Abiotic and biotic factors influencing host-plant use of a generalist herbivore through plant-mediated interactions: oviposition and larval performance by the bertha armyworm, *Mamestra configurata* Walker (Lepidoptera: Noctuidae) on canola

by

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Abstract

The bertha armyworm (BAW), *Mamestra configurata* Walker (Lepidoptera: Noctuidae), is a native, polyphagous herbivore which feeds on a variety of plants in different families. In the Prairie Provinces, canola, *Brassica napus* L. (Brassicaceae), is a preferred host, making BAW a significant pest. Canola is impacted by a variety of abiotic and biotic stressors that could directly compete with BAW or indirectly influence the performance of BAW through plant-mediated interactions. In this study, I tested for plant-mediated interactions of canola plants that were exposed to three different stressors: 1. various levels of nutrition; 2. oviposition and herbivory by a specialist herbivore; and 3. root pathogen infection; on subsequent host use by BAW.

Crop fertilization may have an effect on efficacy of pest control in Integrated Pest Management (IPM) programs. Fertilization of crop plants and the use of resistant canola varieties may directly influence the performance of BAW larval feeding and development. I altered the fertilization level on three different canola varieties: an early flowering variety: ‘5535 CL’; a late flowering variety: ‘6060RR’; and a control variety: ‘Q2’ for experiments. Bertha armyworm females laid more eggs and larvae performed better on plants treated with moderate and high versus low fertilizer treatments. Plant leaf tissue nutrient content (nitrogen, phosphorous, potassium) and plant growth were also higher in plants that received moderate and high levels of fertilization.

The diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Plutellidae), is a specialist herbivore on plants in the Brassicaceae and one of the most significant pests of canola in western Canada. Diamondback moth co-occurs with BAW in canola fields but DBM herbivory occurs prior to BAW herbivory. In this study, I tested the effect of DBM oviposition and herbivory on subsequent host use by BAW. Bertha armyworm larvae fed more on plants with DBM eggs. Bertha armyworm adults laid fewer eggs when exposed to canola plants with DBM eggs. Plant hormone analyses suggested that DBM oviposition suppresses canola defenses. I also subjected DBM-infested, mechanically damaged and uninfested canola plants to subsequent BAW herbivory. Bertha armyworm larvae were negatively influenced by DBM feeding damage whereas BAW oviposition was not affected. Taken together, all these results suggest that herbivory by the specialist, DBM, negatively influences subsequent host use and larval performance by the generalist, BAW on canola.

Clubroot disease, caused by a soil borne protist, *Plasmodiophora brassicae* Woronin, has recently emerged as an important agricultural pest that impacts canola production and yield. Both *P. brassicae* and BAW infestation occur in the agroecosystems of Alberta canola fields. Thus, it is important to study the potential interaction between *P. brassicae* diseased plants and BAW to properly manage both threats. The effect of *P. brassicae* disease infection on subsequent BAW oviposition and larval development was tested using two hybrid canola varieties that varied in resistance to the pathogen ('45H26' [susceptible to *P. brassicae*] and '45H29' [resistant to *P. brassicae*]). Adult female moths preferentially layed eggs on the susceptible canola in the absence of *P. brassicae* inoculation. Inoculation of resistant canola with *P. brassicae* did not influence oviposition of BAW. Inoculation with *P. brassicae* influences larval development as larvae weigh more when reared on non-inoculated as compared with inoculated resistant canola. In contrast, heavier larvae resulted from feeding on inoculated as compared with non-inoculated susceptible canola. Plant hormone analyses suggest that canola defenses are induced in response to *P. brassicae* inoculation. Large amounts of salicylic acid (SA) conjugates are present in the inoculated susceptible canola that may influence BAW oviposition. In addition, inoculation with

P. brassicae changes the volatile profile of chemicals released by canola plants which likely mediate oviposition responses by BAW females.

These results show that interactions between BAW and canola are affected by host plant nutrition, pathogen infection and previous damage by a specialist herbivore. The importance of canola-mediated responses to abiotic and biotic stressors on subsequent host use by the generalist herbivore, BAW, improves our understanding of canola agroecosystems and provides a foundation for the improvement of IPM of canola in Alberta.

Preface

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Dedication

This thesis is dedicated

To my beloved parents Weeraddana Pablias De Silva and W.W.A.M. Molie Silvia, who inspired me throughout my life; my lovely wife Ramya, my lovely sons Abhitha and Adhisha

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

BAW- bertha armyworm

DBM- diamondback moth

VOC- volatile organic compound

MS- methyl salicylate

JA- jasmonic acid

GCxGC TOFMS- two-dimensional gas chromatography-time-of-flight mass spectrometry

ANOVA- Analysis of variance

GLMM- General linear mixed-effects model

Chapter 1: General Introduction

Plants cope with different environmental stressors in agroecosystems, which can have a negative impact on plant growth and reproduction, as well as an impact on agricultural production (Madlung and Comai 2004). Different abiotic and biotic stressors occur simultaneously or in rapid succession in agricultural environments. Biotic stressors include herbivory, pathogen attack and both intra- and interspecific competition. Abiotic stressors to plants include unfavorable environmental conditions such as water, light, temperature, nutrient availability, and light (Madlung and Comai 2004). These biotic and abiotic stressors influence plant quality and defense responses, potentially affecting other biotic stressors like herbivores (Awmack and Leather 2002, Donaldson et al. 2006, Hakes and Cronin 2011). The “Plant stress hypothesis” states that stressed plants are better resources for insect herbivores because stressed plants mobilize nutrients and reduce the production of toxic chemicals (White 1984), however, studies show conflicting results (Koricheva et al. 1998) suggesting that the effect of plant stress on the herbivore varies with the feeding guild of the insects (Larsson 1989).

In many phytophagous insects, adult females select between many host plants for suitable oviposition sites. The ‘preference-performance’ or ‘mother knows best’ hypothesis states that adult female insects choose a host plant on which their offspring will perform best (Jaenike 1978). Most studies conclude that females select their host based on host quality (Gripenberg et al. 2010), however, some studies conclude that female insects choose a host plant for protection or to avoid exposure to natural enemies, which is called “enemy free space” (Jeffries and Lawton 1984). Host plant quality is an important factor for female insects that lay eggs in clusters, as the plant needs to be of high enough quality to support a large number of larvae (Mangel 1987). The quality of the host plant needs to support larval survival and allocation of resources toward the development of insect eggs in insects that initiate egg development before eclosion (Jervis and Ferns 2004). Poor-quality hosts can lead to host rejection by the ovipositing female and result in egg resorption. For example, in the absence of good quality host plants, the Mormon fritillary butterfly, *Speyeria mormonia* (Boisduval) (Lepidoptera: Nymphalidae) resorbs oocytes and reallocates resources to somatic maintenance and dispersal (Boggs and Ross 1993, Boggs 1997). When larvae consume poor quality food, there can be negative effects on adult reproduction. There is a positive relationship between host plant quality that larvae of the European grapevine moth, *Lobesia botrana* (Denis & Schiffermueller) (Lepidoptera: Tortricidae) feed on with the size of eggs produced by the resulting female moth (Moreau et al. 2006).

Plant defenses

Plants respond to insect attacks through direct and indirect defenses. Direct defenses include plant traits that directly influence the physiology and/or behaviour of the attacking insect. Chemicals secreted by trichomes that are repellent to herbivores are referred to as direct chemical defenses whereas leaf toughness is a direct physical defense (Howe and Jander 2008). Indirect plant defense involves communication between the plant and the third trophic level, the natural enemies of the attacking herbivore. Plants emit volatile organic compounds (VOCs) that signal the presence of the herbivore on the plant and attract the natural enemies of the herbivore (Kessler and Baldwin 2002). Both direct and indirect defenses provide vital protection to plants against large numbers of herbivores (Kessler and Baldwin 2002).

Some of these plant traits can be present constitutively or they can be induced by herbivory (Bostock 2005). Constitutive defenses are present in plants whether the herbivore is present or not (Kaplan et al. 2008). Induced defenses are less costly to the plant than constitutive defenses because they are only activated when necessary (Bostock 2005). Induction of specific plant responses can vary with the insect herbivore species. The components of insect oral secretions, saliva and oviposition secretions are recognized as elicitors that trigger plant defenses, however, only a few herbivore-associated molecular patterns (Howe and Jander 2008) have been identified to date. For example, inceptin was isolated from the oral secretions of the fall armyworm, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) larvae. Inceptin induces phytohormones: ethylene, salicylic acid (SA) and jasmonic acid (JA) and increases the emission of plant volatiles: (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and cinnamic acids (Schmelz et al. 2006). Bruchins, in the oviposition fluid of the pea weevil, *Bruchis pisorum* L. (Coeoptera: Chrsomelidae) cause neoplasmic growth in the pea pods, which deters the entry of larvae into the pea pod (Doss et al. 2000).

Nitrogen fertilizer application to wheat plants increases essential and non-essential amino acids (Zhang et al. 2017), and makes the plants more nutritious to insect herbivores. Similarly, nitrogen fertilizer application to rice plants increases soluble proteins in plant tissues and subsequent increased feeding of the brown planthopper, *Nilaparvata lugens* (Stål), (Hemiptera: Delphacidae) (Rashid et al. 2016). Nitrogen fertilizer applications also increase glucosinolates in brassicaceous plants (Grant et al. 2011), which can increase plant defenses against generalist herbivores (Jeschke et al. 2017).

Carbon-nutrient balance hypothesis

One of the primary goals in the study of plant-herbivore interactions is to predict phenotypical, genetic and geographical variation using different theories of plant defenses (Stamp 2003). Among several other hypotheses, the carbon-nutrient balance hypothesis (CNB) attempts to explain reasons for variation of plant resistance to herbivory (Tuomi et al. 1991). The carbon-nutrient balance hypothesis explains the concentration of secondary compounds as a function of carbon and nitrogen reserves in the plant, however, carbon and nitrogen accumulated beyond growth can be used for either defense or storage (Tuomi et al. 1991). For an example, nitrogen fertilization and shading are expected to lower C/N ratio and decrease carbon-based defenses such as phenolics and terpenes. Although some reports support this hypothesis, many do not (Koricheva et al. 1998). Furthermore, the CNB can make valid predictions for carbon allocated to to total carbon-based secondary compounds but not with individual carbon compounds. Despite the shortcomings of CNB, the hypothesis continues to predict the outcome of some plant-herbivore experiments (Koricheva et al. 1998).

Regulation of plant defenses through jasmonic acid pathway

Jasmonic acid (JA) is a plant hormone that plays an important role in plant development and defenses (Turner et al. 2002). Plant defense stimulated through the JA pathway is mostly induced by nectrotrophic pathogens and chewing insects (Smith et al. 2009). Jasmonic acid and its derivatives are oxygenated fatty acids or oxylipins, which are produced through the octadeconoid pathway (Wasternack 2007). Insect derived elicitors such as fatty acid-amino acid conjugates and inceptin induce JA synthesis when applied to artificial wounds (Kessler and Baldwin 2002, Schmelz et al. 2006). Larvae feeding on plant tissues damage membrane lipids and release free fatty acid and linoleic acids. These molecules enter the octadeconoid pathway and are then enzymatically transformed into active JA (Ishiguro et al. 2001). Insect feeding induces defense genes, proteinase inhibitors, plant volatiles and secondary metabolites such as nicotine, phenolics and phytoalexins (Balbi and Devoto 2008). Jasmonic acid induces proteinase inhibitors that inhibit insect feeding (Farmer and Ryan 1992). Methyl jasmonate induces nicotine production in

Nicotiana sylvestris Speg. & Comes and increases resistance to the pallid-winged grasshopper, *Trimerotropis pallidipennis* (Baldwin 1998).

Jasmonic acid can be converted into derivatives which include: methyl jasmonate (MeJA) and jasmonic acid isoleucine (JA-Ile). Methyl jasmonate is used as an airborne signal produced by herbivore-damaged plants to undamaged plants to inform conspecifics of potential future herbivory (Seo et al. 2001) whereas JA-Ile is considered an active metabolite in the JA signaling pathway (Staswick and Tiryaki 2004). Plant-induced VOCs indirectly attract natural enemies of herbivores. As an example, large amounts of terpenoids are emitted following herbivory by beet armyworm, *Spodoptera exigua* (Hiibner) (Lepidoptera: Noctuidae), that attract the parasitic wasp, *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) (Turlings et al. 1990). Depending on the plant species, VOCs can include multiple chemical classes such as: green leaf volatiles, phenylpropanoids, indoles (nitrogen containing compounds), dimethyl disulphides (sulphur containing compounds), isothiocyanates (sulphur and nitrogen containing compounds) and terpenoids (Aartsma et al. 2017).

Regulation of plant defenses through the salicylic acid pathway

Salicylic acid (SA) is a plant defensive hormone which is mostly induced by biotrophic pathogens and phloem feeding insects (Kaloshian and Walling 2005, Schweiger et al. 2014). Salicylic acid induces pathogen resistance genes through both local and systemic acquired resistance (Shah 2003). Salicylic acid synthesis occurs through the shikimate pathway. The first step in defense against a pathogen is a hypersensitive response and cell death which limits further spread of the infection (Greenberg et al. 1994). Hypersensitive response includes production of reactive oxygen compounds such as superoxides, hydrogen peroxide, hydroxy radicals and nitric oxides (Mur et al. 2008). These reactive oxygen species interact with membrane lipids leading to cell death and necrotic lesions (Mur et al. 2008). In addition to the hypersensitive response, pathogenesis-related (PR) genes are also induced during pathogen infection, both locally and systemically (Glazebrook 2005). Pathogenesis-related gene activation is associated with induction of the SA pathway. Pathogenesis-related proteins induce resistance to plant viral, bacterial, fungal pathogens (Vlot et al. 2009) and phloem feeding insects (Walling 2008).

Salicylic acid can be converted into different conjugates in plants. The most abundant conjugate of SA is O-β-glucoside, a less abundant conjugate is salicyloyl glucose ester (Song 2006). Both of these conjugates are inactive forms of SA which can be converted back to active SA following pathogen infection (Hennig et al. 1993). Methyl salicylate (MeSA) and glucosylated conjugates are also biologically inactive forms of SA., MeSA is considered an airborne signal for plant-plant communication (Koo et al. 2007).

Cross talk between SA and JA pathways

Salicylic acid and JA signaling pathways interact with each other through cross talk to fine tune the defensive response of the plant. Generally, the SA and JA pathways are antagonistic which allows only one pathway to be induced while the other is suppressed. The SA pathway is activated by biotrophic pathogens, viruses and phloem feeding insects whereas the JA pathway is activated against necrotrophic pathogens and chewing insects (Glazebrook 2005). For example, infection by the biotrophic pathogen, *Pseudomonas syringae* in *Arabidopsis* induces the SA pathway and suppresses the JA pathway which increases the plant susceptibility to a nectrophic pathogen, *Alternaria brassicicola* (Spoel et al. 2007). In a fewer cases, these two pathways can be synergistically induced. Co-treatment with low concentrations of SA and JA to tobacco, *Nicotiana tabacum* L. synergistically induces genes in both the JA and SA pathways. At higher concentrations, however, JA and SA have an antagonistic interaction (Mur et al. 2005). Despite the cross talk that can occur between signaling pathways to fine-tune the plant defensive response, some attackers can manipulate plant defenses for their own benefit. For instance, elicitors derived from the eggs of the cabbage whitefly, *Pieris rapae* (L.) (Lepidoptera: Pieridae) induce the SA pathway and suppress the JA pathway for the benefit of developing its larvae (Little et al. 2006).

Canola

Rape seed originated from the Mediterranean area and Northern Europe. Historically, rapeseed was identified as an oil that contains > 40% erucic acid. Rapeseed oil was used as an industrial lubricant during World War II (Rakow 2000). Especially in Canada, demand for a Canadian supply of industrial lubricants was reduced after World War II, and plant breeders began to focus on the development of alternatives for industrial lubricant oil. In 1978, the first low erucic acid and low-glucosinolate *Brassica napus* variety was released in Canada which was marketed as "canola". By definition, canola has <2% erucic acid and <30 µmol/g glucosinolate (Mag 1983). Today, canola has been cultivated widely in North America, Europe, China, India and Australia. The canola plant is in the family Brassicaceae that consists of a large number of plant species, including 338 genera and 3709 species distributed worldwide (Al-Shehbaz et al. 2006). The family Brassicaceae includes three major oilseed crops: *B. napus* L., *B. rapa* L. and *B. juncea* (L.) Czern & Coss but *B. napus* is the most widely cultivated oilseed crop in Canada. In 2017, Canadian canola contributed $26.7 billion to the Canadian economy, with most economic benefits to Western Canada where most canola is grown in Canada (www.canolacouncil.org).

Soil nutrition is an important factor for better yield in canola crops, however, the fertilizer recommendation is based on soil test analyses conducted where canola is grown. Nitrogen is the most common limiting nutrient in the soil for canola production. High-yielding canola hybrids need more nitrogen than traditional canola varieties, however, they are better at absorbing nutrients from the soil (Karamanos et al. 2005). In addition, phosphorous and sulphur are important for yield increase in canola crops, but potassium fertilization is not needed to increase canola yield in western Canada because the soils have adequate potassium in this region (Karamanos et al. 2005). Sulphur requirements for canola are higher than any other crops in western Canada (Karamanos et al. 2005).

Herbicide resistant canola systems such as Roundup Ready and Clearfield are popular among Western Canadian canola growers due to higher yield and other economic benefits including the ability to spray herbicides to crops. Clearfield varieties are tolerant to imidazolinone herbicides (Tan et al. 2005) whereas Roundup Ready is resistant to the activity of the herbicide, glyphosate (Funke et al. 2006). Eighteen new canola varieties were introduced to canola growers in 2017. (www.grainews.ca). For example, the canola variety, PV 580 GC is resistant to the clubroot pathotype 5X strain found in some fields in Alberta (https://canoladigest.ca).

Glucosinolates in Brassica

Glucosinolates are the major secondary metabolites present in plants in the Brassicaceae. Three functional groups: 1) β-thioglucose; 2) sulfonated aldoxime moiety; and 3) a variable side chain derived from amino acids make up the basic chemical structures of glucosinolates (Redovnikovic et al. 2008). Glucosinolates are divided into three major classes based on the structure of the amino acid precursors: 1) aliphatic glusinolates; 2) aromatic glucosinolates; and 3) indole glucosnolates (Redovnikovic et al. 2008). Although intact glucosinolates are not toxic to insects, the hydrolysis products of glucosinolates are toxic. Plant damage releases the enzyme myrosinase that hydrolyzes glucosinolates to produce isothiocyanates, nitriles and oxazolinethiones (Redovnikovic et al. 2008). These hydrolysis products are harmful to generalist herbivores but are exploited as feeding and oviposition stimulants for insects that specialize on the Brassicaceae. For instance, the performance of generalists, African cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) and cabbage moth *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae) is negatively affected by glucosinolate content in host plants (Jeschke et al. 2017). Oviposition by the specialist, diamondback moth, *Plutella xylostella* (L.) is stimulated by glucosinolates isolated from different *Brassica* species (Reed et al. 1989). Specialists are adapted to glucosinolate defenses through detoxification, excretion and sequestration (Mainguet et al. 2000). A specific gut enzyme, glucosinolate sulfatase, present in the gut of *P. xylostella* larvae, outcompetes myrosinase and converts glucosinolates to desuloglucosinolates instead of toxic nitriles or isothiocyanates (Ratzka et al. 2002). The green peach aphid, *Myzus persicae* (Sulzer) excretes glucosinolates with their honeydew (Merritt 1996). Larvae of the white butterfly, *Pieris brassicae* (Lepidotera: Pieridae) sequester glucosinolates as allylisothiocyanates (Aplin and Ward 1975). The variation of distribution of glucosinolates within a plant (Merritt 1996) provides specialists and generalists the opportunity to change feeding behaviours according to glucosinolate concentration. For example, larvae of the specialist, *P. brassicae* prefer to feed more on glucosinolate-rich flowers rather than leaves of *Brassica nigra L*. feeding more on flowers results in higher larval growth rates (Merritt 1996).

Disturbance agents in canola in the Prairie Provinces

Insect pests

The major insect pests of canola on the Canadian Prairies are the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae), flea beetles (Coleoptera: Chrysomelidae), lygus bugs (Hemiptera: Miridae), the bertha armyworm (BAW) *Mamestra configurata* Walker (Lepidoptera: Noctuidae), diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), alfalfa looper, *Autographa californica* (Lepidoptera: Noctuidae) and several species of cutworms (www.canolacouncil.org), and root maggot (Diptera: Anthomyiidae: *Delia* spp.) (Dosdall et al. 2012).

Diseases

The major fungal diseases of canola in western Canada are the alternaria black spot, *Alternaria brassicae*, blackleg disease, *Leptosphaeria maculans*, downy mildew, *Peronspora parasitica*, root rot complex, (*Rhizoctonia solani*, *Fusarium* species and *Pythium* species), Sclerotinia stem rot, *Sclerotinia sclerotiorum*, white leaf spot, *Pseudocercosporella capsellae* and white rust, *Albugo candida* (www.canolacouncil.org). In addition to fungal diseases, clubroot is a serious soil-borne disease caused by the fungus-like protist, *Plasmodiophora brassicae* Woronin (www.canolacouncil.org).

Bertha armyworm

Bertha armyworm is a polyphagous insect, which feeds on a variety of host plants in several different families: Brassicaceae, Compositae, Leguminosae, Chenopodiaceae and Linaceae. Larvae can complete development on ~ 40 host plant species which include several economically important crops such as canola, mustard, alfalfa, flax, and pea (Dosdall and Ulmer 2004). Bertha armyworm outbreaks cost millions of dollars in yield loss and control measures (Evenden et al. 2017). Prairie-wide control costs of CAN$16.5 million per year (Mason et al. 1998) and lost yield worth CAN$10-40 million occurred per year in 2005-2007 (Erlandson 2013). The current BAW monitoring program uses sex pheromone-baited traps. The total number of male moths trapped positively correlates to the number of late instar larvae in the field, but this varies between fields within a region as the number of larvae depends on crop phenology and other factors (Turnock 1987).

There are six larval instars, but the sixth instar larvae ingest 70-80% of larval food (Bailey 1976) (Figure 1-1b). If resources are depleted, mature larvae can disperse together to new feeding sites in an army-like procession (Bracken 1984). Larvae take about six weeks to complete development under field conditions. Mature larvae drop from the plant to pupate in the soil where they overwinter in pupal diapause (Wylie and Bucher 1977) (Figure 1-1c).

In the Prairie Provinces of western Canada, adults (Figure 1-1d) eclose between mid-June to early August. Virgin females release a sex pheromone to attract mates during the second or third scotophase (Howlader and Gerber 1986). Mated females have a refractory period of two days before they start to mate (Howlader and Gerber 1986). Seventy-five percent of eggs are laid by the end of the seventh scotophase. When temperatures rise above 35°C, calling and oogenesis are adversely affected (Gerber and Howladert 1987). Each adult female lays approximately 2100 eggs on the abaxial surface of leaves of host plants (Howlader and Gerber 1986) (Figure 1-1 a).

Bertha armyworm females lay eggs mostly in the middle of canola plants (Ulmer et al. 2002). Females prefer to lay eggs on canola plants with flowers as compared to pre-flower and pod stages (Ulmer et al. 2002). Females prefer to lay eggs close to a conspecific female's eggs and this attraction to conspecific eggs might be chemically based (Ulmer et al. 2003). Female BAW appear to choose a host plant for oviposition based on their glucosinolate profile. For instance, females are deterred from laying eggs on *B. juncea* that have higher levels of the glucosinolate sinigrin. In contrast, females are attracted to *Sinapsis alba* that have higher levels of the foliar glucosinolate, sinalbin (Ulmer et al., 2002). The larvae of BAW are deterred from feeding on *B. juncea* (Dosdall and Ulmer 2004). Apart from *Brassica* plants, BAW females prefer to lay eggs and larvae prefer to feed on *Chenopodium album* L. (Chenopodiaceae) (Dosdall and Ulmer 2004).

Diamondback moth

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a specialist herbivore that is oligophagous on plants in the Brassicaceae and is considered one of the most significant worldwide insect pests of Brassicaceae crops (Furlong et al. 2013). In early spring, diamondback moth adults migrate on wind currents from the southern USA and Mexico to the Canadian Prairies Canada (Harcourt 1957, Dosdall 1994). Diamondback moth has four larval instars (Golizadeh et al. 2007). First instar larvae mine the leaves and consume only the spongy mesophyll of the host plant tissue, leaving behind the waxy layer of the epidermis. This feeding behaviour results in characteristic window-like holes in the leaf surface (Talekar and Shelton

1993). Late instar larvae feed on all parts of the leaves and when resources are depleted they can feed on canola pods and stems (Talekar and Shelton 1993). Pupation occurs on the plant in a loosely woven cocoon and can last from two to four weeks, depending on temperature (Harcourt 1957). Adult females produce a sex pheromone (Koshihara and Yamada 1981) to attract mates during the first scotophase after ecolsion (Pivnick et al. 1990). Oviposition occurs for four days, during which females lay between 11-188 eggs. Eggs are laid singly along the concavities on the leaf surface. Gravid females prefer to lay eggs on plants that are damaged by conspecific larvae (Wee 2016). Adults are not known to overwinter in Canada (Harcourt 1957, Talekar and Shelton 1993, Dosdall 1994).

Clubroot

In the canola agroecosystem of the Canadian Prairies, clubroot disease, caused by a soil borne obligate biotrophic protist, *Plasmodiophora brassicae* Woronin, was first reported in 2003 (Tewari et al. 2005). In 2007, it was declared as a pest under the Agricultural Pests Act of Alberta. In Alberta, this disease has spread throughout central Alberta and is now confirmed in Saskatchewan and Manitoba (Strelkov and Hwang 2014). Canola crops with severe *P. brassicae* infections have yield reduced by 30-100% (Strelkov et al. 2007, Hwang et al. 2011). Infection by *P. brassicae* occurs in two stages: 1) root hair infection; and 2) root cortical colonization (Tommerup and Ingram 1971). Infected roots release resting spores that germinate and swim through a film of water in the soil (Macfarlane 1970) until they contact root hairs of a new host plant (Aist and Williams 1971). The primary zoospores encyst and penetrate a root hair to form an amoeba-like cell which undergoes several nuclear divisions to form a multinucleate structure called a plasmodium (Ingram and Tommerup 1972). The plasmodium produces many secondary zoospores that are released back into the soil. These secondary zoospores initiate infection of neighboring host plants (Buczacki and Clay 1984). The secondary zoospores invade the root cortex to produce a secondary plasmodium. These secondary structures alter root physiology and anatomy to produce enlarged root galls or clubs. The clubs are decayed by micro-organisms and release millions of spores into the soil. These resting spores can survive in the soil for at least 20 years, which makes control difficult (Wallenhammar 1996). Infection causes restriction of water and nutrient uptake (Siemens et al. 2011) resulting in host plant stunting and reduced yield (Strelkov and Dixon 2014). The use of resistant cultivars is an important strategy used in *P. brassicae* management (Diederichsen et al. 2009). Resistant cultivars can control the rate of degradation of secondary thickening of xylem and restrict movement of amoeboid forms (Donald et al. 2008).

Thesis objectives

Canola plants cope with different biotic and abiotic stressors in canola fields. These factors may influence plant defense and nutrition traits that can impact the subsequent plant use by associated herbivores. The main goal of this thesis was to gain deeper understanding of interactions between canola and BAW (Figure 1-2). In Chapter 2, I assessed the effect of canola variety and plant nutrition on plant growth parameters and plant elemental analysis. I also assessed the effect of these factors on subsequent oviposition and offspring performance of BAW. Diamondback moth oviposition and larval feeding on canola occurs in the field prior to host use by BAW adults. In Chapter 3, I examine the response of canola plants to the presence of diamondback moth eggs and the subsequent host use by BAW. In Chapter 4, I assessed the effect of DBM herbivory on canola response and the subsequent host use by BAW. In Chapter 5, I assessed the effect of *P. brassicae* disease on the production of plant defenses through SA and JA analyses. I also analyzed volatile organic compounds released by *P. brassicae* inoculated and non-inoculated canola plants. The effect of *P. brassicae* inoculation on subsequent host use by BAW was tested.

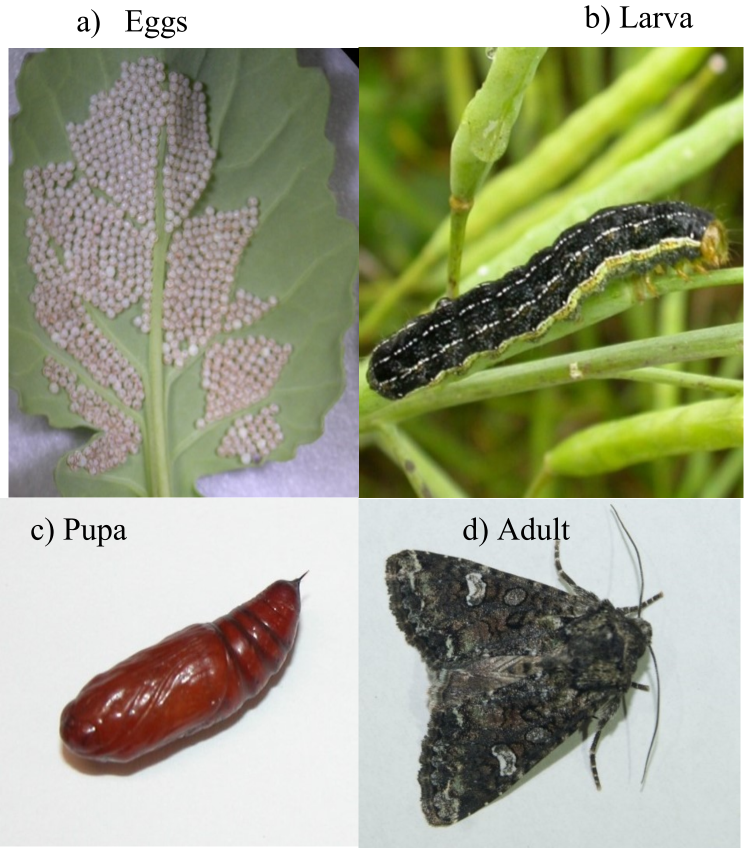


Figure 1-1. Life stages of bertha armyworm, a) eggs b) larva c) pupa d) adult. Mated BAW female lay a single layer of eggs in a honey comb shape on the abaxial leaf surfaces, which hatch to produce larvae. Bertha armyworm overwinter as pupae. The mature larvae can be distinguished by having pale green with yellowish strips on the dorsal part of the body. Bertha armyworm adult moths have a wing span of about 4 cm. A prominent white kidney-shaped mark can be seen near the middle of forewing and an olive color irregular shape marking at the tip of the forewing.

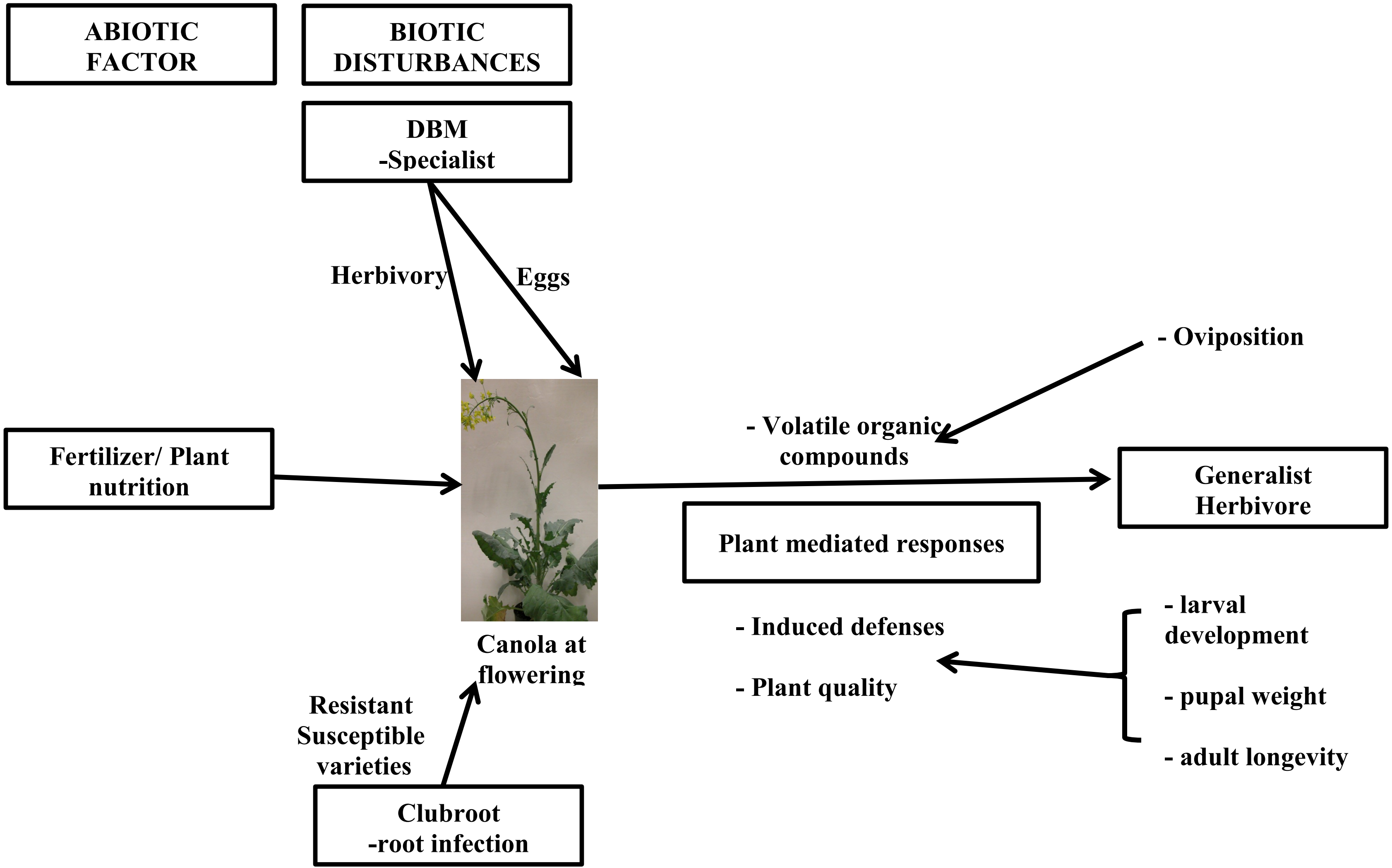


Figure 1-2. Canola plants interact with different abiotic and biotic stressors such as plant nutrition, specialist herbivory and *P. brassicae* infection in agroecosystems. These stressors may induce plant defenses and alter host plant quality, which can influence oviposition and offspring fitness of BAW.

Literature Cited

Aartsma, Y., F. J. J. A. Bianchi, W. van der Werf, E. H. Poelman, and M. Dicke. **2017**. Herbivore-induced plant volatiles and tritrophic interactions across spatial scales. New Phytol. 216: 1054–1063.

Aist, J. R., and P. H. Williams. **1971**. The cytology and kinetics of cabbage root hair penetration by *Plasmodiophora brassicae*. Can. J. Bot. 49: 2023–2034.

Al-Shehbaz, I. A., M. A. Beilstein, and E. A. Kellogg. **2006**. Systematics and phylogeny of the Brassicaceae (Cruciferae): An overview. Plant Syst. Evol. 259: 89–120.

Aplin, R. T., and R. d. Ward. **1975**. Examination of the large white and small white butterflies (*Pieris* spp.) for the presence of mustard oils and mustard oil glycosides. J. Entomol. 50: 73–78.

Awmack, C. S., and S. R. Leather. **2002**. Host plant quality and fecundity in herbivorous insects. Annu. Rev. Entomol. 47: 817–844.

Bailey, C. 1976. A quantitative study of consumption and utilization of various diets in the bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae). Can. Entomol. 108: 1319–1326.

Balbi, V., and A. Devoto. **2008**. Jasmonate signalling network in *Arabidopsis thaliana*: Crucial regulatory nodes and new physiological scenarios. New Phytol. 177: 301–318.

Baldwin, I. T. 1998. Jasmonate-induced responses are costly but benefit plants under attack in native populations. Proc. Natl. Acad. Sci. 95: 8113–8118.

Boggs, C. L. 1997. Reproductive allocation from reserves and income in butterfly species with differeing adult diets. Ecology. 78: 181–191.

Boggs, C. L., and C. L. Ross. **1993**. The effect of adult food limitation on life history traits in *Speyeria Mormonia* (Lepidoptera : Nymphalidae). Ecology. 74: 433–441.

Bostock, R. M. 2005. Signal crosstalk and induced resistance: Straddling the line between cost and benefit. Annu. Rev. Phytopathol. 43: 545–580.

Bracken, G. K. 1984. Within plant preferences of larvae of *Mamestra configurata* (Lepidoptera: Noctuidae) feeding on oilseed rape. Can. Entomol. 116: 45–49.

Buczacki, S. T., and C. M. Clay. **1984**. Some observations on secondary zoospore development in *Plasmodiophora brassicae*. Trans. Br. Mycol. Soc. 82: 339–342.

Canola Council of Canada. What is canola ? Retrived from https://www.canolacouncil.org/oil-and-meal/what-is-canola/.
(accessed 17 March 2018).

Canola Council of Canada. Insects. Retrived from https://www.canolacouncil.org/canola-encyclopedia/insects/
(accessed on 17 March 2018)

Canola Council of Canada. Diseases. Retrived from https://www.canolacouncil.org/canola-encyclopedia/diseases/
(accessed on 17 March 2018)

Canola digest. Clubroot resistance saves canola in Central Alberta. Retrived from
https://canoladigest.ca/science-edition-2017/clubroot-resistance-saves-canola-in-central-alberta/
(accessed on 17 March 2018)

Diederichsen, E., M. Frauen, E. G. A. Linders, K. Hatakeyama, and M. Hirai. **2009**. Status and perspectives of clubroot resistance breeding in crucifer crops. J. Plant Growth Regul.

28: 265–281.

Donald, E. C., G. Jaudzems, and I. J. Porter. **2008**. Pathology of cortical invasion by *Plasmodiophora brassicae* in clubroot resistant and susceptible *Brassica oleracea* hosts. Plant Pathol. 57: 201–209.

