistance of other Lepidopteran species (Olson 2014). This highlights the fact that deeper work needs to be done in order to identify why the host plants used in my experiment resulted in differences in disease but not induction. Therefore, measuring the chemicals present in each plant species and comparing them to alder trees could distinguish these differences, as induction is more highly expressed in trees since they have more to protect than annual crops. Also, looking at immune factors thought to be responsible for viral resistance such as encapsulation, antimicrobial peptides and haemocyte number would give us an indication as to the resistance mechanism which is affected by plant chemistry. Another approach could be to repeat this experiment with entomopathogens that are more widely used as microbial controls and broader in host range such as *Bacillus thuringiensis* (*Bt*) or *Autographa californica* nucleopolyhedrovirus (AcMNPV). This could give us an idea on if these transgenerational responses are unique to TnSNPV, which is a species-specific pathogen, or if they take place with other pathogens.

I am particularly interested in biological pest controls and due to the rise in the use of synthetic chemical pesticides, certain insects have begun to develop resistance with disastrous effect (Naqqash et al. 2016). Recent years have seen a change in the way pest control is approached in many sectors. Microbial biocontrols have seen a rise in use as part of Integrated Pest Management (IPM) strategies, that seek to minimize the effects of synthetic chemical insecticides on the environment, while also complementing other components such as cultural controls and natural enemy conservation (Lacey & Shapiro-Ilan 2008). As some microbial controls are often species-specific, they have minimal effects on non-target insects and other organisms (Barber et al. 1993, Richards et al. 1999; Cory 2000). Thus, it would be beneficial to consider my research in the establishment of a novel viral insecticide as a control for the cabbage looper. With the information established in my experiments, we know that the efficacy of a formulation containing TnSNPV will be impacted depending on the host plant the insect is reared on. Furthermore, these impacts may result in changes to the susceptibility of subsequent generations of cabbage loopers. Therefore, future field trials may be useful in determining the efficacy of TnSNPV.

4.A. References

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