Donaldson, J. R., E. L. Kruger, and R. L. Lindroth. **2006**. Competition- and resource-mediated tradeoffs between growth and defensive chemistry in trembling aspen (Populus tremuloides). New Phytol. 169: 561–570.

Dosdall, L. M. 1994. Evidence for successful overwintering of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), in Alberta. Can. Entomol. 126: 183–185.

Dosdall, L. M., and B. J. Ulmer. **2004**. Feeding, development, and oviposition of bertha armyworm (Lepidoptera : Noctuidae) on different host plant species. Environ. Entomol. 33: 756–764.

Dosdall, L. M., K. N. Harker, J. T. O'Donovan, R. E. Blackshaw, H. R. Kutcher, Y. Gan, and E. N. Johnson. **2012**. Crop sequence effects on root maggot (Diptera: Anthomyiidae: Delia spp.) infestations in Canola. J. Econ. Entomol. 105: 1261–1267.

Doss, R. P., J. E. Oliver, W. M. Proebsting, S. W. Potter, S. Kuy, S. L. Clement, R. T. Williamson, J. R. Carney, and E. D. DeVilbiss. **2000**. Bruchins: Insect-derived plant regulators that stimulate neoplasm formation. Proc. Natl. Acad. Sci. 97: 6218–6223.

Erlandson, M. A. 2013. Biological Control Programmes in Canada 2001–2012, pp. 228–232. *In* Mason, P.G., Gillespie, D.R. (eds.), Biol. Control Program. Canada 2001–2012. CAB International, Wallingford, UK.

Evenden, M. L., R. E. Batallas, and C. Weeraddana. **2017**. Biology and management of the generalist herbivore, the bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), on canola in western Canada., pp. 114–129. *In* Reddy, G.V.P. (ed.), Integr. Manag. Insect Pests Canola Other Brassica Oilseed Crop. CABI, Boston, MA.

Farmer, E. E., and C. A. Ryan. **1992**. Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell. 4: 129–134.

Funke, T., H. Han, M. L. Healy-Fried, M. Fischer, and E. Schönbrunn. **2006**. Molecular basis for the herbicide resistance of Roundup Ready crops. Proc. Natl. Acad. Sci. 103: 13010–13015.

Furlong, M. J., D. J. Wright, and L. M. Dosdall. **2013**. Diamondback moth ecology and management: problems, progress, and prospects. Annu. Rev. Entomol. 58: 517–541.

Gerber, G. H., and M. A. Howladert. **1987**. the Effects of Photoperiod and Temperature on Calling Behaviour and Egg Development of the Bertha Armyworm , *Mamestra configurata* (Lepidoptera: Noctuidae) J. Insect Physiol. 33: 429–436.

Glazebrook, J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. 43: 205–227.

Golizadeh, A., K. Kamali, Y. Fathipour, and H. Abbasipour. **2007**. Temperature-dependent development of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) on two brassicaceous host plants. Insect Sci. 14: 309–316.

Grainnews. New canola varities for 2017. Retrived from https://www.grainews.ca/2016/11/08/new-canolas-for-2017/

(accessed on March 17, 2018)

Grant, C. A., D. A. Derksen, D. L. McLaren, and R. B. Irvine. **2011**. Nitrogen fertilizer and urease inhibitor effects on canola seed quality in a one-pass seeding and fertilizing system. F. Crop. Res. 121: 201–208.

Greenberg, J. T., A. Guo, D. F. Klessig, and F. M. Ausubel. **1994**. Programmed cell death in plants: A pathogen-triggered response activated coordinately with multiple defense functions. Cell. 77: 551–563.

Gripenberg, S., P. J. Mayhew, M. Parnell, and T. Roslin. **2010**. A meta-analysis of preference-performance relationships in phytophagous insects. Ecol. Lett. 13: 383–393.

Hakes, A. S., and J. T. Cronin. **2011**. Environmental heterogeneity and spatiotemporal variability in plant defense traits. Oikos. 120: 452–462.

Harcourt, D. G. 1957. Biology of the diamondback moth, *Plutella maculipennis* (Curt.), in eastern Ontario. 11. Life-history, behaviour, and host relationships. Can. Entomol. 89: 554–564.

Hennig, J., J. Malamy, G. Grynkiewicz, J. Indulski, and D. Klessig. **1993**. Interconversion of the salicylic acid signal and its glucoside in tobacco. Plant J. 4: 593–600.

Howe, G. A., and G. Jander. **2008**. Plant immunity to insect herbivores. Annu. Rev. Plant Biol. 59: 41–66.

Howlader, M. A., and G. Gerber. **1986**. Effects of age, egg development, and mating on calling bahaviour of the bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae). Can. Entomol. 118: 1221–1230.

Hwang, S. F., H. U. Ahmed, Q. Zhou, S. E. Strelkov, B. D. Gossen, G. Peng, and G. D. Turnbull. **2011**. Influence of cultivar resistance and inoculum density on root hair infection of canola (*Brassica napus*) by *Plasmodiophora brassicae*. Plant Pathol. 60: 820–829.

Ingram, D. S., and I. C. . Tommerup. **1972**. The life history of *Plasmodiophora brassicae* Woron. Proc. R. Soc. London. 180: 103–112.

Ishiguro, S., A. Kawai-Oda, J. Ueda, I. Nishida, and K. Okada. **2001**. The defective in anther dehiscence 1 gene encodes a novel Phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation, anther dehiscence, and flower opening in Arabidopsis. Plant Cell. 13: 2191–2209.

Jaenike, J. 1978. On optimal oviposition behavior in phytophagous insects. Theor. Popul. Biol. 14: 350–356.

Jeffries, M. J., and J. H. Lawton. **1984**. Enemy free space and the structure of ecological communities. Biol. J. Linn. Soc. 23: 269–286.

Jervis, M. A., and P. N. Ferns. **2004**. The timing of egg maturation in insects: Ovigeny index and initial egg load as measures of fitness and of resource allocation. Oikos. 107: 449–460.

Jeschke, V., E. E. Kearney, K. Schramm, G. Kunert, A. Shekhov, J. Gershenzon, and D. G. Vassão. **2017**. How glucosinolates affect generalist Lepidopteran larvae: growth, development and glucosinolate metabolism. Front. Plant Sci. 8: 1–12.

Kaloshian, I., and L. L. Walling. **2005**. Hemipterans as plant pathogens. Annu. Rev. Phytopathol. 43: 491–521.

Kaplan, I., R. Halitschke, A. Kessler, S. Sardanelli, and R. F. Denno. **2008**. Constitutive and induced defences to herbivory in above- and below plant tissues. Ecology. 89: 392–406.

Karamanos, R. E., T. B. Goh, and D. P. Poisson. **2005**. Nitrogen, phosphorus, and sulfur fertility of hybrid canola. J. Plant Nutr. 28: 1145–1161.

Kessler, A., and I. T. Baldwin. **2002**. Plant responses to insect herbivory: The emerging molecular analysis. Annu. Rev. Plant Biol. 53: 299–328.

Koo, Y. J., M. A. Kim, E. H. Kim, J. T. Song, C. Jung, J. K. Moon, J. H. Kim, H. S. Seo, S. I. Song, J. K. Kim, J. S. Lee, J. J. Cheong, and Y. Do Choi. **2007**. Overexpression of salicylic acid carboxyl methyltransferase reduces salicylic acid-mediated pathogen resistance in *Arabidopsis thaliana*. Plant Mol. Biol. 64: 1–15.

Koricheva, J., S. Larsson, and E. Haukioja. **1998**. Insect performance on experimentally stressed woody plants: a meta-analysis. Annu. Rev. Entomol. 43: 195–216.

Koricheva, J., S. Larsson, E. Haukioja, and M. Keinanen. **1998**. Regulation of woody plant secondary metabolism by resource availability : hypothesis testing by means of meta-analysis. Oikos. 83: 212–226.

Koshihara, T., and H. Yamada. **1981**. Female sex pheromone of the diamondback moth , *Plutella xylostella*. Japan Agric. Res. Q. 15: 22–28.

Larsson, S. 1989. Stressful times for the plant stress : Insect performance hypothesis. Oikos. 56: 277–283.

Little, D., C. Gouhier-Darimont, F. Bruessow, and P. Reymond. **2006**. Oviposition by *Pierid* butterflies triggers defense responses in Arabidopsis. Plant Physiol. 143: 784–800.

Macfarlane, I. 1970. Germination of resting spores of *Plasmodiophora brassicae*. Trans. Br. Mycol. Soc. 55: 97–112.

Madlung, A., and L. Comai. **2004**. The effect of stress on genome regulation and structure. Ann. Bot. 94: 481–495.

Mag, T. K. 1983. Canola oil processing in Canada. J. Am. Oil Chem. Soc. 60: 380–384.

Mainguet, A. M., A. Louveaux, G. El Sayed, and P. Rollin. **2000**. Ability of a generalist insect, *Schistocerca gregaria*, to overcome thioglucoside defense in desert plants: Tolerance or adaptation? Entomol. Exp. Appl. 94: 309–317.

Mangel, M. 1987. Oviposition site selection and clutch sizes in insects. J. Math. Biol. 25: 1–22.

Mason, P. G., A. P. Arthur, O. O. Olfert, and M. A. Erlandson. **1998**. The bertha armyworm (*Mamestra configurata*) (Lepidoptera: Noctuidae) in Western Canada. Can. Entomol. 130: 321–336.

Merritt, S. Z. 1996. Within-plant variation in concentrations of amino acids, sugar, and sinigrin in phloem sap of black mustard, *Brassica nigra* (L.) Koch (Cruciferae). J. Chem. Ecol. 22: 1133–1145.

Moreau, J., B. Benrey, and D. Thiéry. **2006**. Grape variety affects larval performance and also female reproductive performance of the European grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae). Bull. Entomol. Res. 96: 205–212.

Mur, L. A. J., P. Kenton, R. Atzorn, O. Miersch, and C. Wasternack. **2005**. The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. Plant Physiol. 140: 249–262.

Mur, L. A. J., P. Kenton, A. J. Lloyd, H. Ougham, and E. Prats. **2008**. The hypersensitive response; The centenary is upon us but how much do we know? J. Exp. Bot. 59: 501–520.

Pivnick, K. A., B. J. Jarvis, C. Gillott, G. P. Slater, and E. W. Underhill. **1990**. Daily patterns of reproductive activity and the influence of adult density and exposure to host plants on reproduction in the diamondback moth (Lepidoptera: Plutellidae). Environ. Entomol. 19: 587–593.

Rakow, G. 2000. Downey designer of canola. Plant Breed. Rev. 18: 1–12.

Rashid, M. M., M. Jahan, and K. S. Islam. **2016**. Impact of nitrogen, phosphorus and potassium on brown planthopper and tolerance of its host rice plants. Rice Sci. 23: 119–131.

Ratzka, A., H. Vogel, D. J. Kliebenstein, T. Mitchell-Olds, and J. Kroymann. **2002**. Disarming the mustard oil bomb. Proc. Natl. Acad. Sci. 99: 11223–11228.

Redovnikovic, I. R., T. Glivetic, K. Delonga, and J. Vorkapic-Furac. **2008**. Glucosinolates and their potential role in plant. Period. Biol. 110: 297–309.

Reed, D. W., K. A. Pivnick, and E. W. Underhill. **1989**. Identification of chemical imposition stimulants for the diamondback moth, *Plutella xylostella*, present in three species of Brassicaceae. Entomol. Exp. Appl. 53: 277–286.

Schmelz, E. a, M. J. Carroll, S. LeClere, S. M. Phipps, J. Meredith, P. S. Chourey, H. T. Alborn, and P. E. a Teal. **2006**. Fragments of ATP synthase mediate plant perception of insect attack. Proc. Natl. Acad. Sci. 103: 8894–8899.

Schweiger, R., A. M. Heise, M. Persicke, and C. Muller. **2014**. Interactions between the jasmonic and salicylic acid pathway modulate the plant metabolome and affect herbivores of different feeding types. Plant, Cell Environ. 37: 1574–1585.

Seo, H. S., J. T. Song, J. J. Cheong, Y. H. Lee, Y. W. Lee, I. Hwang, J. S. Lee, and Y. D. Choi. **2001**. Jasmonic acid carboxyl methyltransferase: a key enzyme for jasmonate-regulated plant responses. Proc. Natl. Acad. Sci. 98: 4788–93.

Shah, J. 2003. The salicylic acid loop in plant defense. Curr. Opin. Plant Biol. 6: 365–371.

Siemens, J., M. C. González, S. Wolf, C. Hofmann, S. Greiner, Y. Du, T. Rausch, T. Roitsch, and J. Ludwig-Müller. **2011**. Extracellular invertase is involved in the regulation of clubroot disease in *Arabidopsis thaliana*. Mol. Plant Pathol. 12: 247–262.

Smith, J. L., C. M. De Moraes, and M. C. Mescher. **2009**. Jasmonate- and salicylate-mediated plant defense responses to insect herbivores, pathogens and parasitic plants. Pest Manag. Sci. 65: 497–503.

Song, J. T. 2006. Induction of a salicylic acid glucosyltransferase, AtSGT1, is an early disease response in *Arabidopsis thaliana*. Mol Cells. 22: 233–238.

Spoel, S. H., J. S. Johnson, and X. Dong. **2007**. Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. Proc. Natl. Acad. Sci. 104: 18842–18847.

Staswick, P. E., and I. Tiryaki. **2004**. The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in Arabidopsis. Plant Cell. 16: 2117–2127.

Stamp, N. 2003. Out of the quagmire of plant defense hypotheses. Q. Rev. Biol. 78: 23–55.

Strelkov, S. E., and G. R. Dixon. **2014**. Clubroot (*Plasmodiophora brassicae*) on canola and other Brassica species – disease development, epidemiology and management. Can. J. Plant Pathol. 36: 1–4.

Strelkov, S. E., and S. F. Hwang. **2014**. Clubroot in the Canadian canola crop: 10 years into the outbreak. Can. J. Plant Pathol. 36: 27–36.

Strelkov, S. E., V. P. Manolii, T. Cao, S. Xue, and S. F. Hwang. **2007**. Pathotype classification of *Plasmodiophora brassicae* and its occurrence in *Brassica napus* in Alberta, Canada. J. Phytopathol. 155: 706–712.

Talekar, N. S., and A. M. Shelton. **1993**. Biology, ecology, and management of the diamonback moth. Annu. Rev. Entomol. 38: 275–301.

Tan, S., R. R. Evans, M. L. Dahmer, B. K. Singh, and D. L. Shaner. **2005**. Imidazolinone-tolerant crops: history, current status and future. Pest Manag. Sci. 61: 246–257.

Tewari, J. P., S. E. Strelkov, D. Orchard, M. Hartman, R. M. Lange, and T. K. Turkington. **2005**. Identification of clubroot of crucifers on canola (*Brassica napus*) in Alberta. Can. J. Plant Pathol. 27: 143–144.

Tommerup, I. C., and D. S. Ingram. **1971**. The life cycle of *Plasmodiphora brassicae* Woron. in tissue cultures and in intact roots. New Phytol.

Tuomi, J., T. Fagerstrom, and P. Niemela. 1991. Carbon allocation, phenotypic plasticity, and induced defenses. In: Phytochemical induction by herbivores (eds D.W. Tallamy and M.J. Raupp). John Wiley & Sons, New York, NY, U.S.A., pp. 85-104.

Turlings, T. C. J., J. H. Tumlinson, and W. J. Lewis. **1990**. Exploitation of herbivore-induced Plant odors by host-seeking parasitic wasps. Adv. Sci. 250: 1251–1253.

Turner, J. G., C. Ellis, and A. Devoto. **2002**. The jasmonate signal pathway. Plant Cell. 14 Suppl: S153–S164.

Turnock, W. J. 1987. Predicting larval abundance of the bertha armyworm, *Mamestra configurata* Wlk., in Manitoba from catches of male moths in sex attractant traps. Can. Entomol. 119: 167–178.

Ulmer, B., C. Gillott, and M. Erlandson. **2002**. Oviposition preferences of bertha armyworm *Mamestra configurata* Walker (Lepidoptera: Noctuidae) on different crucifer cultivars and growth stages. Environ. Entomol. 31: 1135–1141.

Ulmer, B., C. Gillott, and M. Erlandson. **2003**. Conspecific eggs and bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), oviposition site selection. Environ. Entomol. 32: 529–534.

Vlot, A. C., D. A. Dempsey, and D. F. Klessig. **2009**. Salicylic Acid, a multifaceted hormone to combat disease. Annu. Rev. Phytopathol. 47: 177–206.

Wallenhammar, A. C. 1996. Prevalence of *Plasmodiophora brassicae* in a spring oilseed rape growing area in central Sweden and factors influencing soil infestation levels. Plant Pathol. 45: 710–719.

Walling, L. L. 2008. Avoiding effective defenses: strategies employed by phloem-feeding insects. Plant Physiol. 146: 859–866.

Wasternack, C. 2007. Jasmonates: An update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann. Bot. 100: 681–697.

Wee, S. 2016. Effects of conspecific herbivory and mating status on host searching and oviposition behavior of *Plutella xylostella* (Lepidoptera: Plutellidae) in relation to its host, *Brassica oleracea* (brassicales: Brassicaceae). Florida Entomol. 99: 159–165.

White, T. C. R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. Oecologia. 63: 90–105.

Wylie, H. G., and G. E. Bucher. **1977**. The bertha armyworm, *Mamestra configurata* (Lepidotera: Noctuidae). Mortality of immature stages on the rape crop. Can. Entomol. 109: 823–837.

Zhang, P., G. Ma, C. Wang, H. Lu, S. Li, Y. Xie, D. Ma, Y. Zhu, and T. Guo. **2017**. Effect of irrigation and nitrogen application on grain amino acid composition and protein quality in winter wheat. PLoS One. 12: 1–15.

Chapter 2: Canola nutrition and variety affect oviposition and offspring fitness in the generalist herbivore, *Mamestra configurata* (Lepidoptera: Noctuidae)

Abstract

Bertha armyworm(BAW) *Mamestra configurata* Walker, is a generalist herbivore that feeds on several crops in different plant families. Canola *Brassica napus* L., is one of its more favored host plants making the bertha armyworm a significant insect pest in the Canadian Prairies. The performance of the bertha armyworm on canola may vary with the quality of the host plant. I tested the impact of plant nutrition and canola variety on oviposition and subsequent larval development of the BAW. A range of fertilization levels (0.0, 1.0, 3.0, 5.0 g/pot) were applied to three different canola varieties: '5535 CL'; '6060RR'; and 'Q2'. The total number of eggs laid on plants in a no-choice experiment increased with fertilizer level but a similar number of eggs was laid on plants that received moderate and high fertilizer treatments. Larvae reared on plants that received the moderate fertilizer application yielded heavier pupae than those reared on plants with low and high fertilizer treatments. Most eggs were laid on the Q2 variety when compared with '5535 CL' and '6060 RR'. Bertha armyworm females preferred to lay eggs on plants that received moderate or high fertilization over plants with the low fertilizer treatment. Plant leaf tissue nutrient content and plant growth were highest in plants that received moderate and high fertilizer treatments. I conclude that BAW oviposition behaviour and larval performance are influenced by plant nutrition and canola variety.

Introduction

The bertha armyworm (BAW) *Mamestra configurata* Walker (Lepidoptera: Noctuidae), is a generalist herbivore native to North America. Bertha armyworm is considered a serious pest of canola and rapeseed crops (*Brassica napus* L. and *Brassica rapa* L.), and is also known to infest flax (*Linum usitatissimum* L.), alfalfa (*Medicago sativa* L.) and other crops (Mason et al. 1998). Population outbreaks of BAW in the Prairie Provinces of Canada are economically costly as canola production contributes billions of dollars to the Canadian economy each year (http://www.canolacouncil.org). Bertha armyworm overwinter as pupae in the soil. After eclosion in early summer, moths mate and females locate suitable host plants for oviposition. Female oviposition decisions are critical because young larvae feed on or near the host plant on which they hatch (Ulmer et al. 2002) and do not disperse in search of other hosts until later in larval development if resources are limiting (Dosdall 2010). Larvae are primarily foliage feeders and at high population densities can cause complete defoliation of the host plant and damage to the developing pods (Bucher and Bracken 1976).

Control of BAW above threshold densities on the Canadian Prairies is primarily achieved with synthetic insecticides (Evenden et al. 2017), and this practice may have ecological consequences in agroecosystems (Mason et al. 1998). It is therefore important to develop environmentally safe and cost-effective pest management tactics to control BAW. Plant resistance has been explored as a potential cultural control tactic against the BAW. Despite the polyphagous feeding behaviour of BAW, it displays a host preference hierarchy both among and within plant species (Turnock 1985, Ulmer and Gillott 2001, Dosdall and Ulmer 2004), suggesting that plant traits could be managed to reduce the impact of BAW populations.

Several factors are known to impact the oviposition behaviour of this species. Bertha armyworm females show preference for different species of host plant within the family Brassicaceae (Ulmer et al. 2002) and among species in different plant families (Dosdall and Ulmer 2004). Female BAW prefer to oviposit near other conspecific egg masses and detection of these eggs appears to be chemically based (Ulmer et al. 2003). Females likely use olfaction to orient to oviposition hosts and differences in the volatile compounds released by hosts may be used by females in host selection. The foliar glucosinolate sinigrin, found in *Brassica juncea* (L.) Czern., which is resistant to BAW feeding (Ulmer and Gillott 2001), deters ovipositing female moths (Ulmer et al. 2002) White mustard *Sinapsis alba* L. (Brassicaceae) contains another foliar glucosinalate, sinalbin, which negatively affects larval BAW performance (Ulmer and Gillott 2001) but enhances oviposition by female moths (Ulmer et al. 2002).

Different canola varieties release different volatile organic compounds (Robertson et al. 1993) which may influence the oviposition preference of female BAW (Ulmer et al. 2002). Oviposition on canola plants is highest at full flower and egg laying is concentrated in the middle portion of the plant on the abaxial leaf surfaces (Ulmer et al. 2002). Exploitation of these preferences through the use of early or late flowering varieties may help to reduce BAW oviposition and subsequent larval feeding damage.

Bertha armyworm host use is also influenced by crop phenology. It is not known if plant nutrition influences oviposition decisions by bertha armyworm, but fertilization alters the volatile profile of canola mainly through a change in the quantity of compounds released (Veromann et al. 2013). In other systems, soil nutrients affect the oviposition behaviour of insect herbivores through plant-mediated interactions. For instance, the beet armyworm *Spodoptera exigua*

(Hübner) (Lepidoptera: Nocuidae) (Chen et al. 2008), the buckeye butterfly *Junonia coenia* Hübner (Lepidoptera: Nymphalidae) (Prudic et al. 2005), and the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Jauset et al. 1998), all prefer to lay eggs on fertilized over unfertilized plants. Leaf nitrogen levels in canola also positively increase with soil fertilization, and make plants not only more attractive, but also more nutritious for herbivores that specialize on plants in the Brassicaceae (Sarfraz et al. 2009).

The growth rate of juvenile insects is impacted by nutrients in the host plant. Insects that feed on plants with low nitrogen content can experience prolonged development (Chen et al. 2008), and may be more vulnerable to mortality by natural enemies (Loader and Damman 1991). Reduced plant growth due to low nitrogen availability can benefit plants by exacting mortality on associated herbivores in what is known as the 'slow growth-high mortality' hypothesis (Clancy and Price 1987). The impact of fertilizer application to canola on host use by the BAW is not known but damage caused by other canola feeders like the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is better tolerated by large plants (Sarfraz et al. 2009).

In this study, I compared oviposition behaviour and larval performance of the BAW on three different canola varieties exposed to varying levels of fertilization. Herbicide-resistant canola crops are popular in Canada due to effective weed control, favorable crop characteristics and other economic benefits (Beckie et al. 2006). It is generally believed that herbicide-resistance proteins do not influence insect performance (Sarfraz et al. 2007). Sufficient evidence does not exist, however, to compare insect performance on herbicide-resistant canola compared with other conventional canola varieties (Sarfraz et al. 2007). The canola varieties tested here included '5535 CL', '6060 RR', and a control variety, 'Q2'. In conventional breeding, two parents of the same variety are crossed to produced offspring with the desired characters while hybrid varieties are the result of crosses with different plant lines to produce offspring with desired characters. In contrast, genetically modified plants are produced through insertion of genes through genetic engineering (www.royalsociety.org). ‘5535 CL’ was developed using traditional breeding methods as a variety that is tolerant to imidazolinone herbicides (Tan et al. 2005). Early maturation of ‘5535 CL’ allows the crop to flower earlier and to benefit from the cool humid environment to improve yield (Phillips 2003). The variety ‘6060 RR’ is a genetically modified canola variety that is resistant to the herbicide, glyphosate (Funke et al. 2006). This variety is late maturing and has delayed flowering which may impact its susceptibility to insect herbivory. Lastly, ‘Q2’, is an old canola variety that was well-adapted to agroecosystems in western Canada. Characteristics of ‘Q2’ include high seed yield, resistance to blackleg disease, reduced susceptibility to lodging compared with other varieties (Stringam et al. 1999). Despite its suitability for the Prairie environment, ‘Q2’ is not commonly used in commercial crop production but is often used as an experimental standard for comparison with other canola varieties (Sarfraz et al. 2007). In the current study, I examine the effect of fertilization and canola variety on oviposition and offspring fitness of the BAW. I assess the effect of these variables on plant growth, plant tissue nutrient content, and how these factors impact BAW oviposition and offspring fitness. This study provides insights to understand the ecological interactions involved in host plant selection and nutrient utilization of the BAW.

Materials and Methods

Insects

Insects were originally obtained from an established BAW colony at Agriculture and Agri-food Canada, Saskatoon Research Centre.   Insects were maintained in a growth chamber at 21°C with a 16L:8D photoperiod on an artificial diet (Bucher and Bracken 1976). Bertha armyworm eggs were transferred to the artificial diet and larvae were not exposed to plant material until adult emergence. Pupae were separated by sex and placed into 500 ml transparent plastic containers with screen lids until adult emergence. Moths were collected within one day of eclosion and individual pairs of adults were placed in separate 500 mL transparent plastic containers with screen lids for two days to ensure mating prior to the start of the experiments. Five percent (weight/volume) honey and sugar solution was provided through a dental wick for adult feeding. After the 48 h mating-period, adults were released into experimental wooden (39.5 X 39.5 X 80.5 cm) or nylon mesh cages (47.5 X 47.5 X 93.0 cm) containing canola for three to six days depending on the experiment.

Host plants and fertilization treatment

Seeds of three varieties of Canola: ‘Q2’ (University of Alberta), ˋ5535 CL’(DL Seeds, Morden research station, MB, Canada) and ˋ6060 RR’ (DL Seeds, Morden research station, MB, Canada) were sown into 15.3 cm-diameter plastic pots filled with Sunshine potting mix-4 (Sun Gro horticulture Canada Ltd, Alberta, Canada). Plants were grown at 21°C under growth lights to provide a 16L:8D photoperiod in a growth room. The light intensity at the plant level ranged from 20000 to 23,000 lux. Canola plants were watered daily. One of four fertilizer treatments: 0, 1.0, 3.0, 5.0 g/pot of 20:20:20 (nitrogen: phosphorus: potassium (NPK); Plant products Co, Ltd., Brampton, Ontario, Canada) was applied to plants over a six weeks period. Soil fertilizer was added according to a protocol described by (Sarfraz et al. 2009) with modification. Fertilizer treatments were applied starting when the plants were three weeks old and fertilization was conducted three times, two weeks apart to avoid phytotoxicity. Plants were used in experiments at the flowering stage as BAW lay more eggs on flowering canola plants than on plants in the pre-flowering or pod stages (Ulmer et al. 2002).

Oviposition no-choice experiment

A no-choice experiment was conducted using three different canola varieties (Clearfield 5535 CL, Roundup Ready 6060 RR and 'Q2') (n=9 per treatment). Plants of each variety were treated with one of four fertilizer levels (20-20-20 NPK: 0, 1.0, 3.0, 5.0 g/pot). Three pairs of mated moths were released into wooden cages (39.5 X 39.5 X 80.5 cm) containing a single canola plant. Each moths were exposed to the plants for three days and then plants were replaced with another similarly treated canola plant for an additional three days of oviposition in the field adult female BAW mate multiple times (Turnock 1985) and ~75 % of eggs are laid between the 2nd-7th scotophase after emergence (Howlader and Gerber 1986). Here no-choice oviposition experiment lasted until the 6th scotophase to allow moths to lay a maximum number of eggs on plants. Throughout the experiment, a 5% (weight/volume) honey and sugar water solution was provided to moths and refreshed at 48 h intervals. The number of eggs laid on each plant, the number of leaves per plant and the plant fresh weight were assessed at the end of the no-choice oviposition experiment.

Plant leaf tissue was collected from the variously treated plants three days after oviposition in the no-choice experiment. Leaves for elemental analysis were selected without eggs. Leaves were dried at 35°C and ground into a fine powder for tissue nutrient analysis (n=3 per treatment). The total nitrogen content was measured using the dry combustion method (AOAC 2000) with a Costech Model EA 4010 elemental analyser (Florence, Italy). Phosphorus, potassium, magnesium, sodium and sulphur were analysed using a Thermo iCAP6300 Duo inductively coupled plasma-optical emission spectrometer (ICP-OES; Thermo Fisher Corp, Cambridge, United Kingdom (Skoog et al. 2007). Tissue analyses were conducted in the analytical service laboratory at the University of Alberta, Edmonton, Canada.

Oviposition choice experiment

A choice experiment was conducted using the 'Q2' variety only treated with one of three fertilizer levels (20-20-20 NPK: 1.0, 3.0, 5.0 g/pot) (n=15). Plants not treated with fertilizer were not used for this experiment as non-fertilized plants were too small as compared to other treatments. Five pairs of mated adult moths were introduced into 47.5 X 47.5 X 93.0 cm nylon mesh cages (model BD44590F; MegaView Science Co., Ltd., Taichung, Taiwan) containing three canola plants, each plant was treated with a different level of fertilizer. Moths remained in the cage for three days when plants were harvested and the total number of eggs was counted.

Offspring development

A third experiment was conducted to measure larval development on the different canola varieties ('5535 CL', '6060 RR' and 'Q2') variously treated with one of the three fertilizer levels

(20-20-20 NPK: 1.0, 3.0, 5.0 g/pot) (n=10 per treatment). A pair of third instar larvae was introduced to each plant and left to develop until pupation. Larval weight was measured before they were introduced to the plants and 7 days after larval introduction. Pupal weight and adult weight were measured with a microbalance to the nearest 0.01 mg (Mettler Toledo XS105, Ontario, Canada). Insects were checked daily, and pupae were collected within two days of pupation at which point mass was recorded. Each pupa was kept in separate containers until eclosion to adults. Adult emergence was recorded daily and moths remained in the cage without access to a 5 % honey and sugar solution until they died. Adult weight and longevity were recorded upon the death of each moth. Adult wings were carefully removed and glued onto paper to measure wing area as an alternative measure of body size using a desktop scanner with Image J software (Sarfraz et al. 2009).

Statistical Analyses

All analyses were conducted using R version 3.4.0 (2017-04-21) (R Core Team). Data transformations were used to achieve normality and homoscedasticity when necessary. Untransformed means are used for the figures and tables. All the results; F and p values are presented using the most parsimonious models.

Oviposition No-choice Experiment

The total number of eggs laid on each plant was the dependent variable in a linear mixed effects model. Fertilizer treatment and canola variety and their interaction were considered as a fixed factors, and time (block) was considered as a random factor. The total egg number was transformed with Y^(0.5) to achieve normality and homoscedasticity. Means were compared using LS means with Tukey HSD post-hoc comparisons.

The total number of leaves and plant fresh weight were the dependent variables used in each of two linear mixed effects models. Fertilizer treatments and canola varieties and their interaction were specified as fixed factors and time (block) was considered as a random factor. Plant fresh weight was transformed to Y^(0.5) and leaf number was transformed to Y^(0.2) to achieve normality and homoscedasticity. Means were compared using LS means with Tukey HSD post-hoc comparisons. Plant tissue nutrient data were compared among the different canola varieties and fertilizer treatments. The level of each nutrient was analyzed in separate models. Data were transformed to Y^(0.5) in the total nitrogen, calcium and magnesium analyses. Log transformation was used in the sulphur analyses to achieve normality and homoscedasticity. Explanatory variables included canola variety, fertilizer treatment and their interaction. Differences in treatments were determined in two-way ANOVA analyses. Means were compared using LS means with Tukey HSD post-hoc comparisons.

Oviposition Choice Experiment

The proportion of the total eggs laid on each plant per cage was used for the analyses with a linear mixed effects model. Fertilizer treatment was considered as a fixed effect and cage was treated as a random factor. The proportion of eggs laid on each plant was specified as the dependent variable. Data were transformed to Y^(0.5) to achieve normality and homoscedasticity. Means were compared using LS means with Tukey HSD post-hoc comparisons.

Offspring Development

Larval weight was measured 7 days after introduction to experimental cages. Larval weight between the two larvae in each cage was averaged within each cage for the analyses. The average weight of the larvae in each experimental cage was treated as the dependent variable in a linear model. Fixed factors were fertilizer treatments, canola varieties and their interaction.

Pupal weight, adult weight and adult wing area were treated as dependent variables in separate linear mixed effects models. Fixed factors were fertilizer treatments, canola varieties and sex. Experimental cage was treated as a random factor. The statistical model included the dependent variable, fixed factors, random factors and interactions between all fixed factors. Data were transformed to Y^(2.5) in pupal weight and Y^(1.5) in adult weight analyses. Adult longevity was treated as the dependent variable in a generalized linear mixed effect model specifying a Poisson error distribution. Fixed factors were fertilizer treatments, canola varieties and sex. Experimental cages were treated as the random factor.

Results

Oviposition No-choice Experiment

The total number of eggs laid on plants in the no-choice experiment was influenced by a significant interaction between fertilizer treatment and canola variety ($\chi^2 = 19.220$, $P = 0.0038$). More eggs were laid on fertilized than unfertilized plants but a similar number of eggs was laid on plants that received moderate and high fertilizer treatments. Most eggs were laid on the 'Q2' variety. The greatest number of eggs was laid on the 'Q2' and '5535 CL' varieties at 3.0 g/pot fertilization application and on the '6060 RR' variety at 5.0 g/pot fertilization application. The egg number were lowest on all varieties without fertilizer application (Figure 2-1.).

In the no-choice oviposition experiment, the fertilization treatments impacted plant fresh weight ($\chi^2 = 757.26$, $P < 0.0001$) (Fig. 2-2A) and the total number of leaves ($\chi^2 = 283.594$, $P < 0.0001$) (Figure 2-2B). There was a significant effect of canola variety on the total number of leaves ($\chi^2 = 32.236$, $P < 0.0001$), as 'Q2' plants had more leaves than the '6060 RR' and '5535 CL' varieties.

Leaf nitrogen content increased with fertilizer rate ($F_{3,30} = 297.696$, $P < 0.0001$) (Table 2-1). Leaf nitrogen content was not influenced by canola variety ($F_{2,30} = 0.789$, $P = 0.4640$). Leaf phosphorus content increased with fertilizer rate until 3.0 g/pot after which it plateaued and was equal in both 3.0 and 5.0 g/pot treatments ($F_{3,32} = 66.833$, $P < 0.0001$) (Table 2-1). Leaf potassium content was affected by both fertilizer treatment ($F_{3,30} = 69.52$, $P < 0.0001$) and canola variety ($F_{2,30} = 19.33$, $P < 0.0001$) (Table 2-1). Similar to phosphorous, potassium content increased until 3.0 g/pot fertilizer rate and plateaued at the 5.0 g/pot fertilizer treatment. The 'Q2' variety had the highest potassium content whereas the Roundup Ready 6060 RR variety had the lowest. Leaf calcium content was affected by fertilizer treatment ($F_{3,30} = 33.882$, $P < 0.0001$) and canola variety ($F_{2,30} = 4.915$, $P < 0.0001$) (Table 2-1). The highest calcium level was found in plants treated with 1.0 g/pot and lowest in plants treated with 5.0 g/pot fertilization applications (Table 2-1). Both the 'Q2' and the '6060 RR' varieties had higher calcium content than the Clearfield variety (Table 2-1). Leaf magnesium content was affected by fertilizer treatment ($F_{3,30} = 29.901$, $P < 0.0001$) and canola variety ($F_{2,30} = 8.428$, $P = 0.0012$) (Table 2-1). The highest magnesium content occurred in plants treated with fertilizer at 1.0 g/pot and the lowest in plants treated with 5.0 g/pot (Table 2-1). Both the ‘Q2’ and ‘6060 RR’ varieties had higher magnesium content than the ‘5535 CL’ (Table 2-1).

There was a significant interaction between fertilizer treatment and canola variety ($F_{6,24} = 2.717$, $P = 0.0370$) that affected the leaf sulphur content. In the ‘Q2’ variety, the highest sulphur levels occurred when plants were fertilized at the low level of 1.0 g/pot. The unfertilized ‘Q2’ plants and those fertilized at the higher rates of 3.0 and 5.0 g/pot had low leaf sulphur levels (Table 2-1). In the ‘5535 CL’, the highest sulphur levels also occurred in plants fertilized with 1.0 g/pot but low sulphur levels were restricted to unfertilized plants (Table 2-1). The highest sulphur levels were observed in 1.0 g/pot whereas the lowest levels were in observed in 5.0 g/pot fertilization treatments in ‘6060 RR’ (Table 2-1).

Oviposition choice experiment

The effect of fertilization on oviposition choice was tested using only the ‘Q2’ variety at three levels of fertilization (1.0, 3.0, and 5.0 g/pot). Fertilizer treatment had a significant effect on the oviposition preference of BAW ($\chi^2 = 42.25$, $P < 0.0001$). Bertha armyworm adult females laid more eggs on plants treated with 3.0 and 5.0 g of fertilizer/pot over those treated with1.0 g of fertilizer/pot (Figure 2-3).

Offspring Development

Larval weight at day 7 after introduction into experimental cages was not affected by fertilizer treatment ($F_{2,86} = 1.0623$, $P = 0.3501$) or canola variety ($F_{2,86} = 0.7209$, $P = 0.4892$). Larvae reared on plants treated with the moderate fertilizer (3.0 g/pot) rate yielded heavier pupae than those reared on plants with low and high fertilizer regimes (Figure 2-4). Pupal weight was affected by fertilizer treatment ($\chi^2 = 11.701$, $P = 0.0028$) and sex ($\chi^2 = 95.351$, $P < 0.0001$). Pupal weight was not influenced by the canola variety that larvae were reared on ($\chi^2 = 1.6136$, $P = 0.4462$). Female pupae were heavier than males. Adult weight was affected by the canola variety ($\chi^2 = 28.7843$, $P < 0.0001$) that larvae were reared on and moth sex ($\chi^2 = 4.0989$, $P = 0.0429$). Heavier adults emerged when larvae were reared on 'Q2' and '5535 CL' as compared to '6060 RR'. Adult weight was not influenced by the fertilizer treatments ($\chi^2 = 0.8022$, $P = 0.6696$). Female adults were heavier than males. The total wing area was not affected by the fertilizer treatment ($\chi^2 = 5.3592$, $P = 0.0686$). The total wing area was affected by the canola variety ($\chi^2 = 11.9251$, $P = 0.0026$) that larvae were reared on and moth sex ($\chi^2 = 36.1956$, $P < 0.0001$). Moths had a larger wing area when reared as larvae on '6060 RR' as compared to 'Q2' and '5535 CL'. Females had a larger wing area as compared to males. Adult moth longevity was not affected by fertilizer treatment ($\chi^2 = 2.2026$, $P = 0.3324$) or the canola variety ($\chi^2 = 1.3623$, $P = 0.5060$), Sex was a determinant of longevity, adult female moths lived longer than males ($\chi^2 = 25.518$, $P < 0.0001$).

Discussion

This study explores the effect of fertilizer treatment and canola variety on host suitability to the generalist herbivore, the BAW, as measured by eggs laid and offspring fitness. The mechanisms underlying these effects were assessed through measures of plant growth and leaf tissue nutrient content. Bertha armyworm preferred to lay eggs and offspring performed better on plants treated with moderate or high fertilizer rates. These plants had higher levels of nitrogen, phosphorous and potassium in the leaves and higher growth rates than unfertilized plants.

When confined to a single plant in the no-choice experiment, BAW female moths laid more eggs on plants that received moderate or high fertilizer regimes than on plants that received a low rate of fertilizer or none at all. A similar oviposition response to fertilized canola occurs in the *Brassica* specialist, *P. xylostella* (Sarfraz et al. 2009). The cabbage white butterfly *Pieris rapae* (L.) (Lepidoptera: Pieridae) lays more eggs on fertilized *Brassica oleracea* L. plants (Myers 1985, Hsu et al. 2009). In the current study, plants grown under the moderate to high fertilization regimes had greater biomass and more leaves which may have provided additional oviposition sites for BAW. The majority of BAW eggs are laid on the underside of leaves (Ulmer et al. 2002). Although I did not count flower numbers, flower number increases with fertilization rate in canola (Veromann et al. 2013) and bertha armyworm prefers to oviposit on plants in bloom (Ulmer et al. 2002). Fertilization increases stem size and root biomass in canola (Sarfraz et al. 2009) as well as seed yield (Brennan et al. 2000). Therefore, fertilized canola plants may attract and support more herbivores but still exhibit increased growth and yield.

Fertilized plants also had a higher concentration of most of the nutrients tested, which may have influenced oviposition choice if volatile emissions from the plants reflect internal nutrient content. Although the response of BAW to host volatiles was not tested here, volatile organic compounds (VOCs) such as green leaf volatiles, monoterpenes, methyl salicylate and acetic acid are related to host attraction in other canola-feeding insects (Veromann et al. 2013). Fertilizer treatment did not affect all plant tissue nutrients in the same manner. Hybrid canola is known to absorb more nutrients, especially nitrogen, as compared with traditional varieties and therefore has higher yield (Smith et al. 2010). In this study, however, I did not find an influence of nitrogen on canola varities. Leaf tissue nitrogen, phosphorous, and potassium levels increased in response to fertilizer treatment but only nitrogen content responded to the highest fertilization regime. There was no clear oviposition pattern associated with micronutrient content of the plant. Similarly, feeding damage from *P. xylostella* on canola is not impacted by micronutrient content (Sarfraz et al. 2009). It is likely that the fertilization regime employed in our study may also increase the need for more micronutrients by the plant (Mortvedt 1985). Further testing with micronutrient amendments in addition to nitrogen, phosphorous and potassium fertilization is appropriate. Sulfur is a key element of the glucosinolate-myrosinase defense system in Brassicaceae plants (Halkier and Gershenzon 2006). Sulphur content might be expected to influence the oviposition behaviour of specialist herbivores because specialists are often cued into respond to distinct VOCs released by specific host plants (Cornell and Hawkins 2003). Bertha armyworm is a generalist herbivore, and I did not see a clear oviposition pattern associated with sulphur content of the plant.

Bertha armyworm preferred to lay eggs on ‘Q2’ plants treated with moderate or high fertilization over those subjected to the low fertilizer rate, when provided with a choice. The generalist herbivore, the beet armyworm *S. exigua* also prefers to lay eggs on fertilized cotton plants (Chen et al. 2008). Both *Pieris rapae crucivora* (Boisduval) and *Pieris canidia canidia* (L.) (Lepidoptera: Pieridae) prefer to oviposit on fertilized cabbage plants over unfertilized plants (Chen et al. 2004). Fertilization of canola affects its volatile profile through an increase in the quantity of compounds released (Veromann et al. 2013). It is likely that BAW uses olfaction, at least in part, in the host selection process as they can detect chemical cues for assessment of the presence of conspecific eggs on host plants (Ulmer et al. 2003). Host plant location and the close range behaviours of acceptance can depend on a variety of cues including visual (Masante-Roca et al. 2007), olfactory (Tasin et al. 2007), gustatory (Amrein and Thorne 2005) and tactile (Rojas et al. 2003) cues. The size of the 'Q2' canola plants in our study increased with fertilization and may have enhanced both visual and olfactory assessment of the host. Increased production of host plant volatiles enhances the contact stimulants, increases landing and oviposition rates of the swallowtail butterfly *Papilio polyxenes* Fabricius (Lepidoptera: Papilionidae) (Feeny et al. 1989). Plant size is known to alter the amount of volatile organic compounds released by plants (Tasin et al. 2007).

Oviposition of BAW was not only affected by fertilizer treatment but also by the variety of canola. Among the canola varieties tested, 'Q2' plants received the highest number of eggs. These differences could be due to different plant morphology, tissue nutrients, secondary plant compounds in plant tissue and volatiles released from the different canola varieties. The 'Q2' variety, however, had more leaves than the '6060 RR' and '5535 CL' varieties. As BAW lay eggs mostly on the undersides of leaves (Ulmer et al. 2002), our findings suggest that leaf surface area is not a limiting factor in BAW oviposition. Oviposition by other pests of *Brassica* can be partially influenced by the number of leaves on the host plant (Badenes-Perez et al. 2005).

The canola varieties I tested may have different levels of foliar glucosinolates that could impact the choice of BAW females if the volatile organic compounds released by plants differ by variety. Bertha armyworm females avoid ovipositing on *B. juncea* plants that have high foliar levels of sinigrin (Ulmer et al. 2002). In contrast, a different foliar glucosinolate, sinalbin, enhances oviposition in BAW on *S. alba* (Ulmer et al. 2002) . This suggests that female BAW make decisions about oviposition on *Brassica* plants based on the glucosinolate profile. *Brassica* varieties have varying levels of glucosinolates (Velasco et al. 2008) and express different VOC profiles (Wright et al. 2002) which may affect attraction or deterrence of BAW females. There is not any information available about the glucosinolate content in the canola varieties that I tested in this research. Bertha armyworm females prefer to oviposit on canola in full bloom (Ulmer et al. 2002). My experiment did not test the effect of plant phenological stage on oviposition, as all experiments were conducted while plants were in the flowering stage. The varieties tested, however, included early and late blooming varieties that may receive different oviposition pressure in the field based on phenological matching of BAW moths with the preferred flowering stage of canola (Ulmer et al. 2002). Oviposition preference studies using these varieties should be conducted under field conditions in the future.

Larval weight measured 7 days after feeding on the variously treated canola plants did not differ with fertilizer treatment or canola variety. Bertha armyworm reared as larvae on moderately fertilized plants developed into heavier pupae than those reared on plants with the low or high fertilizer treatment. There was no effect, however, of canola variety on pupal weight. Pupal weight is mostly dependent on stored food reserves obtained from larval food sources (Harvey and Gols 2011). Proteins, oil content, chlorophyll and glucosinolate content increase with nitrogen fertilization in canola (Grant et al. 2011). As BAW larvae are deterred from feeding on plants containing sinigrin (Shields and Mitchell 1995), I assume that primary metabolites increase with fertilization and better larval nutrition is reflected by pupal weight. Interestingly, pupal weight of BAW peaks when larvae are reared on plants treated with the moderate fertilization regime of 3.0 g/pot, which also results in the maximum content of phosphorous and potassium in leaf tissue. Phosphorous is involved in nucleic acid and protein synthesis (Sterner and Elser 2002) and impacts fitness in chewing insect herbivores (Waring and Cobb 1992). Potassium is involved in distribution of primary metabolites in plants (Amtmann et al. 2008). High levels of potassium in soybean enhances the yield and also results in heavier soybean looper *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae) pupae (Chen 2014). The moderate and high fertilization treatments tested here increased canola foliar nitrogen, potassium and phosphorous compared with the low fertilization treatment. These treatments enhanced plant growth, but insect performance only responded to the moderate fertilization treatment. Plant fertilization impacts insect herbivores in many systems. Heavier *P. xylostella* pupae were reared from canola plants treated with moderate fertilizer application (Sarfraz et al. 2009). High nitrogen content in corn increases the fecundity of the corn plant hopper *Peregrinus maidis* (Ashmead) (Hemiptera: Delphacinae) (Wang et al. 2006). Nitrogen fertilizer application to rice plants increases nitrogen content in the plant tissue as well as in the herbivore, the brown planthopper *Nilaparvata lugens* (Stål), (Hemiptera: Delphacidae) that attacks rice (Rashid et al. 2016).

As has been illustrated in other studies (Geoffrey and Turnock 1979), I found female BAW pupae were larger than males. Bertha armyworm adult females also live longer than males as occurs in other noctuids (Kazimírová 1996, Montezano et al. 2014). As I found an effect of plant fertilization regime on pupal weight, I anticipated that the body size of adult moths would also respond to the fertilization regime. There was no effect, however, of plant fertilization treatment on bertha armyworm adult weight or wing size. Heavier adults did result from larvae reared on ‘Q2’ and ‘5535 CL’ as compared with the ‘6060 RR’ canola variety. Larger body size mostly correlated with the high potential of reproductive success in many insects (Honěk 1993). In contrast, the wings of adults reared as larvae on '6060 RR' were the largest. Larger wing size is associated with higher dispersal in insects (Stevens et al. 2012). This evidence suggests that BAW has the ability to variably allocate resources to reproduction or dispersal.

Canola yield is affected by crop density and fertilizer application as well as nutrient availability in the soil. In the Canadian Prairies, a plant density of 50 - 100 plants/m^2 (http://www.canolacouncil.org) and nitrogen treatment of 168 - 196 kg/hectare improves yield in canola crops (http://www.canolawatch.org). This allows each plant to receive nitrogen levels of 337 – 395 mg when fields are planted with a density of 50 canola plants/ m^2. In our experiments, actual nitrogen represents only 20% of NPK fertilizer mixture, therefore, NPK application of 1.7-2.0 g/pot would represent the actual field nitrogen application to canola crops. As I found that 3-5 g/pot NPK fertilizer treatment benefits the bertha armyworm, the currently recommended level of nitrogen fertilizer in the field would not be expected to favour bertha armyworm populations.

In conclusion, fertilization of canola plants at the moderate and high levels tested here resulted in plants with higher foliar nitrogen, phosphorus, and potassium content. Fertilization impacted the plant growth characteristics measured here. Perhaps for these reasons, BAW female moths prefer to oviposit on moderate and highly fertilized plants. Larvae performed better on plants treated with moderate levels of fertilizer, as measured by pupal weight. My study demonstrates how plant nutrition and canola varieties affect plant and herbivore performance and suggest that BAW female moths can detect differences in plant quality. These findings will be useful for the refinement of Integrated Pest Management of BAW in canola agroecosystems.

Table 2-1. Mean (± S.E) leaf tissue nutrients in different canola varieties grown in soil that received different fertilizer treatments. Tissues were collected 3 days after the oviposition no-choice experiment

Leaf tissue nutrients (%)		Fertilizer treatment (g/pot)				Fertilizer		Variety		Fertilizer * variety	
		0.0	1.0	3.0	5.0	$F_{3,30}$	P	$F_{2,30}$	P	F	P
Nitrogen	‘5535 CL’	0.63 ± 0.05	3.71 ± 0.37	7.35 ± 0.09	10.2 ± 0.82	297.696	< 0.0001	0.789	0.4640	1.9460	0.1141
	‘6060 RR’	0.77 ± 0.06	3.44 ± 0.26	6.07 ± 0.56	9.71 ± 1.12						
	‘Q2’	0.84 ± 0.11	3.64 ± 0.08	7.90 ± 0.45	8.63 ± 0.39						
Phosphorus	‘5535 CL’	0.33 ± 0.01	0.9 ± 0.12	1.27 ± 0.04	1.17 ± 0.09	71.607	< 0.0001	2.143	0.1350	1.049	0.4200
	‘6060 RR’	0.32 ± 0.03	0.72 ± 0.06	1.20 ± 0.02	1.13 ± 0.06						
	‘Q2’	0.51 ± 0.03	0.93 ± 0.11	1.35 ± 0.16	1.04 ± 0.06						
Potassium	‘5535 CL’	1.06 ± 0.11	2.48 ± 0.23	3.96 ± 0.09	3.77 ± 0.35	69.52	< 0.0001	19.33	< 0.0001	1.245	0.3190
	‘6060 RR’	0.93 ± 0.04	1.63 ± 0.20	2.87 ± 0.40	3.22 ± 0.20						
	‘Q2’	1.64 ± 0.28	2.54 ± 0.25	4.42 ± 0.14	4.78 ± 0.47						
Sulphur	‘5535 CL’	0.89 ± 0.07	2.04 ± 0.18	1.33 ± 0.05	1.16 ± 0.05	34.124	< 0.0001	10.19	< 0.0001	2.717	0.0370
	‘6060 RR’	1.13 ± 0.06	1.52 ± 0.19	1.13 ± 0.07	1.07 ± 0.12						
	‘Q2’	1.21 ± 0.12	2.60 ± 0.14	1.38 ± 0.11	1.31 ± 0.11						
Calcium	‘5535 CL’	1.09 ± 0.08	2.18 ± 0.19	1.10 ± 0.02	0.77 ± 0.07	33.882	< 0.0001	4.915	0.0142	1.980	0.1084
	‘6060 RR’	1.74 ± 0.17	2.32 ± 0.35	1.61 ± 0.13	0.82 ± 0.13						

	‘Q2’	1.41 ± 0.06	2.83 ± 0.25	1.20 ± 0.09	1.19 ± 0.28						
Magnesium	‘5535 CL’	0.40 ± 0.02	0.69 ± 0.09	0.35 ± 0.00	0.25 ± 0.02	29.901	< 0.0001	8.428	0.0013	1.136	0.3722
	‘6060 RR’	0.61 ± 0.10	0.69 ± 0.08	0.49 ± 0.02	0.32 ± 0.04						
	‘Q2’	0.57 ±0.02	0.88 ± 0.06	0.44 ± 0.07	0.42 ± 0.08						

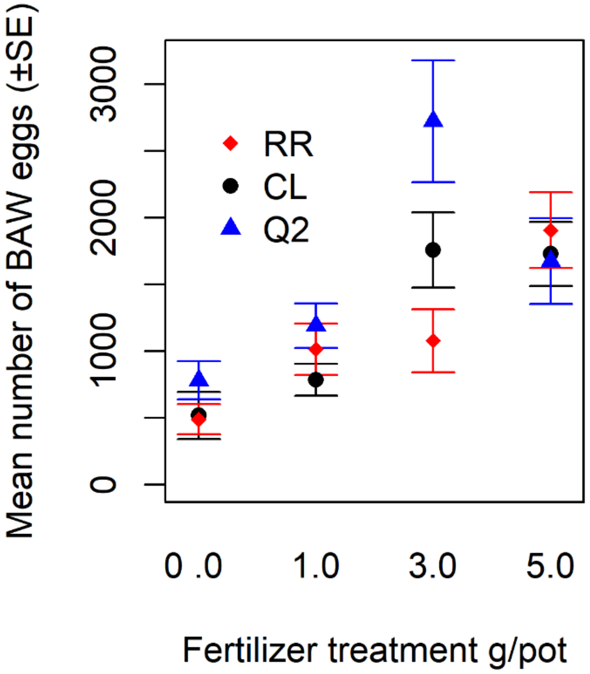


Figure 2-1. The interaction plot indicating the effect of canola variety and fertilization treatment on eggs laid by BAW in the no choice experiment (n=9). Eggs were counted on each plant after six days of oviposition period. Data were analyzed using a linear mixed model (total number of eggs*fertilizer: P = 0.0066). '6060 RR' represents as RR and '5535 CL' represent as CL in the figure.

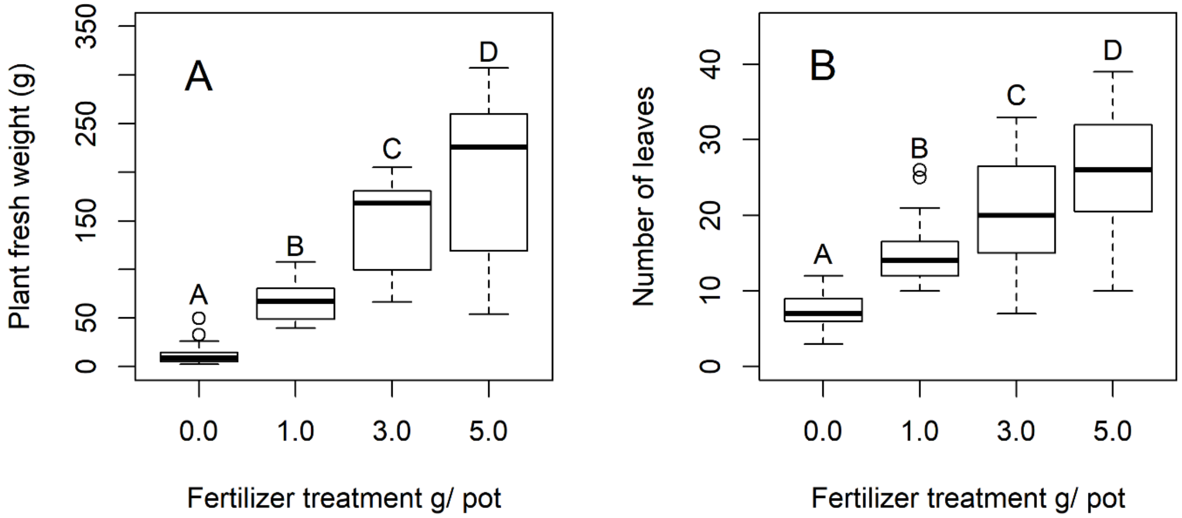


Figure 2-2. The effect of fertilizer treatments on plant fresh weight (A) and leaf number (B) per canola plant (n=9). The midline indicates the median. The bottom and top of the box area indicate the first and third quartiles, respectively. The vertical lines indicate the variability outside the first and third quartiles. Extreme values are indicated as open circles. Fresh plant weight and leaf number were measured three days after moths were introduced into cages in the no-choice experiment. Data were analyzed using a linear mixed model. Bars marked with the different letters are significantly different in figure (A) $P < 0.0001$ and (B) $P < 0.0001$ using LS means with Tukey HSD post-hoc comparisons.

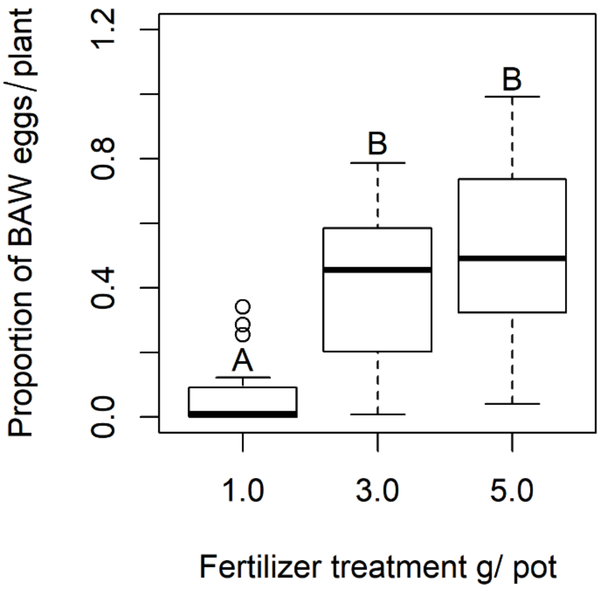


Figure 2-3. The proportion of BAW eggs laid on low (1.0 g/pot), medium (3.0 g/pot) and high fertilized (‘Q2’) plants (5.0 g/pot) in the choice experiment (n=15). The midline indicates the median. The bottom and top of the box area indicate the first and third quartiles, respectively. The vertical lines indicate the variability outside the first and third quartiles. Extreme values are indicated as open circles. Eggs were counted on each plant after three days of oviposition. Data were analyzed using a linear mixed effects model. Bars marked with the different letters are significantly different (LS means Tukey adjusted comparisons) ($P < 0.0001$).

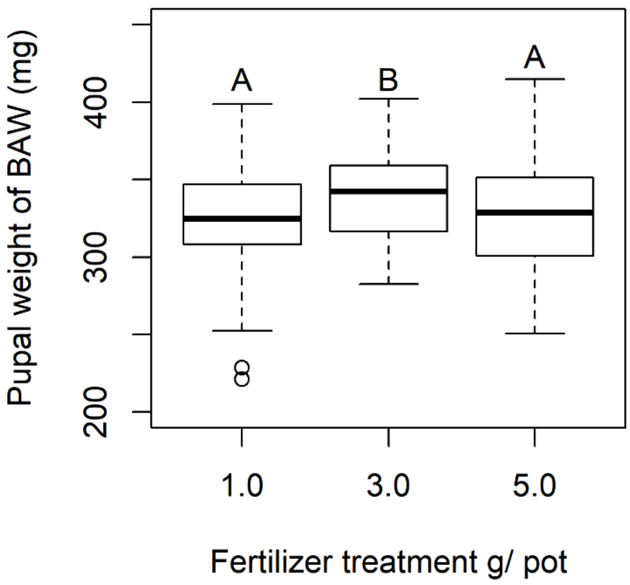


Figure 2-4. Boxplots indicating the effect of fertilizer treatments on the pupal weight of BAW (n=10). The midline indicates the median. The bottom and top of the box area indicate the first and third quartiles, respectively. The vertical lines indicate the variability outside the first and third quartiles. Extreme values are indicated as open circles. Pupal weight was measured at the end of the larval development experiment (n=10). Data were analyzed using a linear mixed model. Bars marked with different letters are significantly different using LSmeans with Tukey HSD post-hoc comparisons (*P* = 0.0028).

Literature Cited

Amrein, H., and N. Thorne. **2005**. Gustatory perception and behavior in Drosophila melanogaster. Curr. Biol. 15: 673–684.

Amtmann, A., S. Troufflard, and P. Armengaud. **2008**. The effect of potassium nutrition on pest and disease resistance in plants. Physiol. Plant. 133: 682–691.

AOAC. **2000**. Official Methods of Analysis of AOAC International. Assoc. Off. Anal. Chem. Int. Method ce 2-66.

Badenes-Perez, F.R, B. Nault, and A. . Shelton. **2005**. Manipulating the attractiveness and suitability of hosts for diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 98: 836–844.

Beckie, H. J., K. N. Harker, L. M. Hall, S. I. Warwick, A. Légère, P. H. Sikkema, G. W. Clayton, A. G. Thomas, J. Y. Leeson, G. Séguin-Swartz, and M. Simard. **2006**. A decade of herbicide-resistant crops in Canada. Can. J. Plant Sci. 86: 1243–1264.

Brennan, R. F., M. G. Mason, and G. H. Walton. **2000**. Effect of nitrogen fertilizer on the concentrations of oil and protein in canola (*Brassica napus*) seed. J. Plant Nutr. 23: 339–348.

Bucher, G. E., and G. K. Bracken. **1976**. The bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae). Artificial diet and rearing technique. Can. Entomol. 108: 1327–1338.

Canola council of Canada. 2017. Industry overview. https://www.canolacouncil.org/markets-stats/industry-overview/

Canolawatch. 2016. How much fertilizer does canola need? http://www.canolawatch.org/2012/02/09/how-much-fertilizer-does-canola-need/

Canola Encyclopedia. 2018. Seeding rate.

https://www.canolacouncil.org/canola-encyclopedia/crop-establishment/seeding-rate/#fnote1

Chen, X. 2014. Effects of induced plant resistance and potassium fertilization rates on soybean looper (*Chrysodeixis includens*) development in Soybean.

Chen, Y., J. R. Ruberson, and D. M. Olson. **2008**. Nitrogen fertilization rate affects feeding, larval performance, and oviposition preference of the beet armyworm, *Spodoptera exigua*, on cotton. Entomol. Exp. Appl. 126: 244–255.

Chen, Y. Z., L. Lin, C. W. Wang, C. C. Yeh, and S. Y. Hwang. **2004**. Response of two Pieris (Lepidoptera: Pieridae) species to fertilization of a host plant. Zool. Stud. 43: 778–786.

Clancy, K., and P. Price. **1987**. Rapid herbivore growth enhances enemy attack: sublethal plant defenses remain a paradox. Ecology. 68: 733–737.

Cornell, H. ., and B. A. Hawkins. **2003**. Herbivore responses to plant secondary compounds: a test of phytochemical coevolution theory. Am. Nat. 161: 507–522.

Dosdall, L. M., and B. J. Ulmer. **2004**. Feeding, development, and oviposition of bertha armyworm (Lepidoptera : Noctuidae) on different host plant species. Environ. Entomol. 33: 756–764.

Dosdall, L.M. 2010. Do Larvae of Bertha armyworm, *Mamestra configurata* (Walker) (Lepidoptera: Noctuidae), express induced feeding responses? J Appl Entomol 134:531–541. doi: 10.1111/j.1439-0418.2009.01434.x

Evenden, M. L., R. E. Batallas, and C. Weeraddana. **2017**. Biology and management of the generalist herbivore, the bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), on canola in western Canada., pp. 114–129. *In* Reddy, G.V.P. (ed.), Integr.

Manag. Insect Pests Canola Other Brassica Oilseed Crop. CABI, Boston, MA.

Feeny, P., E. Städler, I. Åhman, and M. Carter. **1989**. Effects of plant odor on oviposition by the black swallowtail butterfly, *Papilio polyxenes* (Lepidoptera: Papilionidae). J. Insect Behav. 2: 803–827.

Funke, T., H. Han, M. L. Healy-Fried, M. Fischer, and E. Schönbrunn. **2006**. Molecular basis for the herbicide resistance of Roundup Ready crops. Proc. Natl. Acad. Sci. 103: 13010–13015.

Geoffrey, M. K., and W. J. Turnock. **1979**. Growth, development and survival of *Mamestra configurata* Walker, (Lepidoptera : Noctuidae) on various reported food Plants. Dep. Entomol.

Grant, C. A., D. A. Derksen, D. L. McLaren, and R. B. Irvine. **2011**. Nitrogen fertilizer and urease inhibitor effects on canola seed quality in a one-pass seeding and fertilizing system. F. Crop. Res. 121: 201–208.

Halkier, B. A., and J. Gershenzon. **2006**. Biology and biochemistry of glucosinolates. Annu. Rev. Plant Biol. 57: 303–333.

Harvey, J. A., and R. Gols. **2011**. Population-related variation in plant defense more strongly affects survival of an herbivore than its solitary parasitoid wasp. J. Chem. Ecol. 37: 1081–1090.

Honěk, A. 1993. Intraspecific variation in body size and fecundity in insects : A general relationship. Oikos. 66: 483–492.

Howlader, M. A., and G. H. Gerber. **1986**. Effects of age, egg development, and mating on calling behavior of the bertha armyworm, *Mamestra configurata* Walker (Lepidoptera: Noctuidae),. Can. Entomol. 118: 1221–1230.

Hsu, Y.-T., T.-C. Shen, and S.-Y. Hwang. **2009**. Soil fertility management and pest responses: a comparison of organic and synthetic fertilization. J. Econ. Entomol. 102: 160–9.

Jauset, A. M., M. J. Sarasúa, J. Avilla, and R. Albajes. **1998**. The impact of nitrogen fertilization of tomato on feeding site selection and oviposition by *Trialeurodes vaporariorum*. Entomol. Exp. Appl. 86: 175–182.

Kazimírová, M. 1996. Influence of larval crowding and mating on lifespan and fecundity of *Mamestra brassicae* (Lepidoptera: Noctuidae). Eur. J. Entomol. 93: 45–52.

Loader, C., and H. Damman. **1991**. Nitrogen content of food plants and vulnerability of *Pieris rapae* to natural enemies. Ecology. 72: 1586–1590.

Masante-Roca, I., S. Anton, L. Delbac, M. C. Dufour, and C. Gadenne. **2007**. Attraction of the grapevine moth to host and non-host plant parts in the wind tunnel: effects of plant phenology, sex, and mating status. Entomol. Exp. Appl. 122: 239–245.

Mason, P. G., M. A. Erlandson, O. O. Olfert, and A. P. Arthur. **1998**. The bertha armyworm (*Mamestra configurata*) (Lepidoptera: Noctuidae) in western Canada. Can. Entomol. 130: 321–336.

Montezano, D. G., A. Specht, D. R. Sosa-Gómez, V. F. Roque-Specht, T. M. Bortolin, E. Fronza, P. Pezzi, P. C. Luz, and N. M. Barros. **2014**. Biotic potential, fertility and life table of *Spodoptera albula* (Walker) (Lepidoptera: Noctuidae), under controlled conditions. An. Acad. Bras. Cienc. 86: 723–732.

Mortvedt, J. J. 1985. Micronutrient fertilizers and fertilization practices. Fertil. Res. 7: 221–235.

Myers, J. H. 1985. Effect of physiological condition of the host plant on the ovipositional choice of the cabbage white butterfly, *Pieris rapae*. J. Anim. Ecol. 54: 193–204.

Phillips, P. W. B. 2003. The economic impact of herbicide tolerant canola in Canada, pp. 119–140. *In* Kalaitzandonakes, N. (ed.), Econ. Environ. Impacts Agbiotech a Glob. Perspect. Springer US, Boston, MA.

Prudic, K. L., J. C. Oliver, and M. D. Bowers. **2005**. Soil nutrient effects on oviposition preference, larval performance, and chemical defense of a specialist insect herbivore. Oecologia. 143: 578–587.

Rashid, M. M., M. Jahan, and K. S. Islam. **2016**. Impact of nitrogen, phosphorus and potassium on brown planthopper and tolerance of its host rice plants. Rice Sci. 23: 119–131.

Robertson, G. W., D. W. Griffiths, W. M. F. Smith, and R. D. Butcher. **1993**. The application of thermal desorption-gas chromatography-mass spectrometry to the analyses of flower volatiles from five varieties of oilseed rape (*Brassica napus* spp. *oleifera*). Phytochem. Anal. 4: 152–157.

Rojas, J. C., A. Virgen, and L. Cruz-López. **2003**. Chemical and tactile cues influencing oviposition of a generalist moth, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Environ. Entomol. 32: 1386–1392.

Sarfraz, M., L. M. Dosdall, and B. A. Keddie. **2007**. Resistance of some cultivated Brassicaceae to infestations by *Plutella xylostella* (Lepidoptera: Plutellidae). J. Econ. Entomol. 100: 215–224.

Sarfraz, R. M., L. M. Dosdall, and A. B. Keddie. **2009**. Bottom-up effects of host plant nutritional quality on *Plutella xylostella* (Lepidoptera: Plutellidae) and top-down effects of herbivore attack on plant compensatory ability. Eur. J. Entomol. 106: 583–594.

Shields, V. D. C., and B. K. Mitchell. **1995**. Sinigrin as a feeding deterrent in two crucifer-feeding, polyphagous Lepidopterous species and the effects of feeding stimulant mixtures on deterrency. Philos. Trans. R. Soc. B Biol. Sci. 347: 439–446.

Skoog, D. A., F. J. Holler, and S. R. Crouch. **2007**. Principles of Instrumental Analysis, 6th ed, Sect. At. Mol. Spectrosc. Serve as Excell. Introd. to Subj. Thomson Brooks/Cole, Belmont, CA.

Smith, E. G., B. M. Upadhyay, M. L. Favret, and R. E. Karamanos. **2010**. Fertilizer response for hybrid and open-pollinated canola and economic optimal nutrient levels. Can. J. Plant Sci. 90: 305–310.

Sterner, R. W., and J. J. Elser. **2002**. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, New Jersey, USA.

Stevens, V. M., A. Trochet, H. Van Dyck, J. Clobert, and M. Baguette. **2012**. How is dispersal integrated in life histories: A quantitative analysis using butterflies. Ecol. Lett. 15: 74–86.

Stringam, G. R., D. F. Degenhardt, M. R. Thiagarajah, and V. K. Bansal. **1999**. Q2 summer rape. Can. J. Plant Sci. 79: 597–598.

Tan, S., R. R. Evans, M. L. Dahmer, B. K. Singh, and D. L. Shaner. **2005**. Imidazolinone-tolerant crops: history, current status and future. Pest Manag. Sci. 61: 246–257.

Tasin, M., A. C. Bäckman, M. Coracini, D. Casado, C. Ioriatti, and P. Witzgall. **2007**. Synergism and redundancy in a plant volatile blend attracting grapevine moth females. Phytochemistry. 68: 203–209.

The Royal Society. How does GM differ from conventional plant breeding ? https://royalsociety.org/topics-policy/projects/gm-plants/how-does-gm-differ-from-conventional-plant-breeding/

(accessed on May 6, 2018)

Turnock, W. J. 1985. Developmental, survival, and reproductive parameters of bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae) on four plant species. Can. Ent. 117: 1267–1271.

Ulmer, B., and C. Gillott. **2001**. Feeding preferences , growth , and development of *Mamestra configurata* (Lepidoptera : Noctuidae) on Brassicaceae. Can. Entomol. 133: 509–519.

Ulmer, B., C. Gillott, and M. Erlandson. **2002**. Oviposition preferences of bertha armyworm *Mamestra configurata* Walker (Lepidoptera : Noctuidae) on different crucifer cultivars and growth stages. Environ. Entomol. 31: 1135–1141.

Ulmer, B., C. Gillott, and M. Erlandson. **2003**. Conspecific eggs and bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), oviposition site selection. Environ. Entomol. 32: 529–534.

Velasco, P., P. Soengas, M. Vilar, M. E. Cartea, and M. Del Rio. **2008**. Comparison of glucosinolate profiles in leaf and seed tissues of different *Brassica napus* crops. J. Am. Soc. Hortic. Sci. 133: 551–558.

Veromann, E., M. Toome, A. Kannaste, R. Kaasik, L. Copolovici, J. Flink, K. Gabriella, L. Narits, A. Luik, and N. Ülo. **2013**. Effects of nitrogen fertilization on insect pests, their parasitoids, plant diseases and volatile organic compounds in *Brassica napus*. Crop Prot. 43: 79–88.

Wang, J. J., J. H. Tsai, and T. K. Broschat. **2006**. Effect of nitrogen fertilization of corn on the development, survivorship, fecundity and body weight of *Peregrinus maidis* (Hom., Delphacidae). J. Appl. Entomol. 130: 20–25.

Waring, G., and N. Cobb. **1992**. The impact of plant stress on herbivore population dynamics, pp. 167–225. *In* EA, B. (ed.), Insect-Plant Interact. CRC, Boca Raton.

Wright, G. A., B. D. Skinner, and B. H. Smith. **2002**. Ability of honeybee, Apis mellifera, to detect and discriminate odors of varieties of canola (*Brassica rapa* and *Brassica napus*) and snapdragon flowers (*Antirrhinum majus*). J. Chem. Ecol. 28: 721–740.

Chapter 3: Oviposition by a specialist herbivore, the diamondback moth, suppresses plant defenses and increases susceptibility of canola to herbivory by a generalist herbivore, the bertha armyworm

Abstract

Plant-induced defense against insects activates salicylic acid (SA) and/or jasmonic acid (JA) plant hormone signaling pathways. Evidence for the effect of insect oviposition on these pathways is equivocal with some studies reporting induction and others suppression of signaling pathways. Suppression of plant defenses by herbivore activity may alter the susceptibility of the plant to subsequent infestation by other herbivores. In this study, I tested the effect of previous oviposition on canola, *Brassica napus* L., by a *Brassica* specialist, the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), on subsequent herbivory by the generalist feeder, the bertha armyworm (BAW), *Mamestra configurata* Walker (Lepidoptera: Noctuidae). I found a significant increase in BAW larval feeding on plants that had DBM eggs. Further, a decrease in JA level in plants with DBM eggs probably mediates the increased feeding by BAW larvae. A concurrent decrease in SA levels in plants with DBM eggs might be due to emission of SA as methyl salicylate (MeSA) which I did not collect in this study. In no-choice experiments, fewer BAW eggs were laid on plants, which had DBM eggs. In choice experiments, however, oviposition preference of BAW was not altered by DBM eggs on canola plants. These results suggest that oviposition by a specialist herbivore can suppress the JA pathway, which can subsequently increase susceptibility of the host plant to generalist herbivory. These findings provide a novel insight into suppression of plant defense induction in response to insect egg deposition.

Introduction

Plant defenses can be induced by herbivory (Stotz et al. 2000; Mewis et al. 2005) and pathogen infection (Dangl and Jones 2001). Plants can also respond to insect oviposition in the absence of herbivory. Direct plant defenses induced by insect oviposition include the production of ovicidal compounds (Seino et al. 1996) or necrotic lesions at oviposition sites (Shapiro and DeVay 1987) and the production of oviposition deterrents (de Vos et al. 2008). Indirect plant defenses do not directly influence oviposition or larval performance but act via a change in the volatile organic compounds (VOCs) released by plants in order to attract herbivore natural enemies (Girling et al. 2011). The mechanisms by which oviposition elicits the change in plant volatile emissions are not yet fully understood. Elicitors could be present in oviduct secretions that cover the eggs (Hilker et al. 2002), such as glandular secretions or compounds in the egg chorion (Hilker et al. 2008). Plant tissue changes due to egg deposition include hypersensitive responses, the formation of neoplasm, changes in plant surface chemicals and plant volatile emissions (Hilker et al. 2008).

Evidence supporting the induction of plant chemical defenses against insect activity is well documented (Mithöfer and Boland 2012). Less well understood is how these defenses may be suppressed once induced. Herbivore-adapted responses to plant defenses include avoidance, resistance to and suppression of toxins (Utsumi et al. 2011). *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) avoid feeding on leaf areas of *Arabidopsis thaliana* (L.) Heynh. with high glucosinolate levels (Shroff et al. 2008). Plant defense suppression can be facilitated through the presence of salivary components (Consales et al. 2012) or bacteria in herbivores, which counteract plant defenses (Chung et al. 2013).

Several examples exist regarding the suppression of plant defenses through insect oviposition (Bruessow et al. 2010; Sarmento et al. 2011; Kim et al. 2012). Presumably, suppression of plant defenses in response to oviposition is adaptive for the insect, as the larvae that hatch from the eggs and feed on the plant will be exposed to lower levels of plant defense compounds (Bruessow et al. 2010). Plant defense induction or suppression is mediated through salicylic acid (SA) (Walling 2008), jasmonic acid (JA) (Musser et al. 2002) and other hormone signalling pathways (Stotz et al. 2000). The JA pathway induces plant defenses in response to damage created by chewing herbivores and nectrotrophic pathogens (Farmer and Ryan 1992; Howe and Jander 2008), while the SA pathway induces defenses against sucking insects and biotrophic pathogens (Glazebrook 2005). The active compound mediating plant defense in the JA pathway is a jasmonic acid isoleucine conjugate (Staswick and Tiryaki 2004), however, the active form in the SA pathway is SA. Salicylic acid is also active in other conjugated forms, such as salicylic acid glucose ester, salicylic acid O-β-glucoside, methyl salicylate (MeSA) and methyl salicylate O-β-glucoside (Vlot et al. 2009). Salicylic acid glycosides function as inactive storage forms that can be converted readily into SA when needed in plant defenses (Hennig et al. 1993).

Within individual plants, SA and JA interactions are commonly antagonistic (Kunkel and Brooks 2002; Takahashi et al. 2004; Bostock 2005), but, synergistic interactions are also known (Schenk et al. 2000; van Wees et al. 2000; Mur et al. 2005). Suppression of tomato plant defenses through both SA and JA hormone signalling pathways occurs in response to infestation by a spider mite, *Tetranychus evansi* Baker & Pritchard (Tetranychidae: Trombidiformes), which benefits subsequent feeding by conspecifics (Sarmento et al. 2011). The suppression of genes in tomato occurs downstream of SA and JA and is dependent on SA-JA antagonism (Alba et al. 2015). Application of egg extract from a specialist herbivore, the cabbage butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae), or a generalist herbivore, the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) to *Arabidopsis* induces SA-mediated defense (Bruessow et al. 2010). Egg-derived elicitors may suppress plant defenses through the JA pathway and induce the SA signaling pathway. Specialist larvae reared on plants treated with the egg extract of the specialist, *P. brassicae,* are not affected, however, generalist larvae, *S. littoralis* are negatively affected. This suggests that SA accumulation may provide a benefit for generalists but not for specialists and raises the question as to whether ovipositon contributes to the suppression of plant defense response against insect herbivory.

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is an oligophagous species that feeds mostly on Brassicaceae plants that include important crop plants like canola, mustard, cabbage, broccoli and several greenhouse crops (Ulmer 2002). Adult DBM emerge during the photophase (Pivnick et al. 1990) and mating occurs at dusk of the same day (Harcourt 1954). Soon after mating, female DBM lay eggs. Oviposition occurs over approximately 4 days, during which time females lay nearly 200 eggs. Diamondback moth females prefer to lay eggs on the upper surface of leaves as compared with other parts of the plant (Harcourt 1954). Egg hatch occurs 5-6 days after oviposition and is influenced by temperature (Harcourt 1954). Mated DBM females lay more eggs on plants with conspecific larvae, suggesting that infested plants are more attractive to gravid females (Wee 2016). This could be because females are more sensitive to plant odors after mating (Rojas 1999; Mechaber et al. 2002), or because the plant volatiles induced by herbivory attract females. Several orders of insects contain JA and SA in the eggs (Tooker and De Moraes 2005, 2007) and these defense hormones in eggs influence on induced plant defenses.

The bertha armyworm (BAW), *Mamestra configurata* Walker (Lepidoptera: Noctuidae), is a generalist herbivore which feeds on plants in a variety of families including Brassicaceae, Compositae, Leguminosae and Chenopodiaceae (Dosdall 2010). Larvae feed on important crops including canola, alfalfa, flax, and field pea. The BAW larvae can completely deplete entire fields of plant material, forcing mass migration to find new food sources (Dosdall 2010). Oviposition by BAW is influenced by foliar glucosinolates, as sinigrin in *Brassica juncea* (L.) Czern. deters ovipositing females but *Sinapsis alba* L., which contains a different glucosinolate, sinalbin, attracts females (Ulmer et al. 2002a). Bertha armyworm larvae prefer to feed on plants that have sinigrin (Ulmer and Gillott 2001), whereas sinabin negatively influences larval feeding (Ulmer et al. 2002a). Female BAW prefer to lay eggs near other conspecific eggs and this attraction appears to be mediated by chemicals released by the eggs (Ulmer et al. 2003).

In Western Canada, DBM arrive as adults blown on the wind from the southern USA and Mexico in the spring (Harcourt 1957; Dosdall 1994) and are present in canola fields prior to BAW. In this study, I evaluated the effect of oviposition by the specialist DBM on canola on subsequent oviposition and larval feeding by the generalist, BAW. I analyzed both the SA and JA pathways as the two main signaling pathways involved in plant defense against insect herbivores.

Methods

Host plant

In all experiments, canolaplant variety, ('Q2') plants were planted in 15.3 cm diameter pots in Sunshine mix 4 (Sun Gro horticulture Canada Ltd, Alberta, Canada) medium and were housed at 21 °C with a 16h L: 8h D photo regime. Plants were maintained under full spectrum lights that provided light intensity at the plant level from20000 to 23,000 lux. Canola plants were watered daily. Starting 3 weeks after plant germination, plants were fertilized using 20:20:20 (nitrogen:

phosphorus: potassium; Plant products Co, Ltd., Brampton, Ontario, Canada) at a rate of 1.0 g/pot every 2 weeks for 7 weeks. Plants were used at the flowering stage in all the experiments since female BAW adults lay more eggs on flowering canola plants than on plants in the preflowering or pod stages (Ulmer et al. 2002b).

Insects

Bertha armyworm was originally obtained from an established colony housed at Agriculture and Agri-food Canada, Saskatoon Research Centre. Bertha armyworm larvae were reared on a soy bean-based artificial diet (Bucher and Bracken 1976) in a growth chamber maintained at 21°C and 16h L: 8h D photo regime. A 5% (weight/volume) honey and sugar solution was provided through a dental wick to adult BAW. Diamondback moth larvae were collected from infested canola fields near Strathmore, Alberta, Canada in 2015 (51°2'16.27"N and 113°24'1.03"W). Field-collected DBM larvae were reared on canola plants (‘Q2’ variety) housed in wooden cages (39.5 X 39.5 X 80.5 cm) and held at 21°C and 16h L: 8h D photo regime. A 10% (weight/volume) honey solution was provided to adult DBM (Silva and Furlong 2012).

No-choice oviposition experiment

A no-choice experiment was conducted to test the hypothesis that DBM eggs influenced subsequent oviposition by BAW females (n=10 per treatment). To create treated plants with DBM eggs, 30 DBM adults less than 1-day old were introduced to the canola plants at the flowering stage in a 1:1 male: female ratio. Diamondback moths were allowed to mate and oviposit for 48 h in wooden cages (39.5 X 39.5 X 80.5 cm) containing a single canola plant. At the end of the oviposition period, all moths were removed from the cage and the plants with eggs were used as the experimental treatment. Uninfested plants housed in similar cages were used as a control.

Pairs of male and female adult BAW less than 1-day old were allowed to mate for 48 h in transparent 500 mL containers without exposure to plant material. Three pairs of mated moths were introduced into nylon mesh cages (47.5 X 47.5 X 93.0 cm; model BD44590F; MegaView Science Co., Ltd., Taichung, Taiwan) that contained either a single, treated canola plant with DBM eggs or a control plant without DBM eggs. The total number of BAW eggs was counted after 4 days.

Choice oviposition experiment

A choice experiment was conducted to examine the oviposition preference of BAW females for canola plants with or without DBM eggs (n=15 per treatment). Treatment and control plants were generated as described above in the no-choice experiment. The BAW adults were allowed to mate for 48 h before introduction into the bioassay, as described in the no-choice experiment. Five pairs of mated BAW adults were released into nylon mesh cages (47.5 X 47.5 X 93.0 cm; model BD44590F; MegaView Science Co., Ltd., Taichung, Taiwan) containing two canola plants, one with DBM eggs and the other without eggs. Each canola plant was examined and the total BAW egg number on each plant was recorded after 36 h.

Larval feeding no-choice experiment

To test the hypothesis that DBM eggs influence BAW larval feeding behaviour, a no-choice experiment was conducted (n=15 per treatment). Treated and control plants, with and without DBM eggs, respectively, were obtained as explained above for the oviposition experiments. Five, early fourth instar BAW larvae were introduced to the fourth leaf from the bottom on canola plants with or without DBM eggs. The leaf with the BAW larvae was gently covered with a 30 cm X 22 cm sized sieve mesh bag. After 36 h, leaves were harvested from both the treated and control plants and feeding damage was measured using ImageJ software (Version k 1.45s).

Larval feeding choice experiment

A choice experiment tested the feeding preference of BAW larvae for canola plants with and without DBM eggs (n=14 per treatment). Treated and control plants were obtained as explained in the oviposition experiments above. Three BAW larvae at the third instar were released in the middle of two leaves from two separate plants in the standard position four leaves from the bottom of each plant (Figure 3-1). The two leaves were caged together without damage to the leaves using a plastic arena made of two large plastic Petri plates (15 cm diameter) with the lids replaced by sieve mesh. After 48 h, the amount of feeding damage on the leaves from each plant housed in the arena was assessed with ImageJ software (Version k 1.45s).

Phytohormone analyses

Phytohormone analyses were conducted to test the impact of DBM oviposition on plant signaling pathways. Canola plants were manipulated as in oviposition experiments, except that adult DBM were given 72 h instead of 48 h for oviposition. Leaf discs of 2.5 cm diameter containing eggs were collected from treated canola plants. Immediately following harvest, the eggs were gently removed using a fine paintbrush and leaves were snap frozen in liquid nitrogen. As a control, an equal number of leaf discs were collected from canola plants without DBM eggs. Five hundred mg of plant tissue was used in each sample taken from different plants (n=3 per treatment).

Samples were stored in -80 °C and transported with dry ice for tissue analyses to the National Research Council of Canada, Saskatoon, Canada, where the samples and standards were prepared, and analyses were conducted. Analyses were performed using an ultra-performance liquid chromatography-electrospray tandem mass spectrometry system (UPLC/ESI-MS/MS system, Waters Corporation, Milford, USA). Quantification of SA (salicylic acid) and JA (jasmonic acid) followed a method described by (Murmu et al. 2014) with modifications. Analyses utilized the MassLynx v4.1 software (Micromass, Manchester, UK) and trace amounts were quantified with QuanLynx v4.1 software (Micromass, Manchester, UK). Each chromatographic trace was integrated and the resulting ratio of signal (non-deuterated/internal standard) was compared with a calibration curve to yield the amount of analyte present as nanograms per sample. Calibration curves were generated for salicylic acid, jasmonic acid and their conjugates. Samples, internal standards and solvent blanks were also analyzed along with the tissue samples.

Statistical analyses

Analyses were conducted using R version 3.4.3 and R Studio 1.1.423. Data were tested for normality and equal variances using Shapiro-Wilk test and Bartlett's test, respectively. Medians are presented in figures and tables.

Oviposition no-choice experiment

To test for an effect of prior oviposition by DBM on BAW oviposition, the total number of BAW eggs laid on each plant was treated as the dependent variable in a linear model. Plant treatment, with or without DBM eggs, was treated as the fixed factor.

Oviposition choice experiment

To test for an oviposition preference of BAW females for plants with or without DBM eggs, data were analyzed using a paired t-test. The total number of eggs laid on each plant in each cage was treated as the dependent variable. Plant treatment, with or without DBM eggs, was the independent variable.

To determine if BAW larval feeding behaviour was affected by previous oviposition of DBM, feeding damage on each leaf of each plant was treated as dependent variable in a linear model. Plant treatment, with or without DBM eggs, was the independent variable.

Larval feeding choice experiment

To determine if larvae exhibited a choice for feeding on plants with or without DBM eggs, data were analysed using a paired t-test. Larval feeding damage area on each leaf from each plant that was housed in the feeding arena was treated as dependent variable. Plant treatment, with or without DBM eggs, was the independent variable.

Phytohormone analyses

Salicylic acid and conjugated SA were analysed using a linear model. The amount of SA and conjugated SA were treated as the dependent variable in separate linear models. The amount of conjugated SA was transformed to Y^2 to achieve normality and homoscedasticity. Jasmonic acid and JA-Ile were analysed using a linear model. The amount of SA and conjugated SA were treated as the dependent variable in separate linear models.

Results and Discussion

This study illustrates that egg deposition by the specialist herbivore, DBM, suppresses canola defenses and thereby increases susceptibility to generalist herbivory. I found that increased susceptibility to generalist herbivory was correlated with a suppression of both JA and SA hormone signaling pathways. Diamondback moth adults appear in western Canada in the early spring wind currents from the southern USA and Mexico (Harcourt 1957; Dosdall 1994). Diamondback moth larvae are oligophagous and feed on plants in the family Brassicaceae (Talekar and Shelton 1993). In the Prairie Provinces, DBM is a significant pest of canola and can complete up to four generations per year (Dosdall et al. 2011). The first eggs are laid by migratory female DBM on canola plants prior to host location by the native, generalist herbivore, BAW, which colonizes canola at the flowering stage (Philip and Mengersen 1989). I conducted this research to understand the mechanisms of plant defenses induced by oviposition by a specialist on the subsequent performance and preference of a generalist herbivore that appears on the plant after the specialist.

A lower number of BAW eggs was found on plants which had previously received DBM eggs in both choice ($t_{14} = 1.2505$, $P = 0.2316$) and the non-choice experiment ($F_{1,18} = 4.1666$, $P = 0.0562$) (Figure 3-2). In other herbivore systems, generalists do not colonize plants induced due to host use by specialists herbivores (Long et al. 2007). For instance, moths of the generalist feeder, *Mamestra brassicae* L. (Lepidoptera, Noctuidae) lay more eggs on uninfested *Brassica oleracea* L. plants compared with plants previously damaged by the cabbage white butterfly, *Pieris rapae* (L.) (Poelman et al. 2008). In general, early season herbivores will determine the numbers of late season herbivores (Poelman et al. 2008; Miller-Pierce and Preisser 2012; Stam et al. 2014). Early feeding by flea beetles, *Psylliodes affinis* (Paykull) (Coleoptera: Chrysomelidae) reduced the number of eggs laid by the late season tortoise beetle, *Plagiometriona clavata* (Fabricius) (Coleoptera: Chrysomelidae) on bittersweet nightshade, *Solanum dulcamara* L. (Poelman et al. 2008).

The reduced number of BAW eggs on plants with DBM eggs may be due to the emission of MeSA (Park et al. 2007) from plants with DBM eggs. There was no statistical difference between SA ($F_{1,4}$ = 3.9032, P = 0.1194) and SA conjugates ($F_{1,4}$ = 2.1889 , P = 0.2131) in plants with and without DBM eggs (Table 3-1). Overall SA levels appeared to be suppressed in plants with DBM eggs, some of the SA may be lost as the volatile MeSA, which I did not measure. Methyl salicylate production is induced by insect oviposition and herbivory. For instance, MeSA is emitted from *Arabidopsis* plants fed on by *P. rapae* (Van Poecke et al. 2001). Methyl salicylate inhibits oviposition of *M. brassicae* (Ulland et al. 2008), a moth species in the same genus as BAW. In another study, oviposition by BAW females was reduced on canola plants that had higher levels of SA and its conjugates following root pathogen infection (Chapter 5). Oviposition by DBM could enhance the defensive cell death response within canola plants. Egg depositionby *P. brassicae* induces plant defenses and stress-oriented genes that are able to induce plant cell death (Little et al. 2006). Oviposition by *P. brassicae* (Lepidoptera: Pieridae) on *Arabidopsis* induces SA defenses that cause cell death and necrotic lesions near the eggs (Little et al. 2006). An upregulated hypersensitivity response of *Brassica nigra* (L.) Koch. plants to eggs of *P. rapae* and *P. napi* creates necrotic lesions around the eggs, resulting in egg desiccation and detachment (Shapiro and DeVay 1987).

There was no effect, however, of DBM eggs on BAW oviposition preference. It is possible that oviposition by DBM on canola did not significantly alter the profile of volatile organic compounds released by the plant, as female BAW do make oviposition decisions based on the glucosinolate profile and nutrient level in canola plants. High levels of sinigrin, a glucosinolate found in *Brassica juncea* (L.) Czern. plants, repels ovipositing female BAW, whereas the glucosinolate, sinalbin, enhances oviposition of BAW (Ulmer 2002). Bertha armyworm females also show oviposition preference for fertilized canola over non-fertilized canola (Chapter 1), canola in full bloom (Ulmer et al. 2002b) and canola with conspecific eggs on the plant (Ulmer et al. 2003).

In contrast to the lack of preference shown by BAW females to plants with DBM eggs, in both larval feeding no-choice experiment ($F_{1,28}$ = 11.648, P = 0.0020) (Figure 3-3) and larval feeding choice experiment (t_{13} = -2.6119, P = 0.0215) (Figure 3-4). There was no statistical difference between JA ($F_{1,4}$ = 2.3251, P = 0.2020) and JA-Ile ($F_{1,4}$ = 0.9949, P = 0.3750) in plants with and without DBM eggs (Table 3-1). Overall, BAW larvae consumed more leaf area on canola with DBM eggs than on plants without DBM eggs. This response is likely mediated by the plant, as there was a slightly reduced accumulation of free and conjugated JA and SA in leaves harvested from plants with DBM eggs. Cross talk between the two defense pathways in plants can mediate the susceptibility to herbivores in different plant-feeding guilds (Fürstenberg-hägg et al. 2013). Application of the eggs of a specialist, *P. brassicae,* or a generalist, *S. littoralis,* induces SA defense and inhibits JA defenses in *A. thaliana* (Bruessow et al., 2010), which makes plants more susceptible to chewing insects. Weight gain by *S. littoralis* larvae is enhanced when they are fed plants treated with the egg extract of *P. brassicae*. Suppression of JA defenses near oviposition sites helps developing larvae and may be particularly important for generalists feeding on well-defended *Brassica* species (Bruessow et al. 2010). Jasmonic acid treatment of *Arabidopsis* plants reduces subsequent feeding damage and larval growth of the generalist beet armyworm, *Spodptera exigua* (Hübner) (Lepidoptera: Noctuidae) and enhances expression of defensive proteins and glucosinolates (Cipollini et al. 2004).   In contrast to these findings, JA defenses are induced in tomato following oviposition by the generalist herbivore, *Helicoverpa zea* Boddie. (Lepidoptera: Noctuidae). Jasmonic acid was highest just before larval emergence, which suggests tomato plants recognize *H. zea* oviposition and prime to avoid future herbivory (Kim et al., 2012).

These findings do not support the “Plant stress hypothesis” which states that stressed plants are more susceptible to insect feeding because plants mobilize nutrients under stressful conditions (White 1984). Despite efforts to test this hypothesis, several studies show conflicting results (Koricheva et al. 1998). In my study, BAW larvae fed more on plants infested by DBM, which had lower levels of plant defenses compared with uninfested plants.

This study illustrates that oviposition of a specialist herbivore can benefit the developing larvae of a generalist herbivore that subsequently exploits the plant host. Although many studies focus on plant defenses induced by insect oviposition, few studies report suppression of plant defenses in response to insect oviposition (Karban and Agrawal 2002). My research found that oviposition by the specialist herbivore, DBM, suppresses JA and SA signaling pathways, and subsequently influences herbivory by the native, generalist BAW. More research is required to determine if this phenomenon is common in other insects.

Table 3-1. Free/unbound SA, conjugated SA, JA and jasmonic acid isoleucince (JA-lle) concentration in DBM infested and uninfested canola plants determined using UPLC ESI-MS/MS. Leaf tissues were harvested 36 h after introducing DBM adults. Each biological replicate contains 10-20 leaf discs from either canola plants with or without DBM eggs (n=3 per treatment). There was no significant difference between treatments.

Treatment	SA	Conjugated SA	JA	JA-lle
	ng/g fresh weight			
Without DBM eggs	13 ± 1.72	105 ± 3.66	7 ± 3.98	1 ± 0.38
With DBM eggs	9 ± 1.28	77 ± 19.58	<2.6[a]	<0.8[b]

Data are means ± SE. All the samples were analysed using three independent biological replicates, leaves that received DBM eggs measured below the limit of quantification of non-detectable JA and JA-Ile levels. [a] Two samples did not have any detectable level in JA level. [b] One sample that received DBM eggs did does not have any detectable levels of JA-lle.

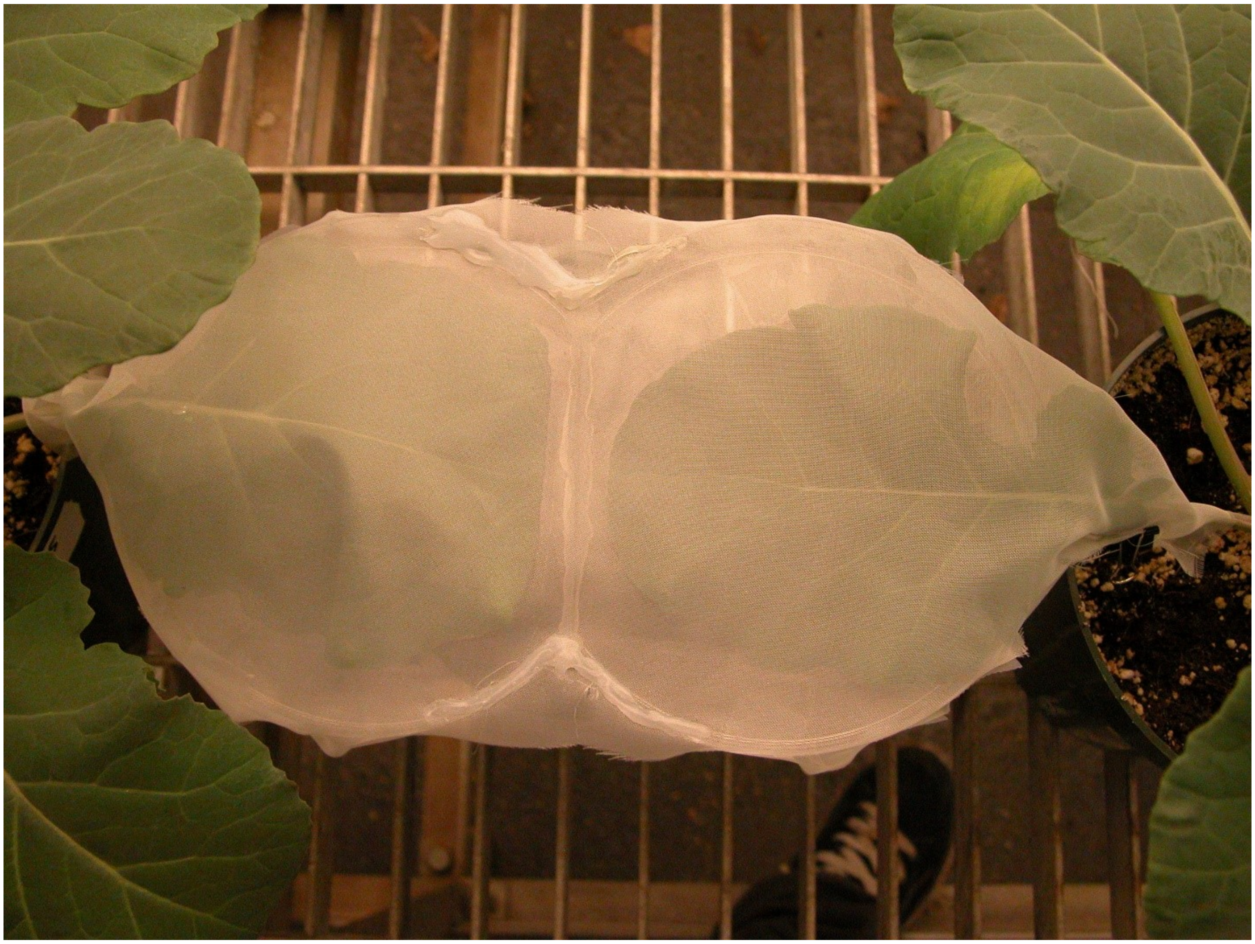


Figure 3-1. Picture of the BAW larvae choice feeding arena. Bertha armyworm larvae were released in the middle of two leaves from two separate plants. The two leaves were caged together using a plastic arena made of two large plastic petri plates with the lids replaced by sieve mesh. After 48 h, the amount of feeding damage on the leaves from each plant housed in the arena was assessed.

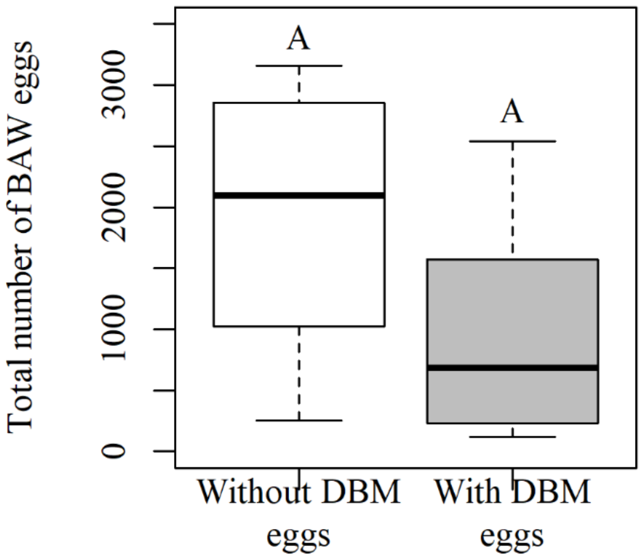


Figure 3-2. Bertha armyworm oviposition on plants with and without DBM eggs in a no-choice oviposition experiment (n=10 per treatment). The midline indicates the median. The bottom and top of the box area indicate the first and third quartiles, respectively. The vertical lines indicate the variability outside the first and third quartiles. Eggs were counted after 4 days of oviposition. Data were analyzed using a linear model. Bars marked with the same letters are not significantly different (*P* = 0.0562).

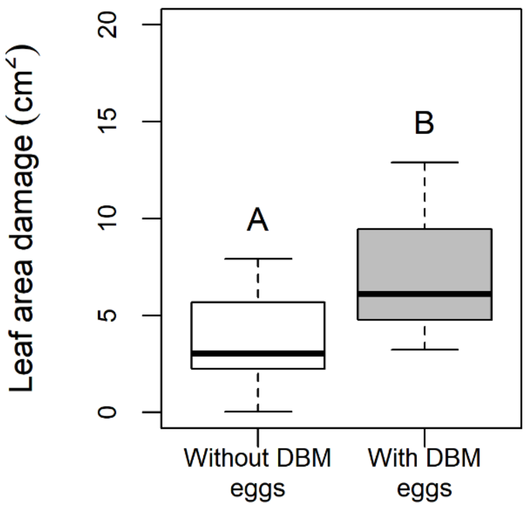


Figure 3-3. Bertha armyworm larval feeding damage (cm^2) on leaves from plants with and without DBM eggs in a no-choice feeding experiment (n=15 per treatment). The midline indicates the median. The bottom and top of the box area indicate the first and third quartiles, respectively. The vertical lines indicate the variability outside the first and third quartiles. Feeding damage was assessed after 72 h of larval feeding. Data were analyzed using linear model. Bars marked with different letters are significantly different ($P = 0.0020$).

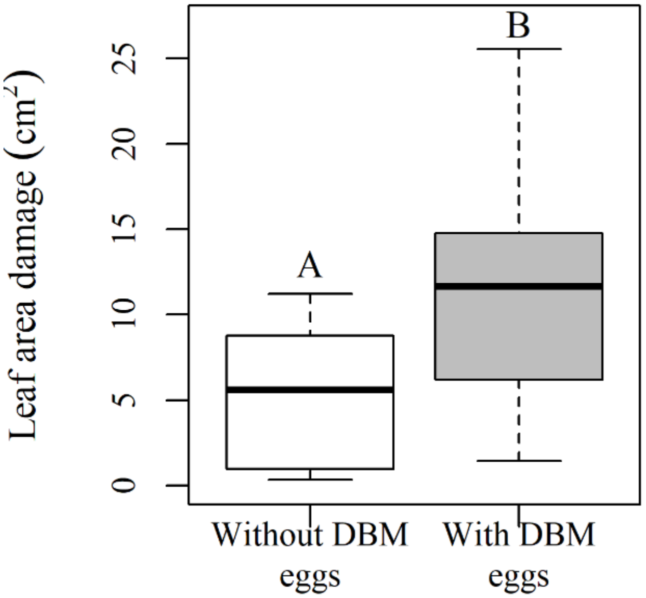


Figure 3-4. Bertha armyworm larval feeding damage (cm^2) on plants with and without DBM eggs in a choice feeding experiment (n=14 per treatment). The midline indicates the median. The bottom and top of the box area indicate the first and third quartiles, respectively. The vertical lines indicate the variability outside the first and third quartiles. Feeding damage was assessed after 48 h in a choice feeding experiment and data were analyzed using a paired t-test. Bars marked with different letters are significantly different (*P* = 0.0215).

Literature Cited

Alba, J. M., B. C. J. Schimmel, J. J. Glas, L. M. S. Ataide, M. L. Pappas, C. A. Villarroel, R. C. Schuurink, M. W. Sabelis, and M. R. Kant. **2015**. Spider mites suppress tomato defenses downstream of jasmonate and salicylate independently of hormonal crosstalk. New Phytol. 205: 828–840.

Bostock, R. M. 2005. Signal crosstalk and induced resistance: straddling the line between cost and benefit. Annu. Rev. Phytopathol. 43: 545–580.

Bruessow, F., C. Gouhier-Darimont, A. Buchala, J. P. Metraux, and P. Reymond. **2010**. Insect eggs suppress plant defence against chewing herbivores. Plant J. 62: 876–885.

Bucher, G. E., and G. K. Bracken. **1976**. The bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae). Artificial diet and rearing technique. Can. Entomol. 108: 1327–1338.

Chung, S. H., C. Rosa, E. D. Scully, M. Peiffer, J. F. Tooker, K. Hoover, D. S. Luthe, and G. W. Felton. **2013**. Herbivore exploits orally secreted bacteria to suppress plant defenses. Proc. Natl. Acad. Sci. 110: 15728–33.

Cipollini, D., S. Enright, M. B. Traw, and J. Bergelson. **2004**. Salicylic acid inhibits jasmonic acid-induced resistance of *Arabidopsis thaliana* to *Spodoptera exigua*. Mol. Ecol. 13: 1643–1653.

Consales, F., F. Schweizer, M. Erb, C. Gouhier-Darimont, N. Bodenhausen, F. Bruessow, I. Sobhy, and P. Reymond. **2012**. Insect oral secretions suppress wound-induced responses in Arabidopsis. J. Exp. Bot. 63: 727–737.

Dangl, J. L., and J. D. G. Jones. **2001**. Plant pathogens and integrated defence responses to infection. Nature. 411: 826–833.

Dosdall, L. M. 1994. Evidence for successful overwintering of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), in Alberta. Can. Entomol. 126: 183–185.

Dosdall, L. M. 2010. Do Larvae of Bertha armyworm, *Mamestra configurata* (Walker) (Lepidoptera: Noctuidae), express induced feeding responses? J. Appl. Entomol. 134: 531–541.

Dosdall, L. M., J. J. Soroka, and O. Olfert. **2011**. The diamondback moth in canola and mustard: current pest status and future prospects. Prairie Soils Crop. J. 4: 66–76.

Farmer, E. E., and C. A. Ryan. **1992**. Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell. 4: 129–134.

Fürstenberg-hägg, J., M. Zagrobelny, and S. Bak. **2013**. Plant defense against insect herbivores. Int. J. Mol. Sci. 14: 10242–10297.

Girling, R. D., A. Stewart-Jones, J. Dherbecourt, J. T. Staley, D. J. Wright, and G. M. Poppy. **2011**. Parasitoids select plants more heavily infested with their caterpillar hosts: a new approach to aid interpretation of plant headspace volatiles. Proceedings. Biol. Sci. 278: 2646–53.

Glazebrook, J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic Pathogens. Annu. Rev. Phytopathol. 43: 205–227.

Harcourt, D. G. 1954. The biology and ecology of the Diamondback moth, *Plutella maculipennis* Curtis, in Eastern Ontario. Cornell Univ.

Harcourt, D. G. 1957. Biology of the diamondback moth, *Plutella maculipennis* (Curt.), in eastern Ontario. 11. Life-history, behaviour, and host relationships. Can. Entomol. 89: 554–564.

Hennig, J., J. Malamy, G. Grynkiewicz, J. Indulski, and D. Klessig. **1993**. Interconversion of the salicylic acid signal and its glucoside in tobacco. Plant J. 4: 593–600.

Hilker, M., C. Kobs, M. Varama, and K. Schrank. **2002**. Insect egg deposition induces *Pinus sylvestris* to attract egg parasitoids. J. Exp. Biol. 205: 455–461.

Hilker, M., O. Rohfritsch, and T. Meiners. **2008**. The plant's response towards insect egg deposition, pp. 205–233. *In* Chemoecology Insect Eggs Egg Depos. Blackwell Publishing Ltd.

Howe, G. A., and G. Jander. **2008**. Plant immunity to insect herbivores. Annu. Rev. Plant Biol. 59: 41–66.

Karban, R., and A. A. Agrawal. **2002**. Herbivore offense. Annu. Rev. Ecol. Syst. 33: 641–664.

Kim, J., J. F. Tooker, D. S. Luthe, C. M. De Moraes, and G. W. Felton. **2012**. Insect eggs can enhance wound response in plants: a study system of tomato *Solanum lycopersicum* L. and *Helicoverpa zea* Boddie. PLoS One. 7: e37420.

Koricheva, J., S. Larsson, and E. Haukioja. **1998**. Insect performance on experimentally stressed woody plants: a meta-analysis. Annu. Rev. Entomol. 43: 195–216.

Kunkel, B. N., and D. M. Brooks. **2002**. Cross talk between signaling pathways in pathgen defense. Curr. Opin. Plant Biol. 5: 325–331.

Little, D., C. Gouhier-Darimont, F. Bruessow, and P. Reymond. **2006**. Oviposition by Pierid butterflies triggers defense responses in Arabidopsis. Plant Physiol. 143: 784–800.

Long, J. D., R. S. Hamilton, and J. L. Mitchell. **2007**. Asymmetric competition via induced resistance: Specialist herbivores indirectly suppress generalist preference and populations. Ecology. 88: 1232–1240.

Mason, P. G., A. P. Arthur, O. O. Olfert, and M. A. Erlandson. **1998**. The bertha armyworm (*Mamestra configurata*) (Lepidoptera: Noctuidae) in Western Canada. Can. Entomol. 130:

321–336.

Mechaber, W. L., C. T. Capaldo, and J. G. Hildebrand. **2002**. Behavioral responses of adult female tobacco hornworms, *Manduca sexta*, to host plant volatiles change with age and mating status. J. Insect Sci. 2: 5.

Mewis, I., H. M. Appel, A. Hom, R. Raina, and J. C. Schultz. **2005**. Major signaling pathways modulate Arabidopsis glucosinolate accumulation and response to both phloem-feeding and chewing insects. Plant Physiol. 138: 1149–1162.

Miller-Pierce, M. R., and E. L. Preisser. **2012**. Asymmetric priority effects influence the success of invasive forest insects. Ecol. Entomol. 37: 350–358.

Mithöfer, A., and W. Boland. **2012**. Plant defense against herbivores: Chemical aspects. Annu. Rev. Plant Biol. 63: 431–450.

Mur, L. A. J., P. Kenton, R. Atzorn, O. Miersch, and C. Wasternack. **2005**. The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. Plant Physiol. 140: 249–262.

Murmu, J., M. Wilton, G. Allard, R. Pandeya, D. Desveaux, J. Singh, and R. Subramaniam. **2014**. Arabidopsis GOLDEN2-LIKE (GLK) transcription factors activate jasmonic acid (JA)-dependent disease susceptibility to the biotrophic pathogen *Hyaloperonospora arabidopsidis*, as well as JA-independent plant immunity against the necrotrophic pathogen Botryti. Mol. Plant Pathol. 15: 174–184.

Musser, R. O., S. M. Hum-Musser, H. Eichenseer, M. Peiffer, G. Ervin, J. B. Murphy, and G. W. Felton. **2002**. Herbivory: caterpillar saliva beats plant defences. Nature. 416: 599–600.

Park, S.-W., E. Kaimoyo, D. Kumar, S. Mosher, and D. F. Klessig. **2007**. Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. Science (80-.). 318: 113–116.

Philip, H., and E. Mengersen. **1989**. Insect pests of the Prairies. University of Alberta Press, Edmonton.

Pivnick, K. A., B. J. Jarvis, C. Gillott, G. P. Slater, and E. W. Underhill. **1990**. Daily patterns of reproductive activity and the influence of adult density and exposure of hosts plants on repdoctuion in the diamondback moth (Lepidoptera: Plutellidae). Environ. Entomol. 19: 587–593.

Van Poecke, R. M. P., M. A. Posthumus, and M. Dicke. **2001**. Herbivore-induced volatile production by *Arabidopsis thaliana* leads to attraction of the parasitoid *Cotesia rubecula*: Chemical, behavioral, and gene-expression analysis. J. Chem. Ecol. 27: 1911–1928.

Poelman, E. H., C. Broekgaarden, J. J. A. Van Loon, and M. Dicke. **2008**. Early season herbivore differentially affects plant defence responses to subsequently colonizing herbivores and their abundance in the field. Mol. Ecol. 17: 3352–3365.

Rojas, J. C. 1999. Electrophysiological and behavioral responses of the cabbage moth to plant volatiles. J. Chem. Ecol. 25: 1867–1883.

Sarmento, R. A., F. Lemos, P. M. Bleeker, R. C. Schuurink, A. Pallini, M. G. A. Oliveira, E. R. Lima, M. Kant, M. W. Sabelis, and A. Janssen. **2011**. A herbivore that manipulates plant defence. Ecol. Lett. 14: 229–236.

Schenk, P. M., K. Kazan, I. Wilson, J. P. Anderson, T. Richmond, S. C. Somerville, and J. M. Manners. **2000**. Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. Proc Natl Acad Sci. 97: 11655–11660.

Seino, Y., Y. Suzuki, and K. Sogawa. **1996**. An ovicidal substance produced by rice plants in response to oviposition by the Whitebacked planthopper, *Sogatella furcifera* (Horvath) (Homoptera: Delphacidae). Appl. Entomol. Zool. 31: 467–473.

Shapiro, A. M., and J. E. DeVay. **1987**. Hypersensitivity reaction of *Brassica nigra* L. (Cruciferae) kills eggs of Pieris butterflies (Lepidoptera: Pieridae). Oecologia. 71: 631–632.

Shroff, R., F. Vergara, A. Muck, A. Svatos, and J. Gershenzon. **2008**. Nonuniform distribution of glucosinolates in *Arabidopsis thaliana* leaves has important consequences for plant defense. Proc. Natl. Acad. Sci. 105: 6196–6201.

Silva, R., and M. J. Furlong. **2012**. Diamondback moth oviposition: Effects of host plant and herbivory. Entomol. Exp. Appl. 143: 218–230.

Stam, J. M., A. Kroes, Y. Li, R. Gols, J. J. A. van Loon, E. H. Poelman, and M. Dicke. **2014**. Plant interactions with multiple insect herbivores: from community to genes. Annu. Rev. Plant Biol. 65: 689–713.

Staswick, P. E., and I. Tiryaki. **2004**. The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in Arabidopsis. Plant Cell. 16: 2117–2127.

Stotz, H. U., B. R. Pittendrigh, J. Kroymann, K. Weniger, J. Fritsche, A. Bauke, and T. Mitchell-Olds. **2000**. Induced plant defense responses against chewing insects. Ethylene signaling reduces resistance of Arabidopsis against Egyptian cotton worm but not diamondback moth. Plant Physiol. 124: 1007.

Takahashi, H., Y. Kanayama, M. S. Zheng, T. Kusano, S. Hase, M. Ikegami, and J. Shah. **2004**. Antagonistic interactions between the SA and JA signaling pathways in Arabidopsis modulate expression of defense genes and gene-for-gene resistance to cucumber mosaic virus. Plant Cell Physiol. 45: 803–809.

Talekar, N. S., and A. M. Shelton. **1993**. Biology, ecology, and management of the diamondback moth. Annu. Rev. Entomol. 38: 275–301.

Tooker, J. F., and C. M. De Moraes. **2005**. Jasmonate in lepidopteran eggs and neonates. J. Chem. Ecol. 31: 2753–2759.

Tooker, J. F., and C. M. De Moraes. **2007**. Jasmonate, salicylate, and benzoate in insect eggs. J. Chem. Ecol. 33: 331–343.

Ulland, S., E. Ian, R. Mozuraitis, A. K. Borg-Karlson, R. Meadow, and H. Mustaparta. **2008**. Methyl salicylate, identified as primary odorant of a specific receptor neuron type, inhibits oviposition by the moth *Mamestra brassicae* L. (Lepidoptera, Noctuidae). Chem. Senses. 33: 35–46.

Ulmer, B., and C. Gillott. **2001**. Feeding preferences , growth , and development of *Mamestra configurata* (Lepidoptera : Noctuidae) on Brassicaceae. Can. Entomol. 133: 509–519.

Ulmer, B., C. Gillott, and M. Erlandson. **2002**. Oviposition preferences of bertha armyworm *Mamestra configurata* Walker (Lepidoptera : Noctuidae) on different crucifer cultivars and growth stages. Environ. Entomol. 31: 1135–1141.

Ulmer, B., C. Gillott, and M. Erlandson. **2003**. Conspecific eggs and bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), oviposition site selection. Environ. Entomol. 32: 529–534.

Ulmer, B. J. 2002. Crucifer host plant suitability for bertha armyworm (*Mamestra configurata*) and diamondback moth (*Plutella xylostella*).

Utsumi, S., Y. Ando, and T. Ohgushi. **2011**. Evolutionary consequence of indirect interactions among insect herbivores through herbivore-induced plant regrowth. J. Plant Interact. 6: 171–172.

Vlot, A. C., D. A. Dempsey, and D. F. Klessig. **2009**. Salicylic acid, a multifaceted hormone to combat disease. Annu. Rev. Phytopathol. 47: 177–206.

de Vos, M., K. L. Kriksunov, and G. Jander. **2008**. Indole-3-acetonitrile production from indole glucosinolates deters oviposition by *Pieris rapae*. Plant Physiol. 146: 916–926.

Walling, L. L. 2008. Avoiding effective defenses: strategies employed by phloem-feeding insects. Plant Physiol. 146: 859–866.

Wee, S. 2016. Effects of conspecific herbivory and mating status on host searching and oviposition behavior of *Plutella xylostella* (Lepidoptera: Plutellidae) in relation to its host, *Brassica oleracea* (brassicales: Brassicaceae). Florida Entomol. 99: 159–165.

van Wees, S. C., E. a de Swart, J. a van Pelt, L. C. van Loon, and C. M. Pieterse. **2000**. Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. U. S. A. 97: 8711–8716.

White, T. C. R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. Oecologia. 63: 90–105.

Chapter 4: Plant-mediated interactions between specialist and generalist herbivores on canola (Brassicaceae)

Abstract

Canola is one of the major oilseed crops grown on the Canadian Prairies. The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is oligophagous on plants in the Brassicaceae, and is considered one of the most significant insect pests of canola. The bertha armyworm (BAW), *Mamestra configurata* Walker (Lepidoptera: Noctuidae), is a generalist herbivore that feeds on canola plants. Diamondback moth herbivory of canola occurs in the field prior to BAW on the Prairies. In this study, I tested the effect of DBM herbivory on subsequent host use by BAW. Oviposition by female BAW was not influenced by previous DBM-herbivory or mechanical damage of canola plants. Bertha armyworm larvae were deterred from feeding on canola previously damaged by DBM and larvae developed into lighter pupae when reared on DBM damaged plants. Bertha armyworm pupae that developed from larvae fed on mechanically damaged plants had similar pupal weights to those fed DBM-damaged plants. Adult BAW reared on canola with previous DBM feeding damage had marginally smaller wings than moths reared on the other treatments. The combination of these results suggests that herbivory by the Brassicaceae specialist, DBM, negatively influences host use and larval performance by the generalist, BAW on canola.

Introduction

Plants encounter multiple herbivore attacks during a growing season (Stam et al., 2014) and have evolved constitutive and induced defenses to protect themselves from herbivores. Constitutive defenses include: a thick cuticle, a thick cell wall, trichomes and toxins (War et al., 2012). Induced defenses include the production of secondary compounds (Fürstenberg-hägg et al., 2013), which can influence the activity of the attacking herbivore and other species that subsequently use the herbivorized plant (Viswanathan et al., 2005). In general, the feeding activity of early season herbivores influences late season herbivore activity. Initial herbivory can deplete host resources through plant biomass reduction and induction of toxic defenses that can negatively influence subsequent herbivores (Agrawal, 2000). Plants damaged early in the season by the potato flea beetle, *Psylliodes affinis* Payk (Coleoptera: Chrysomelidae), caused reduced oviposition by the clavate tortoise beetle, *Plagiometriona clavata* (Fabricius) (Coleoptera: Chrysomelidae), on *Solanum dulcamara* L. late in the season (Viswanathan et al., 2005). In contrast, early season herbivory by *Pieris rapae* (L.) (Lepidoptera: Pieridae) increased susceptibility to flea beetle, *Phylotreta* spp. on wild radish, *Raphanus raphanistrum* (Brassicaceae) (Agrawal & Sherriffs, 2001).

Insects with different feeding strategies may change levels of induction through activation of defensive hormone pathways (Heidel & Baldwin, 2004). The jasmonic acid (JA) and salicylic acid (SA) pathways are induced by herbivores with different feeding strategies and interact differently against different plant-feeding insect guilds (Heidel & Baldwin, 2004). The JA pathway is induced for protection against chewing insects and nectrotrophic pathogens, whereas the SA pathway is induced for protection against sap-sucking insects and biotrophic pathogens (Walling, 2000). Plants fine-tune defenses against specific attackers through cross-talk between JA and SA pathways (Koornneef & Pieterse, 2008). The trade-off plants experience when employing these defenses results in reduced plant growth and insect reproduction (Züst & Agrawal, 2017).

The ecological cost of plant defenses is differentially influenced by the insect feeding guild (Poelman et al., 2008). For instance, infestation of *Brassica oleracea* (L.) with the sap-feeding cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphidinae), enhances the performance of the leaf-chewing larvae of the cabbage moth, *Pieris brassicae* (L.) (Lepidoptera: Pieridae) on the herbivorized host (Soler et al., 2012). This negative interaction likely occurs through the upregulation of SA pathway during the feeding activity of the sucking insects. By the time the chewing insect attacks, the plant does not have the energy to upregulate the JA pathway, so the subsequent attacker profits from the feeding activity of the initial herbivory. In contrast, poor performance by the tobacco cutworm, *Spodoptera litura* (Fabricius) occurs after larvae feed on host plants previously attacked by another leaf chewing insect, the diamondback moth, *Plutella xylostella* (L.) (Mathur et al., 2013). If both attackers are chewing insects, the subsequent attacker is predicted to do poorly as the JA pathway will be induced by the initial herbivore.

The response of insect herbivores to the induced defenses of their plant host may differ depending on the degree of diet specialization of the herbivore (Agrawal, 2000). Generalist herbivores may be negatively influenced by chemical defenses induced by herbivory by a specialist species (Müller et al., 2010). Larvae of the generalist, *S. litura*, feed less and experience less weight gain on *Brassica juncea* (L.) Czern. plants that have been previously damaged by the specialist, diamond back moth, *P. xylostella* (Mathur et al., 2013). In contrast, the hydrolysis of these induced plant defense chemicals can act as feeding and oviposition stimulants for specialist herbivores (Müller et al., 2010). Induced cucurbitacins, in *Cucurbita moschata* Duch. (Cucurbitaceae) plants stimulate feeding of the specialist cucumber beetle, *Acalymma vittata* (Fab.) (Coleoptera: Chrysomelidae) and prey upon by the squash lady beetle, *Epilacha tredecimnotata* (Latreille) (Coleoptera: Coccinelidae) (Agrawal, 2000). Specialists evolve mechanisms that neutralize the defenses through detoxification (Ratzka et al., 2002; Wittstock et al., 2004) or avoidance (Shroff et al., 2008). Defensive volatile compounds, such as isothiocyanates, iberin (3-methylsulfinylpropyl isothiocyanate) and sulforaphane (4-methylsulfinyl-3-butenyl isothiocyanate), are stimulated by the feeding of *P. xylostella* (Renwick et al., 2006). The release of glucosinolate metabolites, such as isothiocyanates, may deter generalists from laying eggs or feeding upon induced host plants (Poelman et al., 2008).

Bertha armyworm (BAW), *Mamestra configurata* Walker (Lepidoptera: Noctuidae), is a generalist herbivore that prefers to feed on crucifers including crop plants such as: canola or rape seed (*Brassica napus* L., *Brassica rapa* L.), mustard [*Brassica juncea* (L.)], and cabbage (*B. oleracea*). Larvae feed after egg hatch on the same plant where eggs are laid, but, mature larvae can disperse across fields when food sources are depleted (Ulmer et al., 2002; Dosdall, 2010). On the Canadian Prairies, BAW females eclose in the early summer and lay eggs on canola plants during flowering (Ulmer et al., 2002). Bertha armyworm oviposition is influenced by host plant nutrition (Chapter 1), foliar glucosinolates (Ulmer, 2002) and the presence of conspecific eggs (Ulmer et al., 2003).

Diamond back moth (DBM) is a specialist that is considered one of the most significant worldwide insect pests of cruciferous crops (Talekar & Shelton, 1993). In early spring, DBM adults migrate on wind currents from the southern USA and Mexico to western Canada (Hopkinson & Soroka, 2010). Diamondback moth larvae develop through four larval instars

(Golizadeh et al., 2007). The first instar are leaf miners that consume the spongy mesophyll of the host plant tissue. This feeding makes characteristic window-like holes in the leaf surface (Talekar & Shelton, 1993). Late instar larvae feed externally on any part of the leaf tissue (Talekar & Shelton, 1993). Mostly the first generation of diamondback moth larvae feed in the field prior to host location by BAW adults on the Prairies. To understand the interaction between these species in canola fields, I tested the effect of herbivory by DBM on the oviposition and offspring fitness of BAW. This study attempts to further understand the effects of specialist damage on host use by a generalist species.

Methods

Host plant

Canola plants of the ‘Q2’ variety were used in all experiments. Canola was seeded in 15.3 cm diameter plastic pots containing Sunshine Mixture 4 (Sun Gro horticulture Canada Ltd, Alberta, Canada) potting medium. Plants were grown at 21°C under a16h: 8h D photoregime with light intensity from 20000 to 23,000 lux . Plants were watered daily. Plants were fertilized with 1/g per pot of 20:20:20 (nitrogen: phosphorus: potassium; Plant products Co, Ltd., Brampton, Ontario, Canada) three times throughout the duration of the experiment. Fertilization started on the third week after germination and continued every two weeks until the seventh week post germination. Flowering canola plants were used for all the experiments as BAW females lay more eggs on canola at the flowering stage as compared to preflower or pod stages (Ulmer et al., 2002).

Insects

The BAW colony was originally obtained from an established colony reared at Agriculture and Agri-food Canada, Saskatoon Research Centre. Larvae were reared on an artificial diet (Bucher and Bracken 1976) in a growth chamber at 21 °C under a 16h: 8h D photo regime. A 5% (weight/volume) honey (Meadow Sweet Honey, Alberta, Canada) and sucrose solution was supplied to adult BAW moths.

Diamondback moth larvae were originally collected from fields near Strathmore, Alberta, Canada (51°2'16.27"N and 113°24'1.03"W) in 2015. Larvae were reared on canola variety (‘Q2’) in wooden cages (39.5 X 39.5 X 80.5 cm) at 21°C under a 16h L: 8h D photo regime. A 10% (weight/volume) honey solution was supplied as a feeding source for adult DBM (Silva & Furlong, 2012).

Experiments

No-choice oviposition experiment

A no-choice experiment (Exp. 1) was conducted to test if DBM larval feeding on canola influenced subsequent oviposition by BAW (n=15; per treatment) as compared to intact and mechanically damaged plants. Canola plants were damaged according to a method described by Silva & Furlong et al. (2012) with modifications. Five early second instar DBM were introduced on the 6th leaf from the bottom of each canola plant (Silva & Furlong, 2012). The leaf and DBM larvae were covered with a mesh cage (30 cm X 22 cm). The mechanical damage treatment was applied by making 10 holes with a 0.5 cm diameter cork borer to the 6th leaf from the bottom of each canola plant (Silva & Furlong, 2012). Undamaged canola plants were used as the control. The 6th leaf from the bottom of control and mechanically damaged canola plants was also covered with a mesh bag. All manipulated plants were kept for 24h in wooden cages (39.5 X 39.5 X 80.5 cm) before the introduction of three pairs of BAW adults for oviposition, but the DBM larvae remained on the plants for the duration of the experiment. Bertha armyworm adults were housed as individual pairs in transparent 500 ml containers, 24h after eclosion. Each pair of male and female BAW adults remained together for 48h to ensure mating prior to introduction to the variously treated canola plants. Three pairs of mated moths were introduced in nylon mesh cages (47.5 X 47.5 X 93.0 cm; model BD44590F; MegaView Science Co., Ltd., Taichung, Taiwan), that contained a single manipulated canola plant. The BAW did not have access to the 6th leaf as this leaf was covered in the treated plants. The number of BAW eggs was counted after four days.

Choice oviposition experiments

Subsequent experiments were conducted as choice experiments. Experiment 2 compared BAW oviposition choice between canola plants fed on by DBM to undamaged canola plants (n=21; per treatment). Experiment 3 compared BAW oviposition on plants fed on by DBM and mechanically damaged canola plants (n=20 per treatment). Canola plants were damaged as in the no-choice oviposition experiment, above. Bertha armyworm adults were allowed to mate for 48h before release into experimental cages. Five pairs of mated BAW adults were introduced into nylon mesh cages (47.5 X 47.5 X 93.0 cm; model BD44590F; MegaView Science Co., Ltd., Taichung, Taiwan) containing one canola plant fed on by DBM and one undamaged plant (Exp. 2) or one plant fed on by DBM and one mechanically damaged plant (Exp. 3). Each canola plant was examined after 36 h exposure to the moths and the total number of eggs was counted.

Offspring development experiment

To test the impact of feeding by DBM on the development of larval BAW, canola plants were damaged as in the no-choice oviposition experiment above with minor modifications in Experiment 4. The DBM larvae were introduced to the whole canola plant instead of being restricted to the 6th leaf from bottom. After 24h of feeding by the DBM larvae, two BAW third instar larvae were introduced onto each plant. The DBM larvae remained on the plant until pupation after which the DBM pupae were replaced with additional DBM larvae. The BAW larvae completed development on the variously manipulated canola plants that had: 1) DBM larval feeding damage; 2) been mechanically damaged; or 3) were left untouched (n=16 per treatment). DBM feeding pressure was maintained throughout the experiment by replacement of DBM larvae as pupation occurred. Bertha armyworm larvae were weighed using a microbalance (Mettler Toledo XS105, Ontario, Canada) two weeks after the introduction onto the plant. Insects were checked daily until pupation, and pupae were collected within 48h of pupation. Pupal weight was measured using a microbalance to the nearest 0.01 mg. Each pupa was kept in a separate container until adult eclosion. Insects were checked daily for adult eclosion and adults were kept without access to a feeding solution until death. Adults were checked daily and weight and longevity were measured at the end of the experiment. Adult forewings were carefully glued (Grand & Toy Limited, Ontario, Canada) onto paper to measure the forewing area as a proxy for body size using a scanner with ImageJ software (Version k 1.45s).

Larval feeding no-choice experiment

To test the hypothesis that DBM larval feeding influences BAW feeding behaviour, a no-choice feeding experiment was conducted (n=10; per treatment) in Experiment 5. The three treatments:

1) DBM damage; 2) mechanical damage; and 3) unifested control plants were obtained as explained in the oviposition experiments above. Five, early third instar BAW larvae were introduced onto the 4th leaf from the bottom of the variously treated canola plants. The leaf with BAW larvae was covered gently using a sieve mesh bag (30 cm X 22 cm). After 72h of feeding activity, the leaves were harvested and actual damage was assessed using a scanner with ImageJ software (Version k 1.45s).

Larval feeding choice experiment

Choice experiments tested the feeding preference of BAW larvae. In Experiment 6, larvae were presented with a choice between canola plants with DBM feeding damage and undamaged canola plants (n=14; per treatment). Experiment 7 tested a choice between canola plants with DBM feeding and mechanically damaged canola plants (n=16; per treatment). DBM damaged, undamaged and mechanically damaged plants were obtained as in the above no-choice oviposition experiment. The choice arena was created by caging two leaves from the fourth position of two differently manipulated plants into a plastic Petri plate (15 cm diam.) with a lid. Three, fourth instar BAW larvae were released into the arena between the leaves and were allowed to feed for 48 h. At the end of the assay, the feeding damage was assessed using a scanner with ImageJ software (Version k 1.45s).

Statistical analysis

Analyses were conducted using R version 3.4.3. Normality and equal variances were tested using Shapiro-Wilk normality test and residuals vs. fit plots, respectively. Non-normal data were transformed to achieve normality and homogeneity of residuals. For all analyses, the significance level was set as α=0.05. Untransformed data are presented in tables and figures. F and p values presented are from the most parsimonious models. Nonsignificant factors were removed from the ANOVA analyses ($p < 0.05$) to achieve model simplification in full and reduced models using AIC values until the most parsimonious model was achieved.

Oviposition no-choice experiment

The total number of eggs laid on canola plants in Exp.1 was treated as the dependent variable in a linear model. Plant manipulation (control, DBM damaged, mechanically damaged) was considered the fixed factor.

Oviposition choice experiments

The total number of eggs laid on either DBM-damaged *vs* control plants in Exp. 2 or DBM-damaged vs mechanically damaged plants in Exp. 3 were compared using a paired t-tests.

Offspring development

Bertha armyworm larval weight was measured at day 14 after introduction to the experimental cages in Exp. 4. The weight of the two larvae in each experimental cage was averaged for the analyses. The average larval weight in each experimental cage was considered as the dependent variable in a linear model. Data were transformed to Y^0.5 for the analyses.

Pupal weight, adult weight and adult forewing area were considered as dependent variables in separate linear mixed effects models. Plant manipulation (control, DBM damaged, mechanically damaged) and sex were considered as fixed factors in all models. Experimental cage was included as a random factor. The initial statistical model included the interaction between the two fixed factors.

Adult longevity was treated as a dependent variable in a generalized linear mixed effects model with a Poisson distribution. Plant manipulation and BAW sex were the fixed factors whereas experimental cage was included as a random factor.

Larval feeding no-choice experiment

Larval feeding damage to each plant in Exp. 5 was treated as the dependent variable in a linear model. Plant manipulation (control, DBM damaged, mechanically damaged) was considered a fixed factor.

Larval feeding choice experiment

Larval feeding damage to the leaves from the differently manipulated plants that were simultaneously presented to BAW in Exp. 6 and 7 was compared using paired t-test.

Results

Oviposition choice and no-choice experiment

There was no difference in the total number of eggs laid by female BAW in the no-choice oviposition experiment (Exp. 1) based on plant manipulation treatment (control, DBM damaged or mechanically damaged plants) ($F_{2,42} = 0.9081$, P = 0.411). Similarly, in choice oviposition experiments, BAW moths showed no preference for oviposition between control plants and those damaged by DBM (t = -1.6826, d.f. = 20, *P* = 0.108) in Exp. 2 and DBM damaged vs mechanically damaged plants (t = 1.2996, d.f. = 19, P = 0.2093) in Exp. 3.

Offspring development

Larval BAW weight was similar when reared on control, DBM damaged and mechanically damaged plants ($F_{2,44} = 0.3583$, $P = 0.7009$) in Exp. 4. Pupal mass, however, was affected by plant treatment as pupae from larvae reared on DBM damaged plants were lighter ($\chi^2 = 6.2758$, $P = 0.0433$) than pupae reared as larvae on undamaged control plants. Adult forewing area was marginally affected by plant manipulation ($\chi^2 = 5.9021$, $P = 0.0523$). Moths had smaller forewing area when reared on DBM damaged plants. Plant treatments did not influence adult weight ($\chi^2 = 0.9976$, $P = 0.6073$) or adult longevity ($\chi^2 = 4.3141$, $P = 0.1157$) in Exp. 4. Across all treatments, females were heavier as pupae ($\chi^2 = 6.8445$, $P = 0.0089$) and adults ($\chi^2 = 25.0246$, $P < 0.0001$) and had a larger forewing area ($\chi^2 = 8.2414$, $P = 0.0041$) as compared with males. Adult longevity, however, was not influenced by sex (($\chi^2 = 1.9993$, $P = 0.1574$) in Exp. 4.

Larval feeding experiments

Bertha armyworm feeding damage was similar ($F_{2,27} = 0.5408$, $P = 0.5884$) on undamaged control plants, plants damaged with DBM and mechanically damaged canola plants in the no choice experiment (Exp. 5). In choice experiments, BAW larvae consumed more leaf area from control plants as compared with DBM damaged plants ($t = 2.534$, d.f. = 13, $P = 0.02494$, Figure 2) in Exp. 6. Whereas, larval BAW fed equally on leaves from plants damaged by DBM and mechanically damaged plants ($t = 0.2995$, d.f. = 15, $P = 0.7687$) in Exp. 7.

Discussion

As BAW and DBM both damage canola by chewing on foliage, I anticipated that BAW females would detect previous feeding damage and avoid laying eggs on DBM-damaged plants. Bertha armyworm females, however, did not exhibit any oviposition preference toward the variously manipulated canola plants. Many female moths use olfactory cues, at least in part, to locate appropriate host plants for oviposition (Thöming & Norli, 2015). Host plant volatiles induced following herbivory (Robert et al., 2012) can alter both olfactory and tactile cues for host finding and acceptance by adult female moths (Feeny et al., 1989). The foliar glucosinolate, sinigrin, in canola plants deters oviposition by BAW, whereas different foliar glucosinolates enhance female BAW oviposition (Ulmer et al., 2002). Larval feeding by DBM on canola induces the production of volatile glucosinolates (Renwick et al., 2006), however, in this experiment, female BAW moths laid the same number of eggs on plants that were undamaged or damaged by DBM larvae. In the current study, BAW were introduced after 24 h of feeding by DBM larvae, which may have not been enough time to strongly influence BAW oviposition. A longer feeding period by the initial herbivore may allow more time for a plant to mount defenses, which could be depleted by the time the second herbivore arrives at the plant (Karban, 2011; Underwood, 2012). In multiple herbivore interactions on *Brassica oleracea* L., longer duration between caterpillar, *P. xylostella* and aphid, *B. brassicae* attack increased resistance to a third herbivore in the system, *Mamestra brassicae* (L.) (Stam et al., 2017).

Bertha armyworm larvae preferred to consume foliage of undamaged plants as compared with DBM damaged plants, however, there was no feeding preference between the mechanically damaged and undamaged plants. This could be due to a difference in hormone levels or defensive compounds in plants that are fed on by caterpillars compared with those that simply receive mechanical damage (Zebelo et al., 2017). In both herbivory and mechanical damage to nicotine, *Nicotiana sylvestris* Speg. and Comes., plants increase JA and nicotine concentration, however, this increase is greater when a larval regurgitant is applied to the artificial wound

(McCloud & Baldwin, 1997). Effectors or symbiotic microbes present in insect saliva have an influence on plant defenses which is absent in the mechanical damage treatment. Effectors in saliva such as glucose oxidase in *Helicoverpa zea* (Boddie) (Musser et al., 2002), the salivary protein MpCOO2 in *Myzus persicae* (Sulzer) and nonproteinaceous components in *P. brassicae* and *S. littoralis* (Bos et al., 2010) induce defenses in host plants. In contrast, symbiotic bacteria in oral secretions suppress plant defenses and increase larval performance of the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) (Chung et al., 2013). Artificial wounding by mechanical means also differs from actual herbivory because larvae consume foliar tissues as they cause damage (McCloud & Baldwin, 1997).

On damaged canola plants, BAW larvae feed less and develop into smaller pupae compared with larvae raised on undamaged canola plants. Canola plants fed on by DBM may hydrolyze glucosinolates, the most important group of secondary metabolites produced by *Brassica* plants. Intact glucosinolates are not toxic to insects, however, glucosinolate metabolites are toxic to generalists (Ute & Meike, 2011). *Brassica* specialists have mechanisms to detoxify glucosinolate metabolites (Jeschke et al., 2016). For instance, a *Brassica* specialist, the cabbage white butterfly, *Pieris rapae* (L.) (Lepidoptera: Pieridae) is able to convert toxic isothiocyanates produced following the hydrolysis of glucosinolates into less toxic nitriles (Wittstock et al., 2004). Generalist herbivores have not evolved detoxification methods and experience negative consequences as a result of consuming these hydrolysis products (Zou et al., 2016). Bertha armyworm has adapted to detoxify some but not all glucosinolates present in *Brassica* species (McCloskey & Isman, 1993). Benzyl isothiocyanate and benzyl glucosinolate are toxic to the generalist fall armyworm larvae, *Spodoptera frugiperda* (Smith), European corn borer larvae, *Ostrinia nubilalis* (Hübner) (Bartelt & Mikolajczak, 1989) and BAW (McCloskey & Isman, 1993). Glucosinolates produced by *Arabidopsis* plants reduce larval weight and delay the development of two generalist-feeding caterpillars: *S. littoralis* and *M. brassicae* (Jeschke et al., 2017). Delayed larval development of these caterpillars results in longer exposure to natural enemies, resulting in an increased chance of predation (Benrey & Denno, 1997; Clancy & Price, 1987).

Pupal weight of insects mostly depends on larval food sources (Harvey & Gols, 2011). As BAW larvae feed less on canola leaves from DBM-damaged plants than from the undamaged plants, I assume that previous herbivory altered the plant to make it a lower quality larval diet for the generalist BAW. Small pupae of the polyphagous fruit fly, *Ceratitis fasciventriss* (Bezzi) (Diptera: Tephritidae) result from larval feeding on diets with high alkaloid levels (Erbout et al., 2009). In the Noctuidae, pupal weight is correlated with fecundity of females (Spitzer et al., 1984; Honěk, 1993), therefore, I assume in BAW that adult fecundity will be suppressed on plants with DBM herbivory. Not only did BAW larvae perform better on control plants than on those damaged by DBM but larvae showed a feeding preference for undamaged canola. It is possible the BAW moths that emerge from small pupae reared on plants previously infested by the specialist DBM will alter their oviposition preference. Although I did not detect an oviposition preference in diet-fed BAW in this study, physiological state is known to influence the response to host plant volatiles in some species of moths (Lemmen-Lechelt et al. 2018). For instance, a negative feeding experience by larvae of the generalist, *S. littoralis* on cabbage plants causes the resulting adults to avoid oviposition on *Brassica* plants (Proffit et al., 2015). There was no influence of previous DBM damage on the resulting adult body weight of BAW moths, but those that eclosed from larvae reared on DBM-damaged plants had smaller wings, which might reduce their dispersal capacity (Stevens et al., 2012).

Both BAW and DBM are important insect pests in canola fields in the Canadian Prairies. This study illustrates how previous damage by a specialist herbivore influences subsequent host use by a generalist herbivore. Early season herbivores may determine the number of late season herbivores and the extent of damage caused by the plant feeders (Viswanathan et al., 2007; Poelman et al., 2008). The generalist BAW larvae preferred to feed on undamaged plants over those that had previously been damaged by the specialist DBM. This could be due to an upregulated defensive response to the initial feeding that also affects the subsequent herbivore. Further, those BAW that did feed on canola plants previously damaged by DBM fed less and developed into smaller pupae and adults with smaller wings than larvae fed on undamaged plants. This study provides additional support for understanding plant-mediated specialist-generalist herbivore interactions in agroecosystems.

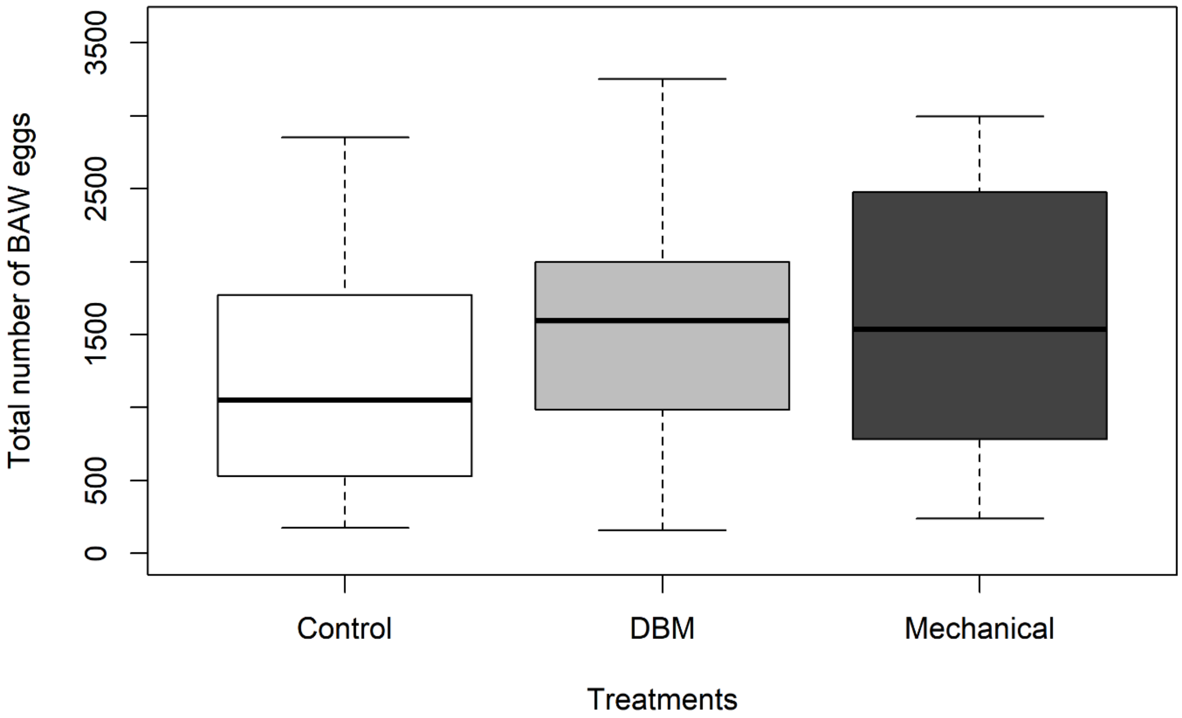


Figure 4-1. The median number of eggs laid by female BAW on variously manipulated canola plants (control, DBM damaged and mechanically damaged) in the no-choice oviposition experiment (Exp. 1) (n=15 per treatment). The midline indicates the median. The bottom and top of the box area indicate the first and third quartiles, respectively. The vertical lines indicate the variability outside the first and third quartiles. Data were analyzed using a linear model. There were no significant differences among treatments (*P* = 0.4110).

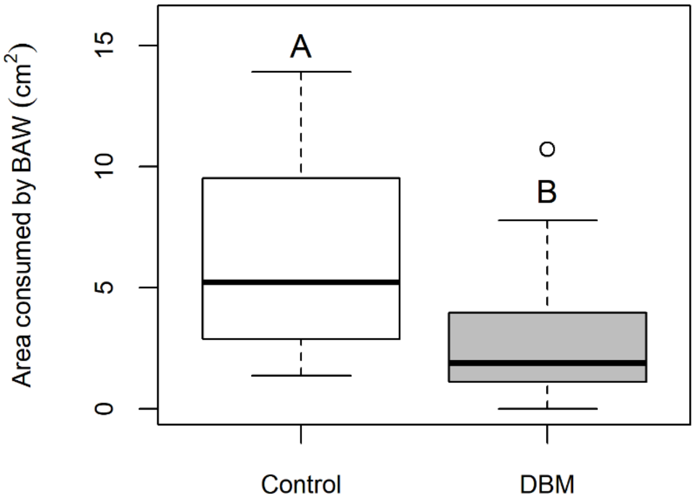


Figure 4-2. Feeding damage by BAW larvae on control (undamaged) and DBM damaged canola plants in the larval feeding choice experiment (Exp. 6) (n=14 per treatment). The midline indicates the median. The bottom and top of the box area indicate the first and third quartiles, respectively. The vertical lines indicate the variability outside the first and third quartiles. Extreme values are indicated as open circles. The midline in the box represents the median. Data were analyzed using a paired t-test. Boxes marked with different letters are significantly different (*P* = 0. 02494).

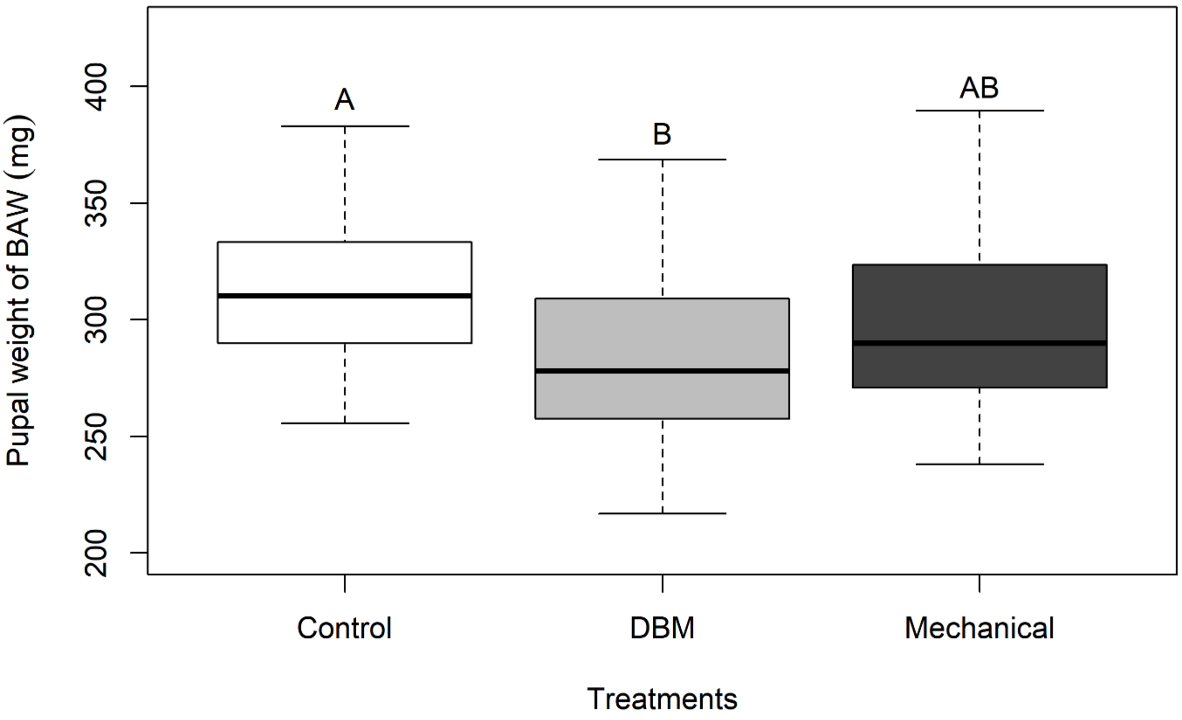


Figure 4-3. The effect of plant host manipulation on the pupal weight of BAW in Exp. 4 (n=16 per treatment). Boxes represent the data distribution within inter-quartile range. The midline indicates the median. The bottom and top of the box area indicate the first and third quartiles, respectively. The vertical lines indicate the variability outside the first and third quartiles. Data were analyzed using a linear mixed model. Boxes marked with different letters represent treatments that are significantly different using Tukey HSD post-hoc comparisons ($P = 0.04337$).

Literature Cited

Agrawal, A. A. 2000. Specificity of induced resistance in wild radish: causes and consequences for two specialist and two generalist caterpillars. Oikos. 89: 493–500.

Agrawal, A. A., and M. F. Sherriffs. **2001**. Induced plant resistance and susceptibility to late-season herbivores of wild radish. Ann. Entomol. Soc. Am. 94: 71–75.

Bartelt, R. J., and K. L. Mikolajczak. **1989**. Toxicity of compounds derived from *Limnanthes alba* seed to fall armyworm (Lepidoptera: Noctuidae) and European corn borer (Lepidoptera: Pyralidae) larvae. J. Econ. Entomol. Econ Entomol. v. 82: 1054–1060.

Benrey, B., and R. F. Denno. **1997**. The slow-growth-high-mortality hypothesis: A test using the cabbage butterfly. Ecology. 78: 987–999.

Bos, J. I. B., D. Prince, M. Pitino, M. E. Maffei, J. Win, and S. A. Hogenhout. **2010**. A Functional genomics approach identifies candidate effectors from the aphid species *Myzus persicae* (Green peach aphid). PLoS Genet. 6: e1001216.

Bucher, G. E., and G. K. Bracken. **1976**. The bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae). Artificial diet and rearing technique. Can. Entomol. 108: 1327–1338.

Chung, S. H., C. Rosa, E. D. Scully, M. Peiffer, J. F. Tooker, K. Hoover, D. S. Luthe, and G. W. Felton. **2013**. Herbivore exploits orally secreted bacteria to suppress plant defenses. Proc. Natl. Acad. Sci. 110: 15728–15733.

Clancy, K. M., and P. W. Price. **1987**. Rapid Herbivore Growth Rapid herbivore growth enhances enemy attack: Sublethal plant defenses remain a paradox. Ecology. 68: 733–737.

Dosdall, L. M. 2010. Do Larvae of Bertha armyworm, *Mamestra configurata* (Walker) (Lepidoptera: Noctuidae), express induced feeding responses? J. Appl. Entomol. 134: 531–

541.

Erbout, N., M. De Meyer, C. Vangestel, and L. Lens. **2009**. Host plant toxicity affects developmental rates in a polyphagous fruit fly: Experimental evidence. Biol. J. Linn. Soc. 97: 728–737.

Feeny, P., E. Städler, I. Åhman, and M. Carter. **1989**. Effects of plant odor on oviposition by the black swallowtail butterfly, *Papilio polyxenes* (Lepidoptera: Papilionidae). J. Insect Behav. 2: 803–827.

Fürstenberg-hägg, J., M. Zagrobelny, and S. Bak. **2013**. Plant defense against insect herbivores. Int. J. Mol. Sci. 14: 10242–10297.

Golizadeh, A., K. Kamali, Y. Fathipour, and H. Abbasipour. **2007**. Temperature-dependent development of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) on two brassicaceous host plants. Insect Sci. 14: 309–316.

Harvey, J. A., and R. Gols. **2011**. Population-related variation in plant defense more strongly affects survival of an herbivore than its solitary parasitoid wasp. J. Chem. Ecol. 37: 1081–1090.

Heidel, A. J., and I. T. Baldwin. **2004**. Microarray analysis of salicylic acid- and jasmonic acid-signalling in responses of *Nicotiana attenuata* to attack by insects from multiple feeding guilds. Plant, Cell Environ. 27: 1362–1373.

Honěk, A. 1993. Intraspecific variation in body size and fecundity in insects : A general relationship. Oikos. 66: 483–492.

Hopkinson, R. F., and J. J. Soroka. **2010**. Air trajectory model applied to an in-depth diagnosis of potential diamondback moth infestations on the Canadian Prairies. Agric. For. Meteorol. 150: 1–11.

Jeschke, V., J. Gershenzon, and D. G. Vassão. **2016**. Insect detoxification of glucosinolates and their hydrolysis products, pp. 199–245. *In* Kopriva, S. (ed.), Adv. Bot. Res. Academic Press.

Jeschke, V., E. E. Kearney, K. Schramm, G. Kunert, A. Shekhov, J. Gershenzon, and D. G. Vassão. **2017**. How glucosinolates affect generalist Lepidopteran larvae: growth, development and glucosinolate metabolism. Front. Plant Sci. 8: 1–12.

Karban, R. 2011. The ecology and evolution of induced resistance against herbivores. Funct. Ecol. 25: 339–347.

Koornneef, A., and C. M. J. Pieterse. **2008**. Cross talk in defense signaling. Plant Physiol. 146: 839–844.

Lemmen-lechelt, J. K., T. J. Wist, and M. L. Evenden. **2018**. State-dependent plasticity in response to host-plant volatiles in a long-lived moth, *Caloptilia fraxinella* (Lepidoptera: Gracillariidae). J. Chem. Ecol. 1–12.

Mathur, V., T. O. G. Tytgat, R. M. de Graaf, V. Kalia, A. Sankara Reddy, L. E. M. Vet, and N. M. van Dam. **2013**. Dealing with double trouble: Consequences of single and double herbivory in *Brassica juncea*. Chemoecology. 23: 71–82.

McCloskey, C., and M. B. Isman. **1993**. Influence of foliar glucosinolates in oilseed rape and mustard on feeding and growth of the bertha armyworm, *Mamestra configurata* Walker. J. Chem. Ecol. 19: 249–266.

McCloud, E. S., and I. T. Baldwin. **1997**. Herbivory and caterpillar regurgitants amplify the wound-induced increases in jasmonic acid but not nicotine in *Nicotiana sylvestris*. Planta. 203: 430–435.

Müller, R., M. de Vos, J. Y. Sun, I. E. Sønderby, B. A. Halkier, U. Wittstock, and G.

Jander. **2010**. Differential effects of indole and aliphatic glucosinolates on lepidopteran herbivores. J. Chem. Ecol. 36: 905–913.

Musser, R. O., S. M. Hum-Musser, H. Eichenseer, M. Peiffer, G. Ervin, J. B. Murphy, and G. W. Felton. **2002**. Caterpillar saliva beats plant defences. Nature. 416: 599–600.

Poelman, E. H., C. Broekgaarden, J. J. A. Van Loon, and M. Dicke. **2008**. Early season herbivore differentially affects plant defence responses to subsequently colonizing herbivores and their abundance in the field. Mol. Ecol. 17: 3352–3365.

Proffit, M., M. A. Khallaf, D. Carrasco, M. C. Larsson, and P. Anderson. **2015**. “Do you remember the first time?” Host plant preference in a moth is modulated by experiences during larval feeding and adult mating. Ecol. Lett. 18: 365–374.

Ratzka, A., H. Vogel, D. J. Kliebenstein, T. Mitchell-Olds, and J. Kroymann. **2002**. Disarming the mustard oil bomb. Proc. Natl. Acad. Sci. 99: 11223–11228.

Renwick, J. A. A., M. Haribal, S. Gouinguené, and E. Städler. **2006**. Isothiocyanates stimulating oviposition by the diamondback moth, *Plutella xylostella*. J. Chem. Ecol. 32: 755–766.

Robert, C. A. M., M. Erb, M. Duployer, C. Zwahlen, G. R. Doyen, and T. C. J. Turlings. **2012**. Herbivore-induced plant volatiles mediate host selection by a root herbivore. New Phytol. 194: 1061–1069.

Shroff, R., F. Vergara, A. Muck, A. Svatos, and J. Gershenzon. **2008**. Nonuniform distribution of glucosinolates in *Arabidopsis thaliana* leaves has important consequences for plant defense. Proc. Natl. Acad. Sci. U. S. A. 105: 6196–6201.

Silva, R., and M. J. Furlong. **2012**. Diamondback moth oviposition: Effects of host plant and herbivory. Entomol. Exp. Appl. 143: 218–230.

Soler, R., F. R. Badenes-Pérez, C. Broekgaarden, S. J. Zheng, A. David, W. Boland, and M. Dicke. **2012**. Plant-mediated facilitation between a leaf-feeding and a phloem-feeding insect in a brassicaceous plant: From insect performance to gene transcription. Funct. Ecol. 26: 156–166.

Spitzer, K., M. Rejmfinek, and T. Soldfin. **1984**. The fecundity and long-term variability in abundance of noctuid moths (Lepidoptera: Noctuidae). Oecologia. 62: 91–93.

Stam, J. M., L. ChrÉtien, M. Dicke, and E. H. Poelman. **2017**. Response of *Brassica oleracea* to temporal variation in attack by two herbivores affects preference and performance of a third herbivore. Ecol. Entomol. 42: 803–815.

Stam, J. M., A. Kroes, Y. Li, R. Gols, J. J. A. Van Loon, E. H. Poelman, and M. Dicke. **2014**. Plant interactions with multiple insect herbivores : from community to genes. Annu. Rev. Plant Biol. 65: 689–713.

Stevens, V. M., A. Trochet, H. Van Dyck, J. Clobert, and M. Baguette. **2012**. How is dispersal integrated in life histories: A quantitative analysis using butterflies. Ecol. Lett. 15: 74–86.

Talekar, N. S., and A. M. Shelton. **1993**. Biology, ecology, and management of the diamonback moth. Annu. Rev. Entomol. 38: 275–301.

Thöming, G., and H. R. Norli. **2015**. Olfactory cues from different plant species in host selection by female pea moths. J. Agric. Food Chem. 63: 2127–2136.

Ulmer, B., C. Gillott, and M. Erlandson. **2002**. Oviposition preferences of bertha armyworm *Mamestra configurata* Walker (Lepidoptera : Noctuidae) on different crucifer cultivars and growth stages. Environ. Entomol. 31: 1135–1141.

Ulmer, B., C. Gillott, and M. Erlandson. **2003**. Conspecific eggs and bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), oviposition site selection. Environ. Entomol. 32: 529–534.

Ulmer, B. J. 2002. Crucifer host plant suitability for bertha armyworm (*Mamestra configurata*) and diamondback moth (Plutella xylostella).

Underwood, N. 2012. When herbivores come back: Effects of repeated damage on induced resistance. Funct. Ecol. 26: 1441–1449.

Ute, W., and B. Meike. **2011**. Glucosinolate breakdown in Arabidopsis: Mechanism, regulation and biological significance. Arab. B. 1–14.

Viswanathan, D. V., O. A. Lifchits, and J. S. Thaler. **2007**. Consequences of sequential attack for resistance to herbivores when plants have specific induced responses. Oikos. 116: 1389–1399.

Viswanathan, D. V., A. J. T. Narwani, and J. S. Thaler. **2005**. Specificity in induced plant responses shapes patterns of herbivore occurrence on *Solanum dulcamara*. Ecology. 86: 886–896.

Walling, L. L. 2000. The myriad plant responses to herbivores. J. Plant Growth Regul. 19: 195–216.

War, A. R., M. G. Paulraj, T. Ahmad, A. A. Buhroo, B. Hussain, S. Ignacimuthu, and H. C. Sharma. **2012**. Mechanisms of plant defense against insect herbivores. Plant Signal. Behav. 7: 1306–1320.

Wittstock, U., N. Agerbirk, E. J. Stauber, C. E. Olsen, M. Hippler, T. Mitchell-Olds, J. Gershenzon, and H. Vogel. **2004**. Successful herbivore attack due to metabolic diversion of a plant chemical defense. Proc. Natl. Acad. Sci. 101: 4859–4864.

Zebelo, S., J. Disi, R. Balusu, B. Reeves, and H. Fadamiro. **2017**. *Spodoptera exigua* modulates gossypol biosynthesis in cotton *Gossypium hirsutum*. J. Plant Interact. 12: 121–127.

Zou, X., Z. Xu, H. Zou, J. Liu, S. Chen, Q. Feng, and S. Zheng. **2016**. Glutathione S-transferase SlGSTE1 in *Spodoptera litura* may be associated with feeding adaptation of host plants. Insect Biochem. Mol. Biol. 70: 32–43.

Züst, T., and A. A. Agrawal. **2017**. Trade-offs between plant growth and defense against insect herbivory: An emerging mechanistic synthesis. Annu. Rev. Plant Biol. 68: 513–534.

Chapter 5: Infection of canola by *Plasmodiophora brassicae*, increases resistance to the generalist herbivore, the bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae)

Abstract

Infection of plants by plant pathogens can result in the upregulation of induced defense responses; plants may be more or less susceptible to attack by insect herbivores following infection. I investigated the interaction between canola, *Brassica napus* L., plants infected with clubroot, *Plasmodiophora brassicae* Woronin, and a generalist herbivore the bertha armyworm (BAW) *Mamestra configurata* Walker using two canola hybrids that varied in susceptibility to *P. brassicae* disease. Analysis of salicylic acid (SA) and jasmonic acid (JA) and its conjugates served as a direct measure of defensive response of the experimental plants. Female adult BAW discriminate between canola plants inoculated with *P. brassicae* and disease-free plants. Adult female moths preferentially lay eggs on the hybrid variety susceptible to *P. brassicae* in the absence of infection. Inoculation of resistant canola with *P. brassicae* , however, does not influence oviposition by female BAW. Differences in the volatile profile released by the different canola varieties with and without inoculation of *P. brassicae* may be driving the oviposition response of female moths. The fitness of BAW larvae is reduced when they are reared on susceptible canola inoculated with *P. brassicae* . The levels of SA and its conjugates in inoculated susceptible canola plants with *P. brassicae* are higher than disease-free susceptible plants. I conclude that suppression of BAW oviposition and offspring fitness may be in part due to an increase in total SA in susceptible canola plants following *P. brassicae* inoculation.

Introduction

Plants are members of complex ecosystems that interact with both belowground and aboveground communities, which can consist of microbes, nematodes, insects and other organisms. Interactions between above and belowground communities may be mediated by changes in the host plant following attack (Ponzio et al. 2013). Initial plant colonization by pathogens or herbivores can influence subsequent attacks by the same or different organisms in either the below or the aboveground communities (Pangesti et al. 2013). Plant use by herbivores and phytopathogens can alter plant resistance to subsequent attacks because the defense pathways that respond to these different guilds are interconnected (Stout et al. 1999). For example, infection with *Fusarium graminearum* Schwabe, which causes fusarium head blight disease in wheat, makes wheat crops repellent to the grain aphid, *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae) (Drakulic et al. 2015). In contrast, infection with *Candidatus* phytoplasma, which causes cassava witches broom disease, positively affects both specialist and generalist mealybug abundance (Homoptera: Pseudococcidae) (Wyckhuys et al. 2017). Above ground herbivore attack by the whitefly, *Bemisia tabaci* (Gennadius) increases resistance to leaf pathogenic bacteria, *Xanthomonas axonopodis* pv. *Vesicatoria*, and the soilborne root pathogen, *Ralstonia solanacearum*, in *Capsicum annuum* L. (Yi et al. 2011). Therefore, plant defense to initial attack may change interactions in multiple guilds of organisms associated with both above and belowground plant communities.

Induced plant defenses in response to insect and pathogen attack can activate the salicylic acid (SA), jasmonic acid (JA), and ethylene-dependent signaling pathways in plants (Thompson and Goggin 2006). The JA pathway is activated by chewing insects (Ye et al. 2012) and necrotrophic pathogens (Koornneef and Pieterse 2008), whereas the SA pathway is activated by biotrophic pathogens and sucking insects (Röder et al. 2011). Because induction of SA and JA pathways is energetically costly for plants (van Hulten et al. 2006), "cross-talk" between these signaling pathways occurs to minimize the energetic costs of induced defenses (Mouttet et al. 2011).

A diverse array of plant pathogens and both generalist and specialist herbivores impact canola plants (*Brassica napus* L.) in managed ecosystems (Médiène et al. 2011). The soil-borne obligate biotrophic protist, *Plasmodiophora brassicae* Woronin, which causes clubroot disease is one of the most damaging pathogens associated with *Brassica* crops worldwide (Dixon 2009). In the Canadian Prairie Provinces, yield in infected canola fields can be reduced by 30-100% (Strelkov et al. 2007, Hwang et al. 2011). Pathogen spores can survive in the soil for up to 20 years, which makes control difficult (Wallenhammar 1996). Infection of canola roots by *P. brassicae* occurs in two stages, first by root hair infection and then root cortical colonization (Tommerup and Ingram 1971). Current canola cultivars that are resistant to *P. brassicae* disease provide the most effective way to control the disease (Diederichsen et al. 2009). Resistant canola cultivars prevent degradation of the secondary wall of the root xylem and restrict movement of the amoeboid forms of the protist during the root colonization stage (Donald et al. 2008). Overuse of resistant cultivars, however, result in the evolution of resistance in the pathogen and increase the susceptibility of canola to *P. brassicae* disease (Diederichsen et al. 2014).

Bertha armyworm (BAW), *Mamestra configurata* Walker, is a generalist herbivore that feeds above ground on canola foliage and stems. Larval feeding in outbreak BAW populations in the Canadian Prairies results in monetary loss through yield loss and the use of costly chemical control measures (Mason et al. 1998). Bertha armyworm adult females oviposit on canola leaves and the larval offspring initially feed on the plant on which they hatched. *Plasmodiophora* *brassicae* and BAW infestations overlap temporally and spatially in canola agroecosystems on the Canadian Prairies. It is therefore important to study the potential canola-mediated interaction between the two types of attackers. Here we examine if canola plants inoculated with *P. brassicae* induce a defensive response in canola that subsequently affects the host use by BAW.

Plasmodiophora brassicae infection induces a defensive response in *Arabidopsis*. Microarray analyses show the SA signaling pathway is more important in resistance to *P. brassicae* infection than the JA pathway during the early root hair infection stage (Mouttet et al. 2011). Accumulation of SA and the methylated form of SA (MeSA) occurs near the inflorescence emergence stage in *Arabidopsis* (Ludwig-Müller et al. 2015). Resistance or susceptibility to *P. brassicae* disease is affected by the genetic background of various *Arabidopsis* lines (Lemarié et al. 2015). Salicylic acid alone is not sufficient for defense of *Arabidopsis* against *P. brassicae* infection (Lovelock et al. 2016). Apart from the SA and JA contribution to resistance against *P. brassicae* in *Arabidopsis*, cytokinin, abscisic acid and auxin are also upregulated in response to *P. brassicae* infection (Devos et al. 2005, Siemens et al. 2006, Jahn et al. 2013). This suggests that *P. brassicae* infection might activate additional defense pathways compared with other biotroph pathogens, or that protists, in general, might activate common pathways in a different manner which is not yet clearly understood (Ludwig-Müller 2009).

In the present study, I focused on induction of the SA and JA pathways in canola plants in response to *P. brassicae* infection, as these pathways affect plant defense against insect herbivory (Schweiger et al. 2014). Apart from the induction of plant hormone signalling pathways, pathogen infection can also alter the profile of volatile organic compounds (VOCs) released from plants (Djonovic et al. 2007). The volatile organic compounds released as a result of root pathogen infection can attract or deter herbivorous insects to their plant hosts (Rostás et al. 2006, Piesik et al. 2011). The goal of this study is to understand the plant-mediated mechanisms involved in interactions between a belowground pathogen and an above ground insect herbivore associated with canola. I assess the effect of *P. brassicae* disease on oviposition and offspring fitness of BAW on both *P. brassicae* -susceptible and resistant canola. This study is the first to show the influence of *P. brassicae* infection on subsequent host use by an insect herbivore on different commercialized host genotypes.

Materials and methods

Plasmodiophora brassicae inoculation

Clubroot galls were collected from single spore-isolates from Alberta, Canada (Xue et al. 2008). The single spore isolates were identified as pathotype 3 (Strelkov et al. 2006), as described by Williams (1966). The galls were air-dried and macerated with sterilized water in a blender. The suspension was passed through several layers of cheesecloth. The *P. brassicae* spore concentration in the suspension was determined with a hemocytometer at 400 X magnification. Depending on the experiment, inoculum concentrations of 10^7, 10^5 or 10^4 spores/ml were prepared and administered to canola seedlings 1-wk after germination. Spore inoculation was performed using a standard method with modifications (Hwang et al. 2011). One-week-old seedlings were dipped in either 10^7, 10^5 or 10^4 spores/ml inoculum suspension for 10 seconds. Treated seedlings were planted individually in pots (15.3 cm-diameter) filled with Sunshine Potting Mix-4 (Sun Gro horticulture Canada Ltd, Alberta, Canada). An additional 1.0 mL of spore suspension was added at the base of the seedling. Non-inoculated control plants were handled in the same manner as inoculated plants, but instead of treatment with the spore solution they received sterilized water.

Plants

Two hybrid canola varieties that differed in their susceptibility to *P. brassicae* were used in the experiments. Seeds of the susceptible variety, Pioneer '45H26', and the resistant variety, Pioneer '45H29' (Pioneer Hi-Bred Limited, Ontario, Canada), were germinated on a moistened filter paper for 1-wk. Seedlings were inoculated with either *P. brassicae* or sterilized water and planted as described above. Plants were held at 21 °C under a 16h L: 8h D photo-regime in a greenhouse. Plants were watered from the bottom of the pots with tap water (pH adjusted with HCL to 6.4). Plants were fertilized with 20-20-20 (nitrogen: phosphorous: potassium; Plant products, Co, Ltd., Brampton, Ontario, Canada) from weeks 3-5 after seed germination. All experiments except the phytohormone analyses were conducted when plants were at the flowering stage because BAW prefers to lay more eggs when plants are in flower (Ulmer et al. 2002). Phytohormone analyses were conducted when plants were 1-wk prior to flowering.

Insects

The BAW colony was originally obtained from Agriculture and Agri-food Canada, Saskatoon Research Centre where it had been maintained for several years. Bertha armyworm larvae were reared on an artificial diet (Bucher and Bracken 1976) under a 16h L: 8h D photoregime at 21°C. Canola plants (variety 'Q2', Edmonton Research Station, Alberta, Canada) (Stringam et al. 1999) were used as an oviposition substrate for adult female moths. Eggs were collected from canola leaves and the emergent larvae were transferred to the artificial diet without further exposure to any plant material. At the end of larval development, pupae were separated by sex and kept in same-sex groups in 500 mL transparent plastic containers with screen lids until adult emergence. Moths were collected within 24 h of eclosion and individual pairs of moths were housed in separate 500 ml plastic containers for two days to ensure females were mated prior to the oviposition experiments. Five percent (weight/volume) honey and sucrose in water was supplied to moth pairs through a dental wick. After a 48 h mating period, three pairs of moths were released into a single plastic nylon mesh cage (47.5 X 47.5 X 93.0 cm) for use in the experiments described below.

Oviposition no-choice experiment

A no-choice oviposition experiment was conducted to determine if *P. brassicae* infection influenced host acceptance by BAW females on susceptible and resistant canola hybrids (n=18 per treatment). *Plasmodiophora brassicae* pathotype 3 inoculum (10^7 spores/mL) was prepared and administered 1-wk post seed germination. At flowering, moths were introduced into cages housing individual plants in one of the following treatments: 1) non-inoculated, susceptible canola; 2) inoculated, susceptible canola; 3) non-inoculated, resistant canola; and 4) inoculated, resistant canola. Moths laid eggs for 4 days after which, the eggs were counted and plant fresh weight, number of leaves and plant height were measured. Plants were uprooted and the clubroot disease severity was assessed based on root galling on a 0-3 rating; 0 = no galls, 1 = a few small galls, 2 = moderate galling, and 3 = severe galling (Kuginuki et al. 1999). In susceptible canola, severely infected plants (rating 3) whereas in resistant canola, rating0 were included for statistical analyses. Resistant canola plants without club symptoms were selected the experiment. The total number of eggs laid on each plant was treated as the dependent variable in a linear mixed effects model (R version 3.4.3). Prior to analyses, data were tested for normality using the Shapiro-Wilk test and for equal variances using visual interpretation of residual versus fitted values plots. The canola variety and *P. brassicae* inoculation and their interaction were considered as fixed factors. Time (block) was included as a random factor. Individual treatment means were compared using LS means with Tukey HSD post-hoc comparisons. The plant fresh weight, total number of leaves and plant height were considered as dependent variables in each of three linear mixed effects models (R version 3.4.3). Canola hybrids, *P. brassicae* inoculation and their interaction were considered as fixed factors, whereas time (block) was considered as a random factor. Means were compared using LS means with Tuckey HSD post-hoc comparisons ($P<0.05$). In all models, simplification was achieved by removal of nonsignificant factors from the model using AIC values.

Oviposition choice experiments

Several oviposition choice experiments tested the ability of BAW females to distinguish inoculated from non-inoculated canola plants. Choice experiments were conducted using 1) *P. brassicae* resistant (n=18 per treatment); or 2) susceptible (n=22 per treatment) canola plants. One of each inoculated (10^7 spores/mL) and non-inoculated canola plants of the same variety were housed together in nylon mesh cages. Five pairs of mated moths were introduced to each cage containing a pair of plants. Moths laid eggs for 3 days, after which eggs were counted. An additional experiment (n=21 per treatment) was conducted using a low spore load (10^4 spores/mL) to inoculate susceptible canola plants to determine if infection with a low spore dose, that is less damaging to the plant, could influence BAW oviposition. The total number of eggs laid on inoculated and non-inoculated plants within each canola variety was compared using paired t-tests (R version 3.4.3).

Larval development

To determine the effect of *P. brassicae* infection on host use by BAW larvae, a larval development experiment was conducted. Two, third-instar larvae were introduced to an individual canola plant in one of the following treatments (n=17 per treatment): 1) non-inoculated, susceptible canola; 2) inoculated, susceptible canola; 3) non-inoculated, resistant canola; and 4) inoculated, resistant canola. Inoculated plants were treated with a *P. brassicae* inoculum of 10^5 spores/mL in this experiment. Larval weight was measured 1-wk after introduction to experimental cages. Larvae fed on the variously treated plants until pupation (2 per plant). Pupae were collected from experimental cages within 48 h of pupation. Each pupa was kept in a separate 118 mL transparent plastic container with screen lids until adult emergence. Emerged adults were kept in separate containers until death. Pupal and adult weights were measured to 0.01 mg using a microbalance (Mettler Toledo XS105, Ontario, Canada). Adult longevity was recorded at death for each individual moth when adult weight was measured. Adult forewings were carefully removed and glued onto paper to measure wing area using a desktop scanner with version 1.45 Image J software (Sarfraz et al. 2009). Larval weight 1-wk after introduction to the variously treated canola plants was averaged between the two larvae in each cage for analysis. Average larval weight was treated as the dependent variable in a linear model (R version 3.4.3). The canola variety and *P. brassicae* inoculation treatments and their interaction were considered as fixed factors. Pupal weight, adult weight, total forewing area and adult longevity were treated as dependent variables in separate linear mixed effects models (R version 3.4.3). The canola variety and *P. brassicae* inoculation and interaction between canola variety and *P. brassicae* inoculation were considered as fixed factors and each experimental cage was treated as a random factor. Adult longevity was transformed to Y^(0.7) to achieve normality. Means comparison was conducted using LS means with Tukey HSD post-hoc comparisons.

Phytohormone analyses

Phytohormone analyses were conducted to determine the level of defense induced by inoculation with *P. brassicae* in both the *P. brassicae* resistant and susceptible canola varieties. The third and fourth leaves from the bottom of 5-wk old canola plants just prior to flowering were harvested from three plants in each of the following treatments: 1) non-inoculated, susceptible canola; 2) inoculated, susceptible canola; 3) non-inoculated, resistant canola; and 4) inoculated, resistant canola. Five hundred mg of foliage was snap frozen immediately in liquid nitrogen. Samples were stored in -80°C until transport on dry ice to the National Research Council of Canada, Saskatoon, Canada for analyses. Quantification of SA, JA and conjugates was performed using a method described by Murmu et al. (2014), with modifications. Preparation of samples, standards and analyses were conducted at the National Research Council of Canada. Deuterated forms of hormones (2,2-d_2-jasmonic acid, 3,4,5,6-d_4-2-hydroxybenzoic acid and 12,12,12-d_3-jasmonic acid isoleucine) were used as internal standards. Ultra-performance liquid chromatography-electrospray tandem mass spectrometry (UPLC/ESI-MS/MS system, Waters Corporation, Milford, USA) was used to analyze hormone levels with MassLynx v4.1 software (Waters Inc.). Trace amounts were quantified with QuanLynx v4.1 software (Waters Inc.). Calibration curves were prepared for SA, JA and conjugates. Samples, internal standard blanks and solvent blanks were also analyzed along with the tissue samples. I conducted statistical analyses for only SA and conjugated SA levels since JA and JA-Ile levels were found to be only minute levels. The amount of SA and conjugated SA were treated as the dependent variable in separate linear models to compare inoculated and non-inoculated susceptible or resistant varieties.

Volatile collection

Volatile organic compounds (VOCs) released from the variously treated canola plants were analyzed to determine if VOC emission is influenced by inoculation with *P. brassicae* in both the *P. brassicae* resistant and susceptible canola varieties. At flowering, volatiles released by inoculated and non-inoculated susceptible and resistant canola plants were collected using SPME fibers. Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) (film thickness 50/30 µm) SPME fibers (Supelco, Bellefonte, PA, USA) were used for volatile collection. Each SPME fiber was conditioned at 270 °C under a constant Helium stream for 30 minutes prior to use for volatile collection. Canola plants were positioned within 100 cm height metal tomato cages (Canadian Tire, Canada) and enclosed with an ostrich oven bag (size: 76 X 122 cm, True Liberty, CA, USA). A single SPME fiber was located above each canola plant in the enclosed cage for 72h (n=3; per treatment). Another SPME fiber was located in an empty bag as mentioned above without the canola plant for treatment comparisons (control). After VOC collection, the SPME fiber was inserted into the GC×GC-TOFMS (Leco Instruments, St. Joseph, MI). injection port, and then thermally desorbed at 250ºC for 3 minutes.

Two-dimensional gas chromatography-time-of-flight mass spectrometry analyses (GC x GC-TOFMS).

VOC profiles were collected using a Leco Pegasus 4D GC×GC-TOFMS (Leco Instruments, St. Joseph, MI). The columns used for the first and second dimensions were a 30 m × 0.25 µm; 1 µm film thickness Rtx-5MS (Chromatographic Specialties) and a 1.8 m × 0.25 mm, 0.5 µm film thickness Rtx-200MS (Chromatographic Specialties), respectively. Helium (5.0 grade; Praxair, Edmonton, AB) was used as the carrier gas with flow controlled at 1.5 mL/min. The analytes were desorbed in the split/splitless injection port of the GC×GC TOFMS using an inlet temperature set at 250 °C, operating in splitless mode. The 69.66 min GC method began with an initial oven temperature of 40 °C for 3 min, followed by a ramp of 3 °C/min up to 240 °C in the first oven. Relative to the primary oven, the secondary oven was programed to have a constant offset of +5 ºC and the modulator a constant offset of +10 ºC. The modulation period was 3.0 s. The TOF-MS had an acquisition rate of 100 Hz and acquired over a mass range of m/z 10-700. The detector voltage was -1350 V, the ion source temperature was 200 ºC, and the MS transfer line temperature was 240 ºC.

GC×GC-TOFMS data were processed using ChromaTOF® (v.4.43; Leco). For processing, the baseline offset was set above the middle of the noise (0.7), the minimum S/N for the base peak and the sub-peaks was set at 6, and the data were auto smoothed by the software. The first dimension peak width was set at 8 s while the second dimension peak width was set at 0.16 s.

The Statistical Compare features of ChromaTOF® were used to align each analyte found commonly across the samples. The peak tables were aligned based on the parameters of retention time (first- and second-dimension) and mass spectra. The first dimension shift tolerance was set to ±1 modulation period (3s) and for the second dimension the tolerance was set to 0 s. For mass spectral matching, the minimum similarity was set at 750 and the mass threshold at 10%. Statistical Compare was constrained to accept only analytes found in at least five samples or analytes that were present in at least 50% of the chromatograms. The aligned peak table was exported as a .csv file for multivariate analysis (Principal Component Analysis; PCA) using custom routines written in MATLAB® R2017a, Windows 64-bit version (The Mathworks Inc., Natick, MA, USA), with multivariate statistical analysis performed using PLS Toolbox 8.5.2 (Eigenvector Research Inc., Wenatchee, WA, USA). An in-house algorithm was used to perform variable selection and model optimization on the peak table. This algorithm is based on a hybrid backward elimination/forward selection (BE/FS) approach that relies on cluster resolution (CR) as a model quality metric/objective function (Sinkov and Harynuk 2011, Sinkov et al. 2014, Adutwum et al. 2017). Retention indices for the first dimension of each compound were calculated using Van den Dool and Kratz definition (1963) for temperature-programming measurements.

Results

Oviposition no-choice experiment

The total number of eggs laid on canola plants in the no-choice experiment was influenced by a significant interaction between *P. brassicae* inoculation and canola variety ($\chi^2 = 9.2501$, P=0.0023) (Figure 5-1). Fewer eggs were laid on susceptible plants inoculated with *P. brassicae* as compared with non-inoculated susceptible plants. Oviposition was not influenced by *P. brassicae* inoculation in resistant plants. Following inoculation with *P. brassicae* , there was no reduced plant growth in the resistant canola. *Plasmodiophora brassicae* inoculation and canola variety impacted plant fresh weight ($\chi^2 = 146.38$, $P<0.0001$), plant leaf number ($\chi^2 = 44.2206$, $P<0.0001$) and plant height ($\chi^2 = 78.096$, $P<0.0001$). *Plasmodiophora brassicae* inoculation reduced the fresh weight, the number of leaves and the plant height of susceptible canola plants.

Oviposition choice experiments

Bertha armyworm females preferred the non-inoculated *P. brassicae* susceptible canola over inoculated susceptible plants (spore concentration: 10^7 spores/ml) ($t_{21} = 6.8595$, $P = 0.0001$) (Fig.2a). There was no oviposition preference between inoculated and non-inoculated *P. brassicae* resistant plants ($t_{17} = -0.2087$, $P = 0.8371$). There was no effect of inoculation of susceptible canola with the lower *P. brassicae* spore inoculation (10^4 spores/mL) on oviposition by BAW females ($t_{20} = 1.5824$, $P = 0.1292$) (Fig. 5-2b).

Insect development

There was a main effect of *P. brassicae* inoculation on larval weight that showed larvae reared on canola inoculated with *P. brassicae* were heavier than on non-inoculated plants ($F_{1,68} = 10.8759$, $P = 0.0016$) (Fig. 3). Pupal weight was impacted by the variety of canola that larvae were reared on ($\chi^2 = 15.996$, $P<0.0001$), *P. brassicae* inoculation ($\chi^2 = 25.8084$, $P<0.0001$) and sex ($\chi^2 = 12.8887$, $P = 0.0003$). Larvae reared on *P. brassicae* inoculated canola, regardless of variety, had smaller pupae than those reared on non-inoculated canola plants (Figure 5-4). Female pupae were heavier than males.

Adult weight was impacted by an interaction between canola variety and *P. brassicae* inoculation ($\chi^2 = 4.5672$, $P = 0.0326$). Heavier adults emerged after feeding as larvae on non-inoculated susceptible plants as compared with those on susceptible plants inoculated with *P.*

brassicae . Adult weight, however, was not influenced by inoculation in resistant plants. Adult forewing area was influenced by an interaction between canola variety and *P. brassicae* inoculation ($\chi^2 = 5.308$, $P = 0.0212$). Smaller forewings occurred on adults reared as larvae on inoculated, susceptible plants as compared to those reared on non-inoculated susceptible plants. Forewing area, however, was not affected by inoculation of resistant plants. Adult moth longevity was not affected by canola variety ($\chi^2 = 2.4126$, $P = 0.1204$) or *P. brassicae* inoculation ($\chi^2 = 2.696$, $P = 0.1006$). Moth sex impacted adult longevity ($\chi^2 = 51.7165$, $P <$ 0.0001) as females lived longer than males.

Plant hormone analyses

Higher amount of SA ($F_{1,4} = 19.114$, $P = 0.0119$) and SA conjugates ($F_{1,4} = 455.46$, $P = 0.0001$) were higherfound in inoculated susceptible plants as compared to non-inoculated susceptible plants (Table 5-1). Similar levels of SA ($F_{1,4} = 0.2841$, $P = 0.6223$) and SA conjugates ($F_{1,4} =$ 0.2203, $P = 0.6632$) were found in both inoculated and non-inoculated resistant plants (Table 5-1). Plant hormone analyses of *P. brassicae* inoculated and non-inoculated canola plants indicated that overall levels of SA in *P. brassicae* inoculated samples from both the *P. brassicae*-susceptible and *P. brassicae*-resistant varieties are higher than those in the non-inoculated samples (Table 5-1). The overall JA content was lower in *P. brassicae* susceptible than *P. brassicae* resistant canola (Table 5-1). The overall JA levels in samples inoculated with *P. brassicae* from both canola varieties were lower than those in the respective non-inoculated samples (Table 5-1.).

Volatile organic compounds

GC×GC-TOFMS is a powerful tool to separate complex mixtures of VOCs. More than 200 peaks were detected in a typical chromatogram. The GC×GC chromatogram of *P. brassicae* inoculated canola plant profile is included in the Appendix A.

Chemometric results

Principal component analysis (PCA) was applied to distinguish the VOC profiles between the three types of treatment comparisons: 1) inoculated vs non-inoculated, 2) susceptible *vs.* resistant) and 3) BAW preference *vs.* non-preference. Bertha armyworm preference *vs*. non-preference groupings were based oviposition rate and larval performance on the variously manipulated plants. A lower number of eggs on inoculated susceptible plants and smaller larval weight on non-inoculated resistant plants resulted in the grouping of these treatments as 'non-prefered'for the analyses. In contrast, a higher number of eggs on non-inoculated susceptible plants and higher larval weight on inoculated resistant plants resulted in the grouping of these treatments as 'prefered'. PCA analyses were performed using all the variables (peaks) recovered and on a subset of variables selected using the in-house feature selection algorithm. All comparisons showed separation of the VOC emissions between treatment comparisons (Appendix A) and a better separation between groups was achieved after the variable selection. Variables selected are shown in (Appendix A). Tentative identifications of the compounds were made based on library matches and comparisons with the first dimension retention index (Appendix A). For the comparison of the VOC profile based on BAW preference (non-inoculated susceptible canola and inoculated resistant canola) *vs.* the BAW non-preference (inoculated susceptible and non-inoculated resistant canola), 22 variables (Figure 5-7) were selected and five compounds were tentatively identified (Appendix A). These compounds were nonane, β-myrcene, (2*E*)-2-nonene, acetic acid and limonene. In the comparison of susceptible and resistant canola, 18 variables were selected (Figure 5-6) and 3 compounds were tentatively identified (AppendixB). These compounds were 1,2-dimethyl-benzene, (e)-β-ocimene, and methoxy-Benzene. Based on the comparison between *P. brassicae* inoculated *vs* non-inoculated canola plants, eight compounds were tentatively identified (Figure 5-5) (Appendix A).

Discussion

Overall, I found that female BAW are able to distinguish between plants inoculated with *P. brassicae* and adjust their subsequent oviposition behaviour. Moth detection of the different VOC profiles emitted from the different canola varieties with varying disease status could be the mechanism driving the alteration of oviposition behaviour. Female BAW layed fewer eggs on the susceptible canola variety when it is inoculated with *P. brassicae* than on non-inoculated susceptible canola plants. Oviposition, however, was not influenced following *P. brassicae* inoculation in resistant plants. Not only could BAW moths distinguish between inoculated and non-inoculated susceptible plants for oviposition, but the resulting offspring were smaller by *P. brassicae* infection when reared on susceptible canola. Overall, lower levels of JA in inoculated plants may influence larval development on *P. brassicae* inoculated canola plants.

Bertha armyworm oviposition is influenced by an interaction between *P. brassicae* inoculation and canola variety. Inoculated susceptible plants have fewer leaves and an overall smaller biomass and receive fewer BAW eggs. There may be fewer oviposition sites on these plants because BAW lay eggs mostly on the underside of leaves (Ulmer et al. 2002). Apart from leaves, *P. brassicae* infection can induce root galling that decreases the surface area of roots, and reduces water and nutrient absorbance (Dixon 2006). Although I supplied similar fertilizer treatments to all plants, *P. brassicae* inoculated susceptible plants could be deficient in nutrients and render these plants less attractive to herbivores. The *Brassica* specialists, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and *Pieris rapae* (L.) (Lepidoptera: Pieridae) lay more eggs on nutritious *Brassica* plants as compared to nutrient-stressed plants (Sarfraz et al. 2009, Myers 1985). Bertha armyworm oviposition is affected by plant nutrition, as moderate and highly fertilized plants receive more eggs in oviposition preference experiments (Chapter 2). Similar to the findings of Hwang et al. (2013), in the current study inoculated susceptible canola plants are shorter than non-inoculated plants, but inoculation does not influence plant biomass in resistant plants. When I inoculated resistant plants, a few plants became mildly symptomatic, however, I omitted those plants from the experiment to keep consistency in the *P. brassicae* severity. In field trials, 14% of inoculated, resistant canola plants had some root galling (Hwang et al. 2013).

Host plant searching behaviour in insects depends on visual, olfactory and tactile cues (Talsma et al. 2008, Rojas and Virgen 2003). *Plasmodiophora brassicae* infection affects the volatile profile of canola through differences in several volatile compounds released (Appendix A). Bertha armyworm uses olfaction, at least in part, for host searching as oviposition increases in the presence of chemical cues associated with conspecific eggs (Ulmer et al. 2003). The larger plant size of non-inoculated susceptible plants may enhance both visual and olfactory cues of the host plant to BAW females. Oviposition behaviour of the specialist butterfly, *Melitaea cinxia* L. (Lepidoptera: Nymphalidae) is influenced by plant size and iridoid glycosides content of the host plant, *Plantago lanceolate* (Talsma et al. 2008). Infection of citrus trees with the bacterium, *Candidatus liberibacter asiaticus* (Las) results in induced release of methyl salicylate, which attracts the herbivorous insect, Asian citrus psyllid *Diaphorina citri* Kuwayama

(Hemiptera: Psyllidae) (Martini et al. 2014). Root pathogens influence the production of a variety of plant secondary metabolites including: 1) green leaf volatiles (Hassan et al. 2015); 2) terpenoids (Wäckers et al. 2004): and 3) glucosinolates (Ludwig-M**ü**ller et al. 1999). These compounds are synthesized in the roots and are transported to the shoots, which can subsequently influence oviposition behaviour of leaf-feeding insects (Qiu et al. 2009, McCallum et al. 2011).

The canola varieties that I used for this experiment may have different levels of foliar glucosinolates that could impact BAW oviposition preference. Bertha armyworm adults lay fewer eggs on plants that have high foliar levels of sinigrin (Ulmer et al., 2002). In contrast, the foliar glucosinolate, sinalbin, enhances BAW oviposition on *Sinapsis alba* L. (Ulmer et al., 2002). These results suggest that BAW females make decisions regarding oviposition on *Brassica* species based on the glucosinolate profile of the plant. Higher total glucosinolate content occurred in roots of two *P. brassicae* susceptible Chinese cabbage varieties as compared with resistant varieties (Ludwig-Müller et al. 1997). Moreover, in the current study, aromatic glucosinolates were enhanced during early infection in *P. brassicae* in susceptible varieties. As the low dose *P. brassicae* inoculum does not severely affect plant morphology, it is likely that BAW uses olfactory cues to detect infected plants and guide oviposition decisions. Oviposition of BAW was not influenced by *P. brassicae* inoculation in resistant plants. The volatile profiles of the non-inoculated susceptible canola did not differ from the inoculated resistant canola in this study.

Larvae reared on susceptible canola plants inoculated with *P. brassicae* were heavier than larvae reared on uninoculated plants. The inoculated susceptible plants have lower levels of JA, which is associated with better larval growth in other insect species (Qiu et al. 2009).

Ultimately, BAW larval performance was negatively influenced by *P. brassicae* infection as larvae reared on inoculated susceptible plants developed into smaller pupae. Larval weight varies until pupation which makes pupal weight a better proxy for offspring fitness (Miller 2005). *Plasmodiophora brassicae* inoculation of the susceptible plants resulted in smaller plants that provide less food for BAW larvae. Although I measured the larval weight of BAW between the 4 and 5th instar, most feeding occurs in the 6th instar. The adults reared as larvae on *P. brassicae* inoculated plants had smaller forewings, which could influence fitness and dispersal of adult BAW, as occurs in other insect species (Honěk 1993, Stevens et al. 2012). Interestingly, inoculation of the resistant variety with *P. brassicae* also resulted in heavier larvae but pupal weight was not affected.

Glucosinolate content in the canola varieties tested here will likely change after herbivory and *P. brassicae* infection. These changes can lead to plant-mediated interactions among the organisms utilizing the plant resource. Black mustard, *Brassica nigra* L. is induced to produce more glucosinolates following *P. rapae* herbivory (Traw 2002). Glucosinolate content is higher in susceptible Chinese cabbage varieties following *P. brassicae* infection (Ludwig-Müller et al. 1997). Indol and allyl glucosinolate (sinigrin) reduces growth and survival, and delays development time of the specialist herbivore, *P. rapae* (Agrawal and Kurashige 2003). Sinigrin, a glucosinolate found in *Brassica*, negatively influences BAW larval herbivory (Shields and Mitchell 1995).

Inoculation with *P. brassicae* influenced plant hormone levels in both the susceptible and resistant canola varieties. The levels of SA and its conjugates are higher in inoculated plants of both the resistant and susceptible varieties compared with non-inoculated plants and this is more pronounced in the susceptible plants. Similar effects of *P. brassicae* inoculation have been reported in studies using *Arabidopsis*, the model Brassicaceae species. Higher SA content occurs in both root and leaf tissues after inoculation with *P. brassicae* in *Arabidopsis*. The accumulation of SA is higher in foliar tissues as compared to roots (Ludwig-Müller et al. 2015). In the current experiment, inoculation with *P. brassicae* resulted in higher levels of SA and its conjugates in foliar tissues of susceptible canola after 5-wks. Methyl salicylate (MeSA) is a conjugate of SA which is involved in systematic signaling in plants (Dempsey and Klessig 2017). A detectable level of MeSA is emitted by *P. brassicae* -inoculated *Arabidopsis* plants. It is possible that SA in inoculated root tissues converts to MeSA via methyltransferase, an enzyme found in the *P. brassicae* pathogen (Ludwig-Müller et al. 2015). Methyl salicylate is transported from root to shoot and converted into SA as storage form or emitted as MeSA (Ludwig-Müller et al. 2015). Gene expression in the SA pathway is higher than the JA pathway during the early stage of *P. brassicae* infection (Mouttet et al. 2011). This evidence suggests that SA is involved in *P. brassicae* infection in *Arabidopsis* which is similar to my finding in canola. In contrast, SA may not be the only pathway activated by *P. brassicae* infection in*Arabidopsis* (Lovelock et al. 2016).

Inoculated, susceptible plants have higher levels of SA and its conjugates than uninoculated, susceptible plants. Increased SA concentration in plants can lead to conversion of SA to MeSA that is emitted for systemic signalling (Park et al. 2007). MeSA is known to influence mating behaviour in some Lepidoptera. Exposure to MeSA results in reduced courtship duration in the green veined white butterfly, *Pieris napi* (L.) (Lepidoptera: Pieridae) (Andersson et al. 2000). *Pieris rapae* males synthesize MeSA and indoles and transfer them to females during mating which suppress the subsequent attractiveness of females (Andersson et al. 2003). Although, I did not directly test oviposition deterrence to MeSA in BAW, oviposition by the congeneric species, *M. brassicae,* is inhibited by MeSA (Ulland et al. 2008). In our study, female BAW lay fewer eggs on inoculated susceptible plants that had higher levels of SA and its conjugates, which suggests that SA may negatively influence oviposition preference of BAW.

Pathogen infection induces production of VOCs in infected plants (Djonovic et al. 2007), however, there is not much information on VOCs induced by root pathogens that attract or deter herbivorous insects (Rostás et al. 2006, Piesik et al. 2011). Plant metabolomic studies provide a comprehensive dissection of plant-biotic interactions (Aliferis and Jabaji 2012, Balmer et al. 2013). In the current study, the plant VOC profile is influenced by *P. brassicae* inoculation (Appendix A, Figure 5-7). Although complete identification of all compounds is needed for further interpretation of these data, several biologically relevant classes of compounds such as monoterpenes (β-myrcene, limonene, β-ocimene), carboxylic acids (acetic acid), aromatics (benzene, 1,2-dimethyl-benzene, methoxy-benzene, 2-methyl-furan,), alkanes [nonane, (2E)-2-nonene, pentadecane, tridecane), aldehydes (nonanal) were recovered in the analyses. It is not clear if BAW uses information from the entire plant emission or if a subset of specific compounds will influence oviposition behaviour. For instance, the flower bouquet of jimsonweed, *Datura wrightii* Regel, contains more than 60 compounds; but three odorants, linalool, benzaldehyde and benzyl alcohol mimic the floral odor responsible for feeding behaviour of *Manduca sexta* L. (Sphighidae: Lepidoptera) (Riffell et al. 2009), and only linalool is needed to induce oviposition in the same species (Reisenman et al. 2010). Bertha armyworm females prefer to lay eggs on flowering plants as compared with the preflower and pod stages (Ulmer et al. 2002), therefore the main host searching cues may be the odour of the flowering plant. Acetic acid may be a key compound that affects oviposition of BAW as large amounts of acetic acid are emitted from canola plants at the flowering stage as compared with preflowering plants (Veromann et al. 2013). A combination of acetic acid with the floral volatile, phenylacetaldehyde enhances attraction of other moths like the alfalfa looper, *Autographa californica* (Speyer) (Lepidoptera: Noctuidae) and the armyworm *Spodoptera albula* (Walker), but reduces attraction of other moths including the cabbage looper, *Trichoplusia ni* (Hübner), the silver Y moth, *Autographa gamma* (L.), *MacDunnoughia confusa* (Stephens) and the soybean looper, *Chrysodeixis includens* (Walker). This indicates that the combination of acetic acid and phenylacetaldehyde can yield variable responses in different moth species (Landolt et al. 2013). As large amounts of SA conjugates were recovered in the *P. brassicae* inoculated samples in the current study, it is likely that MeSA is released as part of the profile from these plants, but I was unable to recover any SA conjugates in the volatile profile analyses. The amount of VOC emission is highly variable under abiotic and biotic stresses (Holopainen and Gershenzon 2010, Toome et al. 2010), and additional biological replicates are necessary to determine the role of MeSA in *P. brassicae* inoculated plants. Complete VOC analyses using plant metabolomic tools will be useful to better understand plant-mediated insect-pathogen interactions (De Vos et al. 2007, Nakabayashi and Saito 2013, Veromann et al. 2013).

This study shows that *P. brassicae* infection not only suppresses the growth of susceptible canola, but also impacts subsequent host use by the generalist herbivore, BAW. Higher levels of SA and its conjugates in susceptible plants may be associated with the poor performance of juvenile BAW. In contrast, *P. brassicae* inoculation in resistant plants did not influence canola growth or BAW oviposition. Interestingly, I found higher larval growth on inoculated resistant plants, which suggests that *P. brassicae* inoculation may induce susceptibility in resistant canola to BAW herbivory. This is an interesting finding as canola growers use *P. brassicae* resistant varieties to overcome *P. brassicae* infection, which may result in canola that is more vulnerable to BAW attacks. Taken together, all this information will be important to further understand the plant mediated interaction between *P. brassicae* and BAW.

Table 5-1. Free/unbound SA, conjugated SA, JA and jasmonic acid isoleucince (JA-lle) content (ng/g fresh weight) in *P. brassicae* inoculated and non-inoculated (*P. brassicae* resistant and susceptible) canola foliar tissue samples (n=3 per treatment). Analysis was conducted using UPLC ESI-MS/MS. Leaf samples were collected 5-wks after inoculation (spore concentration: 10^7). Different uppercase or lowercase letters indicate treatments are significantly different ($P<0.05$). Data are means ± SE.

	P. brassicae inoculation	SA	Conjugated SA	JA	JA-lle
Susceptible canola	Non-inoculated	4.51 ± 0.15^{A}	35.19 ± 3.04^{A}	< 1	0.65 ± 0.09
	Inoculated	14.24 ± 2.36^{B}	503.19 ± 21.81^{B}	< 1.6[α]	≤ 0.4[β]
Resistant canola	Non-inoculated	7.55 ± 2.62^{a}	46.46 ± 7.88^{a}	< 2.0[γ]	< 0.4
	Inoculated	6.12 ± 1.67^{a}	54.03 ± 14.24^{a}	< 1[δ]	0.66 ± 0.16

[α] Two samples had <1 JA levels

[β] One sample did not have detectable level of JA-Ile

[γ] Two samples had <1 JA levels

[δ] One sample did not have detectable level of JA

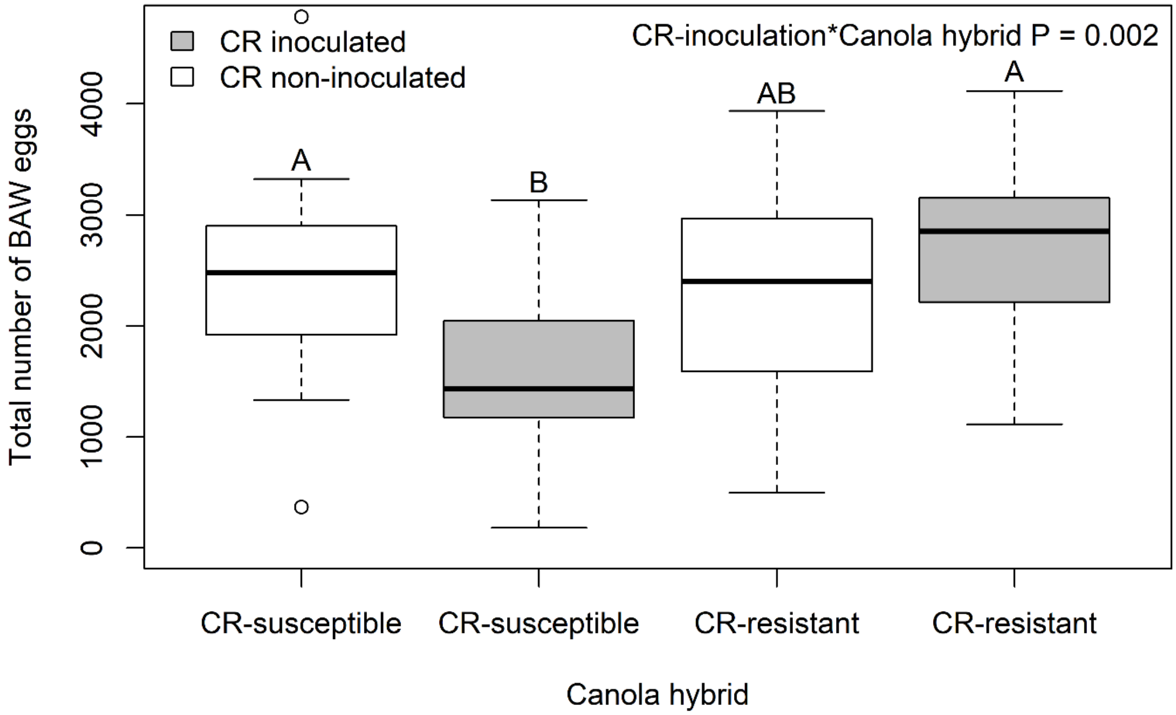


Figure 5-1. Boxplot indicates total number of eggs laid on *P. brassicae* resistant and susceptible plants (*P. brassicae* inoculated and non-inoculated). The midline indicates the median. The bottom and top of the box area indicate the first and third quartiles, respectively. The vertical lines indicate the variability outside the first and third quartiles. Extreme values are indicated as open circles. Eggs were counted after four days of oviposition in a no choice experiment (n=18; per treatment). Data were analyzed using a linear mixed model. Bars marked with different letters are significantly different (*P*<0.05) using LS means with Tukey HSD post-hoc comparisons. In this figure, CR represents *P. brassicae.*

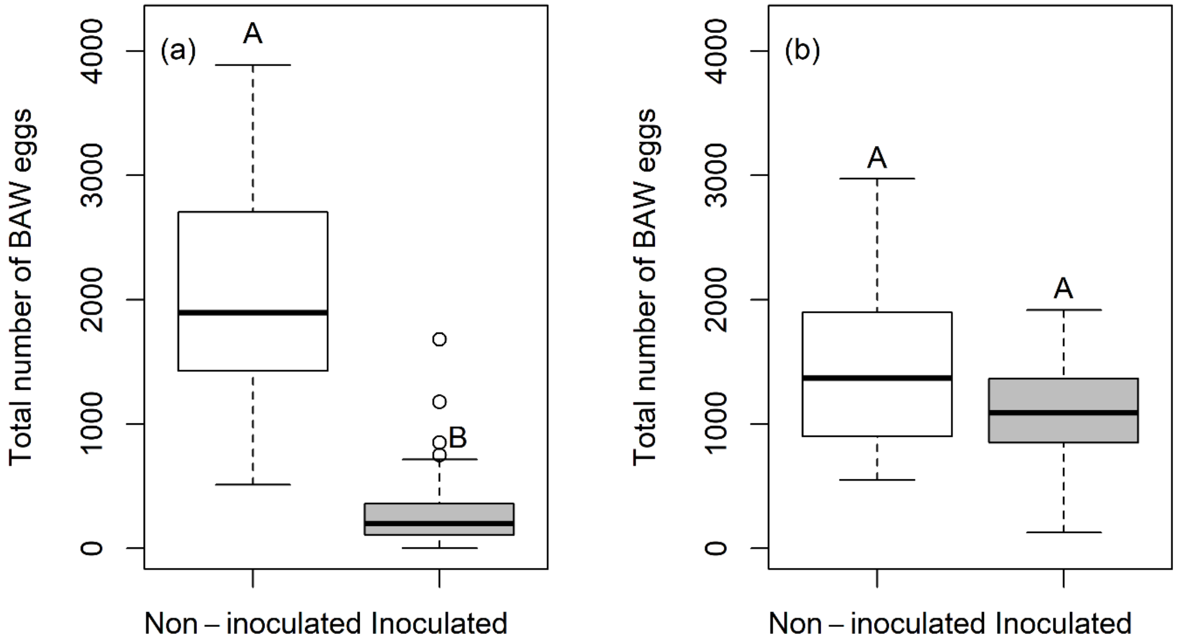


Figure 5-2. Boxplot indicates total number of eggs laid on *P. brassicae* susceptible non-inoculated and inoculated plants in oviposition choice experiment. Plot (a) indicates 10^7 *P. brassicae* spore inoculation (n=22; per treatment) (b) indicates 10^4 *P. brassicae* spore inoculation (n=21; per treatment). The midline indicates the median. The bottom and top of the box area indicate the first and third quartiles, respectively. The vertical lines indicate the variability outside the first and third quartiles. Extreme values are indicated as open circles. Extreme values are indicated as open circles. Eggs were counted after three days of oviposition. Bars marked with the different letters are significantly different in figure (a) ($P = 0.0001$) and (b) ($P = 0.1292$) ($P<0.05$) using paired t-test.

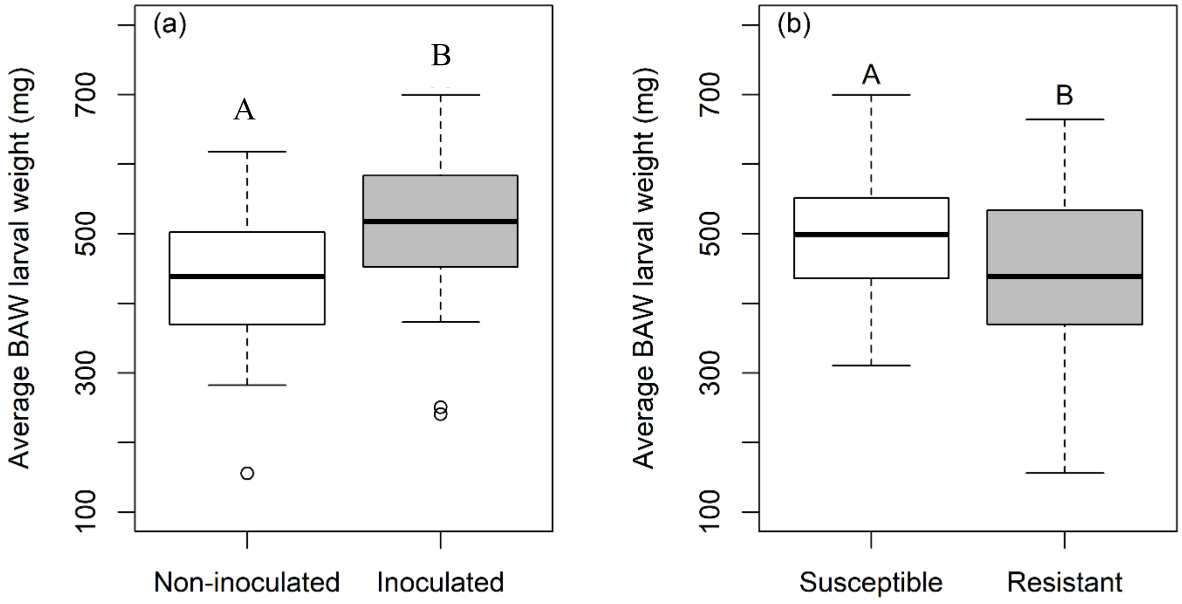


Figure 5-3. Boxplot indicates (a) the effect of *P. brassicae* inoculation on the average bertha armyworm larval weight after 2-wk feeding; and (b) the effect of the canola variety on larval weight. The midline indicates the median. The bottom and top of the box area indicate the first and third quartiles, respectively. The vertical lines indicate the variability outside the first and third quartiles. Extreme values are indicated as open circles. Larval weight was measured at 7 days after larvae introduction to experimental cages (n=17; per treatment). Data were analyzed using a linear mixed model. Bars marked with the different letters are significantly different in figure (a) $P = 0.0016$ and (b) $P = 0.0248$ using LS means with Tukey HSD post-hoc comparisons.

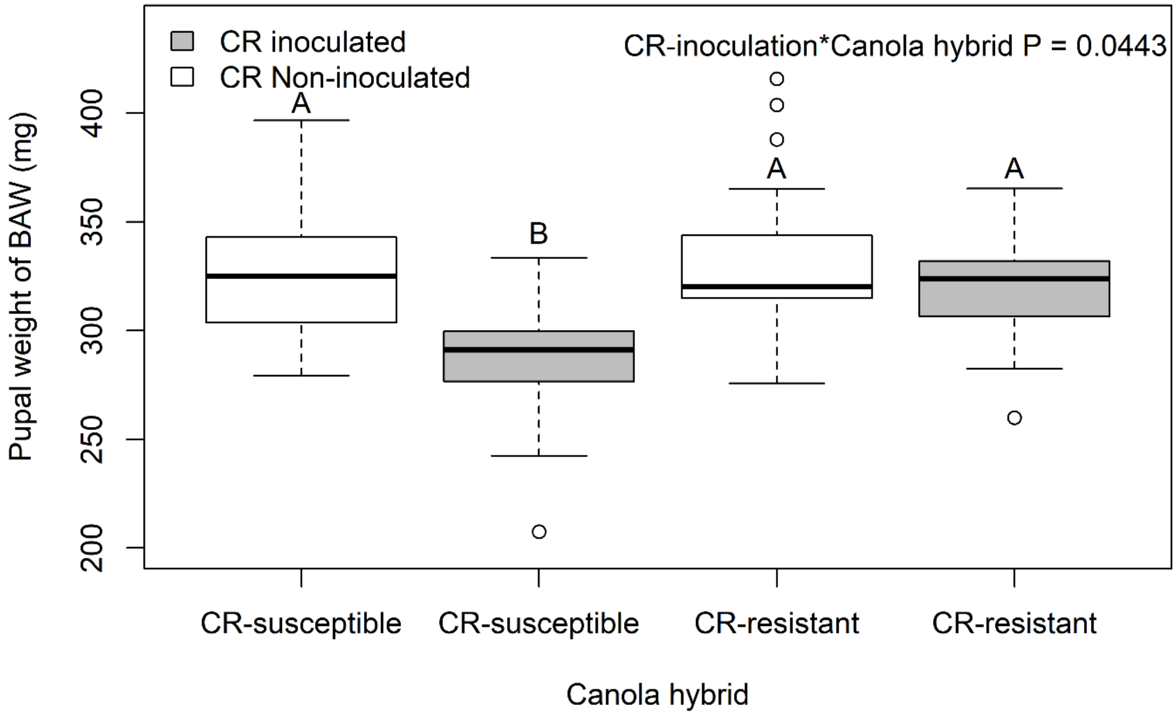


Figure 5-4. Boxplot indicates the effect of *P. brassicae* inoculation on bertha armyworm pupal weight. The midline indicates the median. The bottom and top of the box area indicate the first and third quartiles, respectively. The vertical lines indicate the variability outside the first and third quartiles. Extreme values are indicated as open circles. Pupal weight was measured at the end of the offspring development experiment (n=17; per treatment). Data were analyzed using a linear mixed model. Bars marked with the different letters are significantly different ($P<0.05$) using LS means with Tukey HSD post-hoc comparisons. In this figure, CR represents *P. brassicae.*

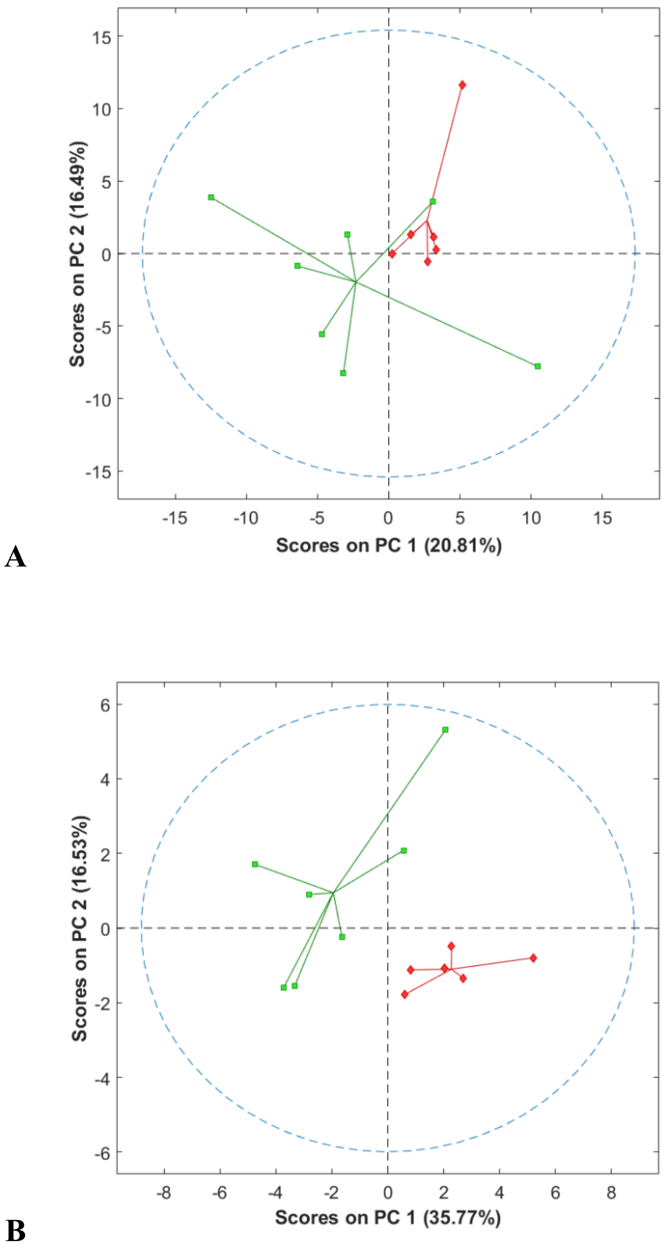
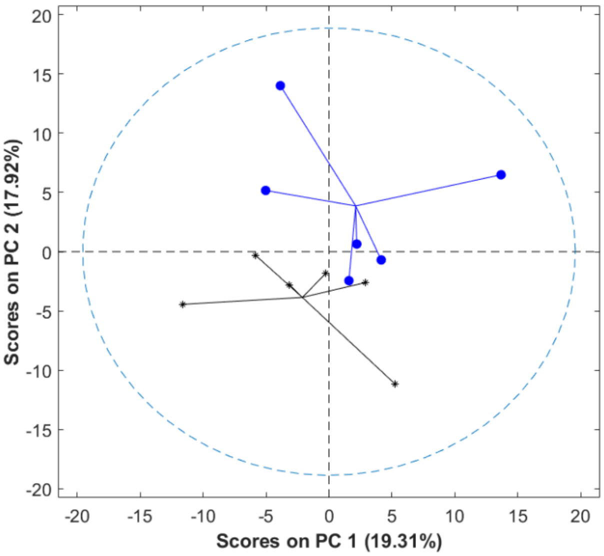
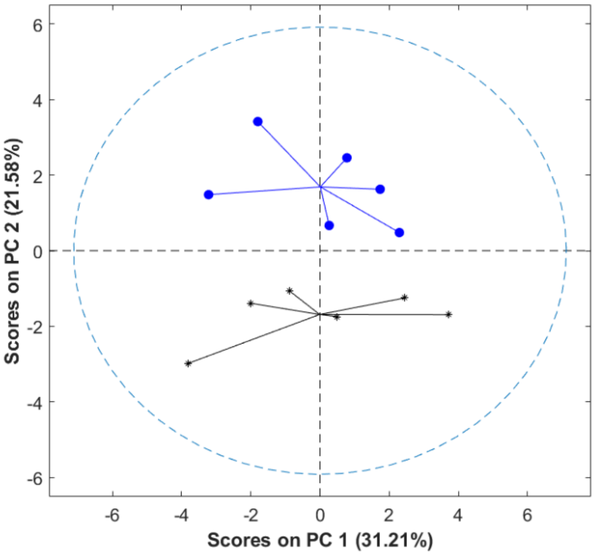


Figure 5-5. PC1 and PC2 score plots of the VOC profile of inoculated and non-inoculated canola. A. 166 variables B. 25 variables.🟩Inoculated ◆non-inoculated



A



B

Figure 5-6. PC1 and PC2 score plots of the VOC profile of susceptible vs resistant canola. A. 222 variables B. 18 variables. ● Susceptible * Resistant

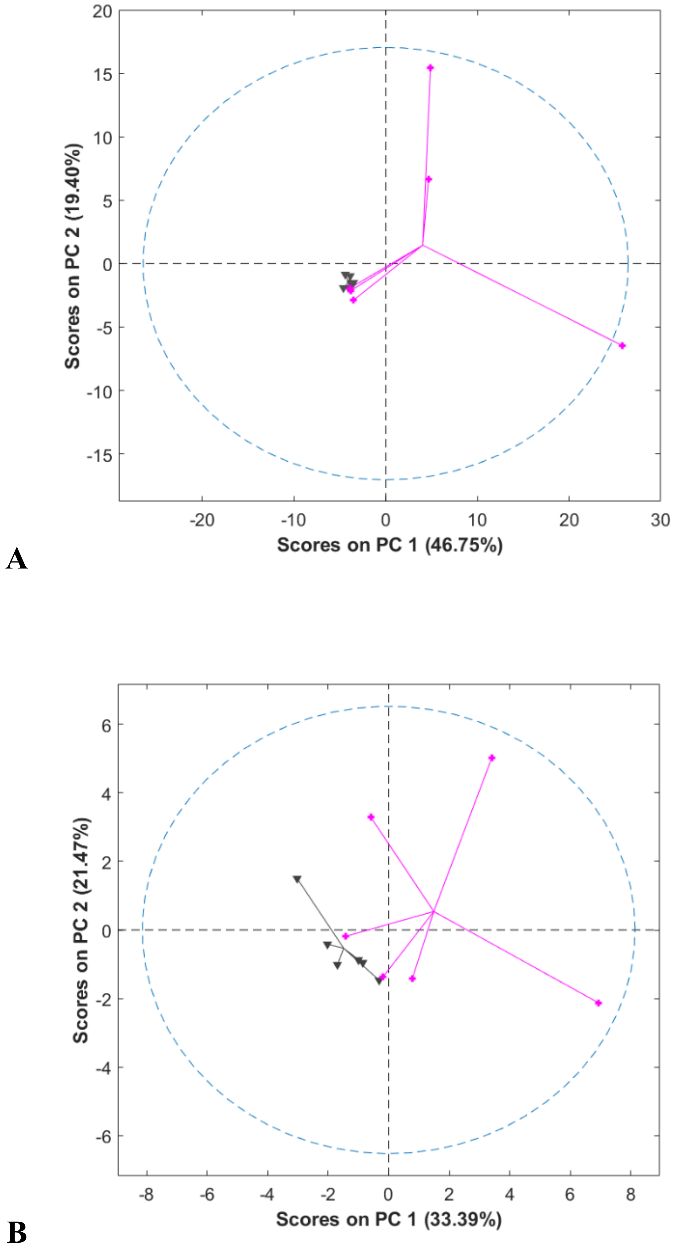


Figure 5-7. PC1 and PC2 score plots of VOC profile of plants preferred by BAW vs non-preferred canola. A. 166 variables B. 22 variables. ■ BAW preference ▼ BAW non-preference

Literature Cited

Adutwum, L. A., A. P. de la Mata, H. D. Bean, J. E. Hill, and J. J. Harynuk. **2017**. Estimation of start and stop numbers for cluster resolution feature selection algorithm: an empirical approach using null distribution analysis of Fisher ratios. Anal. Bioanal. Chem. 409: 6699–6708.

Agrawal, A. A., and N. S. Kurashige. **2003**. A role for isothiocyanates in plant resistance against the specialist herbivore *Pieris rapae.* J. Chem. Ecol. 29: 1403–1415.

Aliferis, K. A., and S. Jabaji. **2012**. FT-ICR/MS and GC-EI/MS metabolomics networking unravels global potato sprout's responses to *Rhizoctonia solani* infection. PLoS One. 7: 1–13.

Andersson, J., A. K. Borg-Karlson, and C. Wiklund. **2003**. Antiaphrodisiacs in pierid butterflies: A theme with variation! J. Chem. Ecol. 29: 1489–1499.

Andersson, J., A. Borg-Karlson, and C. Wiklund. **2000**. Sexual cooperation and conflict in butterflies: a male-transferred anti-aphrodisiac reduces harassment of recently mated females. Proc. R. Soc. London B. 267: 1271–1275.

Balmer, D., V. Flors, G. Glauser, and B. Mauch-Mani. **2013**. Metabolomics of cereals under biotic stress: current knowledge and techniques. Front. Plant Sci. 4: 1–12.

Bucher, G. E., and G. K. Bracken. **1976**. The bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae). Artificial diet and rearing technique. Can. Entomol. 108: 1327–1338.

Dempsey, D. A., and D. F. Klessig. **2017**. How does the multifaceted plant hormone salicylic acid combat disease in plants and are similar mechanisms utilized in humans? BMC Biol. 15: 23.

Devos, S., K. Vissenberg, J. Verbelen, and E. Prinsen. **2005**. Infection brassicae on cell wall of leads Chinese to metabolism cabbage a stimulation and by of *Plasmodiophora* plant growth : impacts hormone balance. New Phytol. 166: 241–250.

Diederichsen, E., M. Frauen, E. G. A. Linders, K. Hatakeyama, and M. Hirai. **2009**. Status and perspectives of clubroot resistance breeding in crucifer crops. J. Plant Growth Regul. 28: 265–281.

Diederichsen, E., M. Frauen, and J. Ludwig-Müller. **2014**. Special Issue: Clubroot disease management challenges from a German perspective. Can. J. Plant Pathol. 36: 85–98.

Dixon, G. R. 2006. The biology of Plasmodiophora brassicae Wor.- A review of recent advances, pp. 271–282. *In* Acta Hortic. International Society for Horticultural Science (ISHS), Leuven, Belgium.

Dixon, G. R. 2009. The occurrence and economic impact of *Plasmodiophora brassicae* and clubroot disease. J. Plant Growth Regul. 28: 194–202.

Djonovic, S., W. A. Vargas, M. V. Kolomiets, M. Horndeski, A. Wiest, and C. M. Kenerley. **2007**. A proteinaceous elicitor Sm1 from the beneficial fungus *Trichoderma virens* is required for induced systemic resistance in maize. Plant Physiol. 145: 875–889.

Donald, E. C., G. Jaudzems, and I. J. Porter. **2008**. Pathology of cortical invasion by *Plasmodiophora brassicae* in clubroot resistant and susceptible *Brassica oleracea* hosts. Plant Pathol. 57: 201–209.

van Den Dool, H., and P. Dec. Kratz. **1963**. A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. J. Chromatogr. A. 11: 463–471.

Drakulic, J., J. Caulfield, C. Woodcock, S. P. T. Jones, R. Linforth, T. J. A. Bruce, and R.

V. Ray. **2015**. Sharing a host plant (Wheat [*Triticum aestivum*]) Increases the fitness of Fusarium graminearum and the severity of fusarium head blight but reduces the fitness of grain aphids (*Sitobion avenae*). Appl. Environ. Microbiol. 81: 3492–3501.

Hassan, M. N. ul, Z. Zainal, and I. Ismail. **2015**. Green leaf volatiles: Biosynthesis, biological functions and their applications in biotechnology. Plant Biotechnol. J. 13: 727–739.

Holopainen, J. K., and J. Gershenzon. **2010**. Multiple stress factors and the emission of plant VOCs. Trends Plant Sci. 15: 176–184.

Honěk, A. 1993. Intraspecific variation in body size and fecundity in insects : A general relationship. Oikos. 66: 483–492.

van Hulten, M., M. Pelser, L. C. van Loon, C. M. J. Pieterse, and J. Ton. **2006**. Costs and benefits of priming for defense in Arabidopsis. Proc. Natl. Acad. Sci. 103: 5602–5607.

Hwang, S. F., H. U. Ahmed, S. E. Strelkov, B. D. Gossen, G. D. Turnbull, G. Peng, and R. J. Howard. **2011**. Seedling age and inoculum density affect clubroot severity and seed yield in canola. Can. J. Plant Sci. 91: 183–190.

Hwang, S. F., H. U. Ahmed, Q. Zhou, A. Rashid, S. E. Strelkov, B. D. Gossen, G. Peng, and G. D. Turnbull. **2013**. Effect of susceptible and resistant canola plants on *Plasmodiophora brassicae* resting spore populations in the soil. 404–412.

Jahn, L., S. Mucha, S. Bergmann, C. Horn, P. Staswick, B. Steffens, J. Siemens, and J. Ludwig-Müller. **2013**. The clubroot pathogen (*Plasmodiophora brassicae*) influences auxin signaling to regulate auxin homeostasis in Arabidopsis. Plants. 2: 726–749.

Koornneef, A., and C. M. J. Pieterse. **2008**. Cross Talk in Defense Signaling. Plant Physiol. 146: 839–844.

Kuginuki, Y., H. Yoshikawa, and M. Hirai. **1999**. Variation in virulence of *Plasmodiophora* *brassicae* in Japan tested with clubroot-resistant cultivars of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). Eur. J. Plant Pathol. 105: 327–332.

Landolt, P. J., M. Tóth, R. L. Meagher, and I. Szarukán. **2013**. Interaction of acetic acid and phenylacetaldehyde as attractants for trapping pest species of moths (Lepidoptera: Noctuidae). Pest Manag. Sci. 69: 245–249.

Lemarié, S., A. Robert-Seilaniantz, C. Lariagon, J. Lemoine, N. Marnet, M. Jubault, M. J. Manzanares-Dauleux, and A. Gravot. **2015**. Both the jasmonic acid and the salicylic acid pathways contribute to resistance to the biotrophic clubroot agent *Plasmodiophora brassicae* in Arabidopsis. Plant Cell Physiol. 56: 2158–2168.

Lovelock, D. A., I. Šola, S. Marschollek, C. E. Donald, G. Rusak, K. H. van Pée, J. Ludwig-Müller, and D. M. Cahill. **2016**. Analysis of salicylic acid-dependent pathways in Arabidopsis thaliana following infection with *Plasmodiophora brassicae* and the influence of salicylic acid on disease. Mol. Plant Pathol. 17: 1237–1251.

Ludwig-Müller, J. 2009. Plant defence - What can we learn from clubroots? Australas. Plant Pathol. 38: 318–324.

Ludwig-Müller, A. J., R. N. Bennett, G. Kiddle, S. Ihmig, M. Ruppel, R. N. Bennett, G. Kiddle, and J. W. G.- Universitdt. **1999**. The host range of *Plasmodiophora brassicae* and its relationship to endogenous glucosinolate content. New Phytol. 141: 443–458.

Ludwig-Müller, J., S. Jülke, K. Geiß, F. Richter, A. Mithöfer, I. Šola, G. Rusak, S. Keenan, and S. Bulman. **2015**. A novel methyltransferase from the intracellular pathogen *Plasmodiophora brassicae* methylates salicylic acid. Mol. Plant Pathol. 16: 349–364.

Ludwig-Müller, J., B. Schubert, K. Pieper, S. Ihmig, and W. Hilgenberg. **1997**. Glucosinolate content in susceptible and resistant Chinese cabbage varieties during development of clubroot disease. Phytochemistry. 44: 407–414.

Martini, X., K. S. Pelz-stelinski, and L. L. Stelinski. **2014**. Plant pathogen-induced volatiles attract parasitoids to increase parasitism of an insect vector. Front. Ecol. Evol. 2: 1–8.

Mason, P. G., A. P. Arthur, O. O. Olfert, and M. A. Erlandson. **1998**. The bertha armyworm (*Mamestra configurata*) (Lepidoptera: Noctuidae) in Western Canada. Can. Entomol. 130: 321–336.

McCallum, E. J., J. P. Cunningham, J. Lucker, M. P. Zalucki, J. J. De Voss, and J. R. Botella. **2011**. Increased plant volatile production affects oviposition, but not larval development, in the moth *Helicoverpa armigera*. J. Exp. Biol. 214: 3672–3677.

Médiène, S., &Muriel Valantin-Morison, J.-P. Sarthou, S. de Tourdonnet, M. Gosme, M. Bertrand, J. Roger-Estrade, J.-N. Aubertot, A. Rusch, N. Motisi, C. Pelosi, and T. Doré. **2011**. Agroecosystem management and biotic interactions: a review. Agron. Sustain. Dev. 31: 491–514.

Miller, W. E. 2005. Extrinsic effects on fecundity-maternal weight relations in capital-breeding lepidoptera. J. Lepid. Soc. 59: 143–160.

Mouttet, R., P. Bearez, C. Thomas, and N. Desneux. **2011**. Phytophagous arthropods and a pathogen sharing a host plant: Evidence for indirect plant-mediated interactions. PLoS One. 6.

Murmu, J., M. Wilton, G. Allard, R. Pandeya, D. Desveaux, J. Singh, and R. Subramaniam. **2014**. Arabidopsis GOLDEN2-LIKE (GLK) transcription factors activate jasmonic acid (JA)-dependent disease susceptibility to the biotrophic pathogen *Hyaloperonospora arabidopsidis*, as well as JA-independent plant immunity against the necrotrophic pathogen *Botryti.* Mol. Plant Pathol. 15: 174–184.

Myers, J. H. 1985. Effect of physiological condition of the host plant on the ovipositional choice of the cabbage White butterfly, *Pieris rapae*. J. Anim. Ecol. 54: 193–204.

Nakabayashi, R., and K. Saito. **2013**. Metabolomics for unknown plant metabolites. Anal. Bioanal. Chem. 405: 5005–5011.

Pangesti, N., A. Pineda, C. M. J. Pieterse, M. Dicke, and J. J. A. van Loon. **2013**. Two-way plant mediated interactions between root-associated microbes and insects: from ecology to mechanisms. Front. Plant Sci. 4: 1–11.

Park, S.W., E. Kaimoyo, D. Kumar, S. Mosher, and D. F. Klessig. **2007**. Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. Science (80-.). 318: 113–116.

Piesik, D., G. Lemńczyk, A. Skoczek, R. Lamparski, J. Bocianowski, K. Kotwica, and K. J. Delaney. **2011**. Fusarium infection in maize: Volatile induction of infected and neighboring uninfected plants has the potential to attract a pest cereal leaf beetle, *Oulema melanopus*. J. Plant Physiol. 168: 1534–1542.

Ponzio, C., R. Gols, C. M. J. Pieterse, and M. Dicke. **2013**. Ecological and phytohormonal aspects of plant volatile emission in response to single and dual infestations with herbivores and phytopathogens. Funct. Ecol. 27: 587–598.

Qiu, B., J. A. Harvey, C. E. Raaijmakers, L. E. M. Vet, and N. M. Van Dam. **2009**. Nonlinear effects of plant root and shoot jasmonic acid application on the performance of *Pieris brassicae* and its parasitoid *Cotesia glomerata*. Funct. Ecol. 23: 496–505.

Reisenman, C. E., J. A. Riffell, E. A. Bernays, and J. G. Hildebrand. **2010**. Antagonistic effects of floral scent in an insect-plant interaction. Proc. R. Soc. B Biol. Sci. 277: 2371–2379.

Riffell, J. A, H. Lei, and J. G. Hildebrand. **2009**. Neural correlates of behavior in the moth *Manduca sexta* in response to complex odors. Proc. Natl. Acad. Sci. U. S. A. 106: 19219–26.

Röder, G., M. Rahier, and R. E. Naisbit. **2011**. Do induced responses mediate the ecological interactions between the specialist herbivores and phytopathogens of an alpine plant? PLoS One. 6: 1–7.

Rojas, J. C., and A. Virgen. **2003**. Chemical and tactile cues influencing oviposition of a generalist moth, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Environ. Entomol. 32: 1386–1392.

Rostás, M., J. Ton, B. Mauch-Mani, and T. C. J. Turlings. **2006**. Fungal infection reduces herbivore-induced plant volatiles of maize but does not affect naïve parasitoids. J. Chem. Ecol. 32: 1897–1909.

Sarfraz, R. M., L. M. Dosdall, and A. B. Keddie. **2009**. Bottom-up effects of host plant nutritional quality on *Plutella xylostella* (Lepidoptera: Plutellidae) and top-down effects of herbivore attack on plant compensatory ability. Eur. J. Entomol. 106: 583–594.

Schweiger, R., A. M. Heise, M. Persicke, and C. Muller. **2014**. Interactions between the jasmonic and salicylic acid pathway modulate the plant metabolome and affect herbivores of different feeding types. Plant, Cell Environ. 37: 1574–1585.

Shields, V. D. C., and B. K. Mitchell. **1995**. Sinigrin as a feeding deterrent in two crucifer-feeding, polyphagous Lepidopterous species and the effects of feeding stimulant mixtures on deterrency. Philos. Trans. R. Soc. B Biol. Sci. 347: 439–446.

Siemens, J., I. Keller, J. Sarx, S. Kunz, A. Schuller, W. Nagel, T. Schmülling, M. Parniske, and J. Ludwig-Müller. **2006**. Transcriptome analysis of Arabidopsis clubroots indicate a key role for cytokinins in disease development. Mol. Plant-Microbe Interact. 19: 480–494.

Sinkov, N. A., and J. J. Harynuk. **2011**. Cluster resolution: A metric for automated, objective and optimized feature selection in chemometric modeling. Talanta. 83: 1079–1087.

Sinkov, N. A., P. M. L. Sandercock, and J. J. Harynuk. **2014**. Chemometric classification of casework arson samples based on gasoline content. Forensic Sci. Int. 235: 24–31.

Stevens, V. M., A. Trochet, H. Van Dyck, J. Clobert, and M. Baguette. **2012**. How is dispersal integrated in life histories: A quantitative analysis using butterflies. Ecol. Lett. 15: 74–86.

Stout, M. J., a L. Fidantsef, S. S. Duffey, and R. M. Bostock. **1999**. Signal interactions in pathogen and insect attack: systemic plant-mediated interactions between pathogens and herbivores of the tomato, *Lycopersicon esculentum*. Physiol. Mol. Plant Pathol. 54: 115–130.

Strelkov, S. E., V. P. Manolii, T. Cao, S. Xue, and S. F. Hwang. **2007**. Pathotype classification of Plasmodiophora brassicae and its occurrence in *Brassica napus* in Alberta, Canada. J. Phytopathol. 155: 706–712.

Strelkov, S. E., J. P. Tewari, and E. Smith-Degenhardt. **2006**. Characterization of *Plasmodiophora brassicae* populations from Alberta , Canada. Can. J. Plant Pathol. 28: 467–474.

Stringam, G. R., D. F. Degenhardt, M. R. Thiagarajah, and V. K. Bansal. **1999**. Q2 summer rape. Can. J. Plant Sci. 79: 597–598.

Talsma, J. H. R., A. Biere, and J. A. Harvey. **2008**. Oviposition cues for a specialist butterfly – plant chemistry and size. J Chem Ecol. 34: 1202–1212.

Thompson, G. A., and F. L. Goggin. **2006**. Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. J. Exp. Bot. 57: 755–766.

Tommerup, I. C., and D. S. Ingram. **1971**. The life cycle of *Plasmodiphora brassicae* Woron. in tissue cultures and in intact roots. New Phytol.

Toome, M., P. Randjärv, L. Copolovici, Ü. Niinemets, K. Heinsoo, A. Luik, and S. M. Noe. **2010**. Leaf rust induced volatile organic compounds signalling in willow during the infection. Planta. 232: 235–243.

Traw, M. B. 2002. Is induction response negatively correlated with constitutive resistance in black mustard? Evolution. 56: 2196–2205.

Ulland, S., E. Ian, R. Mozuraitis, A. K. Borg-Karlson, R. Meadow, and H. Mustaparta. **2008**. Methyl salicylate, identified as primary odorant of a specific receptor neuron type, inhibits oviposition by the moth *Mamestra brassicae* L. (Lepidoptera, Noctuidae). Chem. Senses. 33: 35–46.

Ulmer, B., C. Gillott, and M. Erlandson. **2002**. Oviposition preferences of bertha armyworm *Mamestra configurata* Walker (Lepidoptera: Noctuidae) on different crucifer cultivars and growth stages. Environ. Entomol. 31: 1135–1141.

Ulmer, B., C. Gillott, and M. Erlandson. **2003**. Conspecific eggs and bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), oviposition site selection. Environ. Entomol. 32: 529–534.

Veromann, E., M. Toome, A. Kannaste, R. Kaasik, L. Copolovici, J. Flink, K. Gabriella, L. Narits, A. Luik, and N. Ülo. **2013**. Effects of nitrogen fertilization on insect pests, their parasitoids, plant diseases and volatile organic compounds in *Brassica napus*. Crop Prot. 43: 79–88.

De Vos, R. C. H., S. Moco, A. Lommen, J. J. B. Keurentjes, R. J. Bino, and R. D. Hall. **2007**.

Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. Nat. Protoc. 2: 778–791.

Wäckers, T. M., BezemerR., W. M. van Dam, W. H. van Der Putten, and L. F. Wäckers. **2004**. Above- and below-ground terpenoid aldehyde induction in cotton, *Gossypium herbaceum*, following root and leaf injury. J. Chem. Ecol. 30: 53–67.

Wallenhammar, A. C. 1996. Prevalence of *Plasmodiophora brassicae* in a spring oilseed rape growing area in central Sweden and factors influencing soil infestation levels. Plant Pathol. 45: 710–719.

Williams, P. 1966. A system for the determination of races of *Plasmodiophora brassicae* that infect cabbage and rutabaga. Phytopathology. 56: 624–626.

Wyckhuys, K. A. G., I. Graziosi, D. D. Burra, and A. J. Walter. **2017**. Phytoplasma infection of a tropical root crop triggers bottom-up cascades by favoring generalist over specialist herbivores. 1–18.

Xue, S., T. Cao, R. J. Howard, S. F. Hwang, and S. E. Strelkov. **2008**. Isolation and variation in virulence of single-spore isolates of *Plasmodiophora brassicae* from Canada. Plant Dis. 92: 456–462.

Ye, M., S. M. Luo, J. F. Xie, Y. F. Li, T. Xu, Y. Liu, Y. Y. Song, K. Zhu-Salzman, and R. Sen Zeng. **2012**. Silencing COI1 in rice increases susceptibility to chewing insects and impairs inducible defense. PLoS One. 7: 1–11.

Yi, H. S., J. W. Yang, S. Y. Ghim, and C. M. Ryu. **2011**. A cry for help from leaf to root: Aboveground insect feeding leads to the recruitment of rhizosphere microbes for plant self-protection against subsequent diverse attacks. Plant Signal. Behav. 6: 1192–1194.

Chapter 6: General Conclusion

In this study, I examined how abiotic and biotic factors influence the interaction between canola and the generalist herbivore, the bertha armyworm (BAW) *Mamestra configurata* Walker (Lepidoptera: Noctuidae). First, I tested the effect of plant nutrition on plant growth, tissue nutrients, and the subsequent host use by BAW (Chapter 2) (Figure 6-1). I examined the impact of several biotic stressors of canola on the subsequent host use by BAW. I studied the effect of egg deposition by the specialist herbivore, the diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) on oviposition and larval feeding by BAW (Chapter 3) (Figure 6-2). I also examined the influence of herbivory by DBM larvae on canola plants on subsequent BAW herbivory (Chapter 4) (Figure 6-2). These studies (Chapters 3 and 4) will provide more information on the interactions between specialist and generalist herbivore species that utilize Brassicaceae plants. The final chapter addresses the little studied area of plant-mediated protist-insect interactions (Chapter 5) (Figure 6-3).

Abiotic factors mediate insect herbivore host use

Abiotic factors such as soil and environmental conditions affect plant nutrition and defense traits (Hakes and Cronin 2011). These impacts on plant quality can influence the condition and success of both above-ground (Hahn and Maron 2018) and below-ground insect herbivores (Li et al. 2007). In my study, I tested the influence of plant nutrition on the plant quality for on a leaf-feeding, generalist herbivore, BAW (Figure 6-1). Plant nutrition may influence nutritional quality and defenses in the host plant.

Female adults of BAW laid more eggs on good quality hosts including several canola varieties treated with moderate and high fertilizer rates. In the presence of poor quality host plants, however, egg laying is reduced and females may resorb eggs to provide energy for somatic maintenance (Boggs and Ross 1993, Boggs 1997). High quality host plants attracted and supported more herbivores and exhibit increased growth and yield. Soil nutrients impact oviposition of several insect species (Jauset et al. 1998, Prudic et al. 2005, Chen et al. 2008). Nitrogen makes plants more attractive and nutritious to insects (Sarfraz et al. 2009). Increased attraction of BAW to fertilized canola plants may be driven by the release of different suites of volatile organic compounds (VOCs) by the variously treated plants. Volatile organic compounds such as green leaf volatiles and sesquiterpenes are released in high quantities from highly fertilized oil seed rape (Ibrahim et al. 2008). These plant signals could directly influence herbivore behaviour and host use or indirectly impact herbivores through attraction of natural enemies (Loader and Damman 1991). Higher levels of phosphorus allows plants to make more proteins (Sterner and Elser 2002) and potassium allows plants to mobilize more nutrients (Amtmann et al. 2008). Larval growth of BAW was impacted by nutritional quality of the host plant. Insects that feed on low quality plants can experience prolonged development (Chen et al. 2008), and be more vulnerable to natural enemies (Loader and Damman 1991). Nitrogen fertilization increases plant protein content (Grant et al. 2011). Bertha armyworm larvae had higher fitness as measured by higher pupal weight on moderately fertilized canola plants in this study.

Biotic factors can mediate insect interactions

Plant defense capacity is influenced by biotic factors such as previous herbivore damage (Agrawal 2000) and pathogen infection (Yi et al. 2011). Interestingly, response by the generalist herbivore, BAW, to plants that had previously been used by the specialist, DBM, varied with life stage of DBM. After DBM eggs were deposited on canola plants, there was a significant increase in larval feeding by BAW (Chapter 3) (Figure 6-2). In contrast, BAW larvae preferred to consume foliage of healthy, undamaged plants with compared to plants that experience DBM herbivory (Chapter 4). Bertha armyworm larvae fed on hosts fed on by DBM develop into smaller pupae than larvae fed healthy plants. Plants previously attacked by herbivores may hydrolyze intact glucosinolates, which are the dominant group of secondary metabolites produced by *Brassica* plants (Ute and Meike 2011).

Less is known about microbe-insect interactions mediated by host plants but it is common for plants to be affected by both stressors simultaneously (Fernandez-Conradi et al. 2018). For instance, clubroot, *Plasmodiophora brassicae* Woronin inoculation in susceptible canola affects plant growth and defenses. Female BAW laid fewer eggs on inoculated susceptible plants than on healthy susceptible plants (Chapter 5). This behaviour may be due, at least in part, to the effect of disease on plant morphology, as smaller plants provide fewer oviposition sites for BAW (Ulmer et al. 2002). On the other hand, poor quality host plants can influence insects through suppression of oviposition (Boggs and Ross 1993, Boggs 1997). I found that plant inoculation with the biotrophic pathogen, *P. brassicae* triggers defenses in the plant through the salicylic acid (SA) pathway, which is similar to other findings (Robertson et al. 1993, Ludwig-Müller et al. 1995, 2015, Lemarié et al. 2015). In contrast, inoculated susceptible canola had smaller amounts of jasmonic acid (JA) than healthy susceptible plants. Heavier BAW larvae developed on inoculated susceptible plants, as lower levels of JA favour development of leaf-chewing larvae (Qiu et al. 2009). Altogether, these results revealed that pathogen infection and previous herbivore damage mediate plant-insect interactions in this system.

Coordination of SA and JA signaling pathways

The basis for plant defenses against biotic stressors is mediated by a small set of phytohormone signaling pathways such as SA, JA and ethylene (Bodenhausen and Reymond 2007). Plants fine-tune defenses against specific attackers through cross-talk between pathways (Koornneef and Pieterse 2008). Salicylic acid and JA interactions are mostly antagonistic (Kunkel and Brooks 2002, Takahashi et al. 2004, Bostock 2005). In the current study, higher amounts of SA and SA conjugates occurred in *P. brassicae* inoculated susceptible plants (Chapter 5). This finding is similar to other studies that measure SA in *Arabidopsis* following *P. brassicae* infection (Lemarié et al. 2015, Ludwig-Müller et al. 2015) and other crops (Ludwig-Müller et al. 1995). Induction of the SA pathway suppresses the JA pathway, which results in a reduction of VOC emission from inoculated susceptible plants. Reduced volatiles may suppress the attraction of adult female BAW to canola and result in less oviposition on inoculated, susceptible plants. I found that the VOC profile of inoculated resistant and susceptible is different from non-inoculated canola plants (Chapter 5). *Plasmodiophora brassicae* inoculated susceptible canola will have smaller amounts of JA because the JA pathway is antagonistic with the SA pathway (Schweiger et al. 2014). Lower JA levels are favourable to developing leaf-chewing larvae (Qiu et al. 2009), and may contributed to heavier BAW larvae on inoculated susceptible plants in this study. Reduction in offspring fitness as measured by pupal weight on *P. brassicae* inoculated plants suggests that additional factors may influence this microbe-insect interaction.

There are some instances, in which interactions between SA and JA are synergistic (Schenk et al. 2000, van Wees et al. 2000, Mur et al. 2005). For instance, reduction of SA and JA defenses occurs following plant attack by spider mite, *Tetranychus evansi* Baker & Pritchard (Tetranychidae: Trombidiformes), which helps for feeding of conspecifics (Sarmento et al.

2011). Treatment of tobacco, *Nicotiana tabacum* L., plants with low concentrations of SA and JA induces genes in both the JA and SA pathways whereas, application at higher concentrations results in antagonistic interactions between pathways (Mur et al. 2005). In this study, plants with DBM eggs had a reduced accumulation of free and conjugated JA and SA in leaf tissue. The BAW larvae consumed more leaf area on canola with DBM eggs than on egg-free-plants. Insect egg deposition also reduces plant volatile emissions. Lower emission of leaf volatiles occurs from plants with eggs of fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) as compared to egg-free plants (Hilker and Fatouros 2015). These plant odors are exploited by ovipositing females for host selection (Talsma et al. 2008). I found that BAW females lay a marginally lower number of eggs on plants with DBM eggs. This could be due to reduced emissions of leaf volatiles, which may attract fewer ovipositing females (Talsma et al. 2008).

Preference-performance link

The ‘preference-performance’ (Gripenberg et al. 2010), ‘mother knows best’ (Jaenike 1978) or ‘optimal oviposition’ (Thompson 1988) hypothesis states that adult female insects choose a plant on which their offspring perform best. Most studies conclude that females select their host plant based on the host plant quality (Gripenberg et al. 2010). Some studies state that adult females choose host plants for protection from natural enemies (Bjorkman et al. 1997). The BAW female adults lay eggs as clusters, so plants need to support a large number of larvae, therefore it is predicted that adult females will choose the host based on plant quality for their offspring.

Adult female BAW preferentially choose the moderate and highly fertilized canola plants for oviposition. Furthermore, heavier pupae result from larvae that are reared on moderately fertilized plants (Chapter 2). The BAW adult females also exhibited host choice for healthy canola plants that were not inoculated with *P. brassicae* for oviposition over inoculated susceptible canola plants. The subsequent BAW offspring also performed better on healthy plants (Chapter 5). Bertha armyworm oviposition was not affected by previous host use by the specialist herbivore, DBM. Bertha armyworm larval feeding was impacted negatively by previous herbivory by DBM (Chapter 4). Therefore, the preference-performance hypothesis is not fully supported in BAW response to canola with prior DBM presence. This discrepancy may be due to restricted space inside the cage for host selection by the females. There is evidence that the preference-performance link may be supported under more natural conditions (Gripenberg et al. 2010).

Future directions

This study focused on economically and ecologically important disturbances found in canola agroecosystems. My study contributes to further understanding of the interactions between BAW and canola (Figure 6-4). Bertha armyworm female moths preferred to lay more eggs on moderate and highly fertilized plants. ‘Q2’ canola received the highest number of eggs. Larvae performed better on plants treated with moderate levels of fertilizer. The results from my study demonstrate bottom up effects of canola nutrition on BAW interactions. Not only is plant nutrition important for the fitness of herbivores, but it may also influence natural enemies of herbivores, directly or indirectly. For instance, large herbivores on high quality host plants increase the performance of parasitoids (Mayhew and Godfray 1997). Furthermore, plant VOCs are important mediators of plant-insect interactions, and they can function across trophic levels to attract natural enemies of herbivores. Therefore, it is important investigate community level impacts of plant nutrition in future studies.

I found that egg deposition by DBM altered plant defenses, causing plants to be more susceptible to BAW feeding. This is interesting information as plant defense suppression following insect egg deposition is very rare among insects (Hilker and Fatouros 2015). In contrast, there is much evidence on improved defenses against insect egg deposition. I have observed a trend that shows the suppression of oviposition of BAW when plants had DBM eggs. Studies have reported suppression of VOC emission following insect egg deposition (Peñaflor et al. 2011), therefore, it will be very interesting to further examine this evidence through plant VOC analyses. In addition, defense induction may be much higher when DBM eggs are close to hatching, therefore, a time series analysis of plant defenses could be informative in the canola system studied here. I also found that herbivory by DBM can make canola more resistant to feeding and reduce offspring fitness of BAW, which is an important information on further understanding specialist-generalist insect interactions. This may be due to increased glucosinolate levels in canola following herbivory, therefore, further analysis of glucosinolate should be investigated.

Study of the interaction between *P. brassicae* and BAW shows that the magnitude of BAW response to plant infection by *P. brassicae* varies between canola varieties. *Plasmodiophora brassicae* inoculation increases resistance in susceptible canola to BAW feeding whereas *P. brassicae* inoculation increases susceptibility in resistant canola to BAW feeding. It is evident that changes in insect responses are driven by changes in phytohormones in canola. Although I have filled particular gaps in this interaction, more information on other phytohormone pathways that are impacted by *P. brassicae* infection is required. For instance, in the current study, both SA and JA pathways are examined but the ethylene pathway is not. The ethylene pathway is also important in plant defenses against herbivores (Lu et al. 2014). Time series analysis of phytohormones could be more informative in the canola system studied here. Insect responses to pathogen infected plants can be affected by timing and disease progression (Mouttet et al. 2011, 2013). Therefore, more studies need to be conducted to better understand the insect response across a time gradient and disease progression. I have observed interesting trends with increased oviposition on *P. brassicae* inoculated resistant plants, suggesting that this should be tested under field conditions with different pathogen densities in the soil.

Significance of work to canola industry

Bertha armyworm is a pest of canola fields in the Canadian Prairies. Bertha armyworm outbreaks can cost tens of millions of dollars due to insecticide spraying and yield losses. However, BAW attacks generally appear with some other stressor, either abiotic or biotic, including plant nutrition, below ground infection, or previous herbivory by a specialist insect. These abiotic and biotic stressors might influence BAW infestation in canola fields. My findings on crop nutrition will give insight to canola producers on different crop cultivars and fertilizer applications that may reduce BAW damage. Furthermore, minimizing the use of plant fertilizers and selection of suitable canola varieties will protect the environment from excess chemicals and can also be financially beneficial to canola producers. Diamondback moth migrate from the southern and western US to western Canada each year. How the yield of DBM infested canola responds to BAW should be tested in the field to understand the severity of BAW damage when DBM cooccurs with BAW. *Plasmodiophora brassicae* is one of the major threats to canola. Recently in Alberta, *P. brassicae* disease emerged and has caused significant reductions in canola production. Understanding how *P. brassicae* infected canola plants interact with BAW will give an opportunity for implementing proper control measures. Use of susceptible and resistant canola hybrids explains how different canola varieties are responding to these threats. This could help minimize chemical use to control BAW infestation.

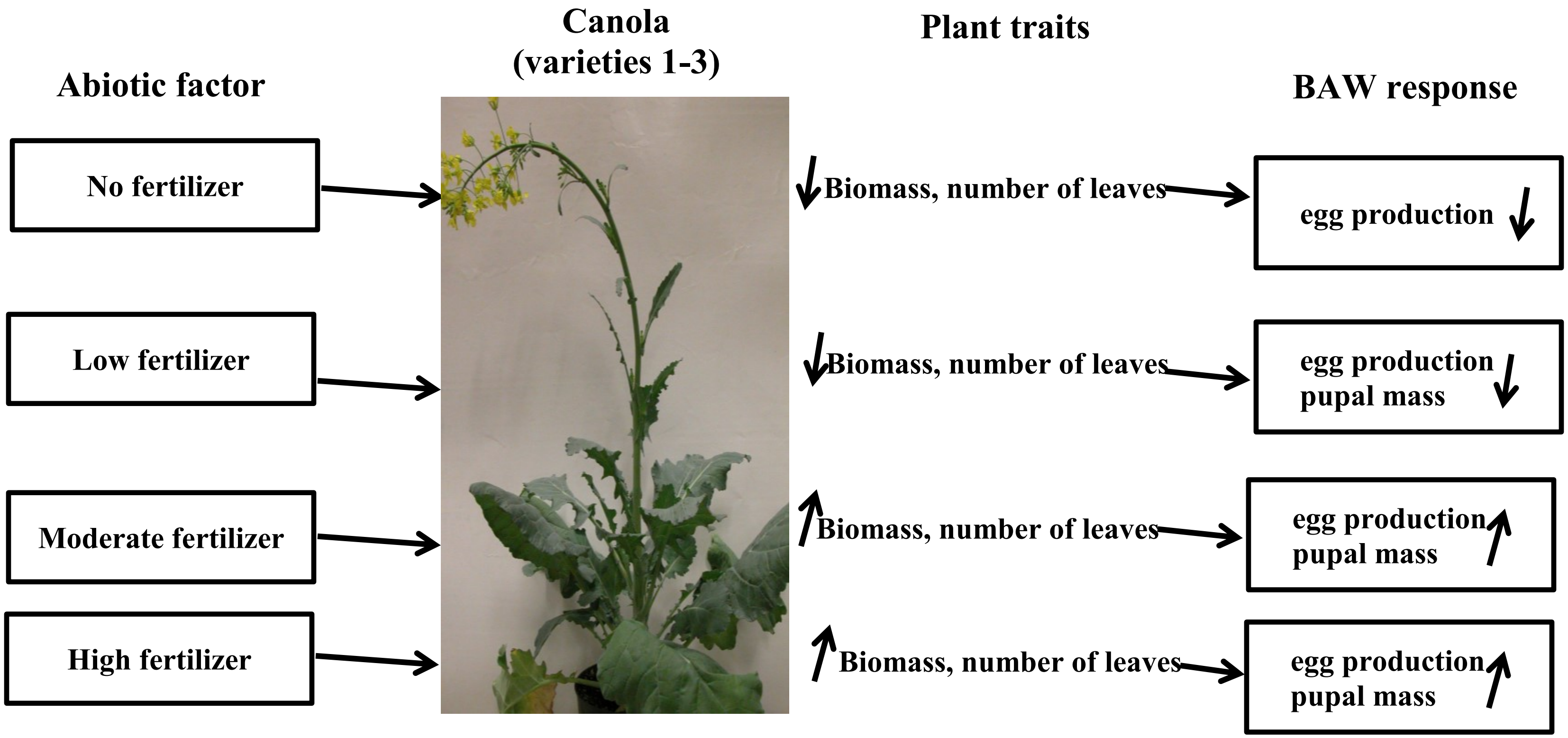


Figure 6-1. Overview of the findings of this study on the effects of plant nutrition - BAW interaction in canola. Moderate fertilizer treatments increase canola growth, pupal mass and egg production of BAW whereas low fertilizer treatments reduce canola growth and pupal mass of BAW.

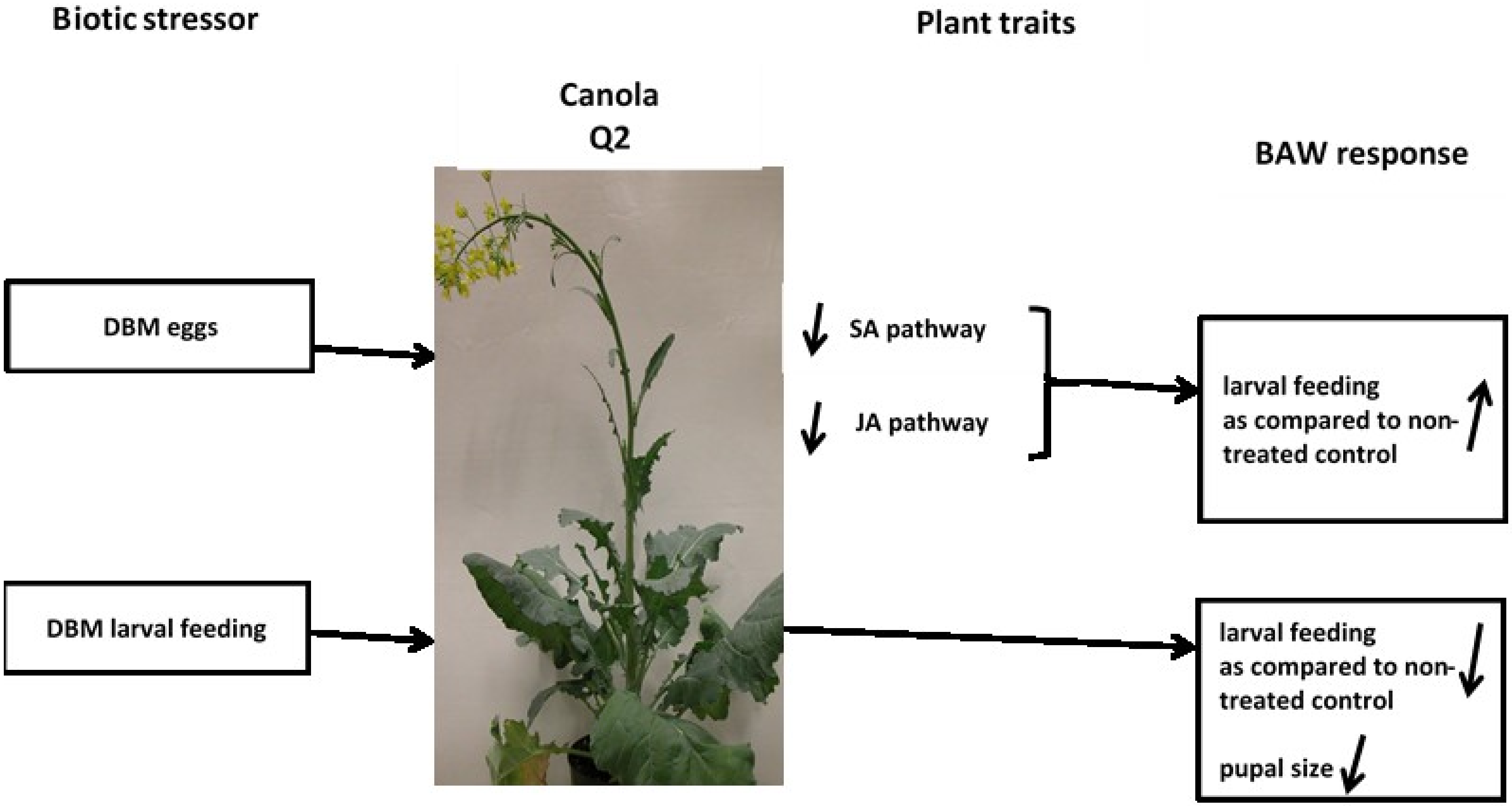


Figure 6-2. Overview of the effects of DBM oviposition and herbivory on BAW in canola. Diamondback moth oviposition suppress SA and JA pathways and increase susceptibility to larval feeding by BAW. The generalist, BAW larvae preferred to feed on non-treated control as compared to plants damaged by specialist DBM. Bertha armyworm fed less on canola plants previously damaged by DBM and developed into smaller pupae.

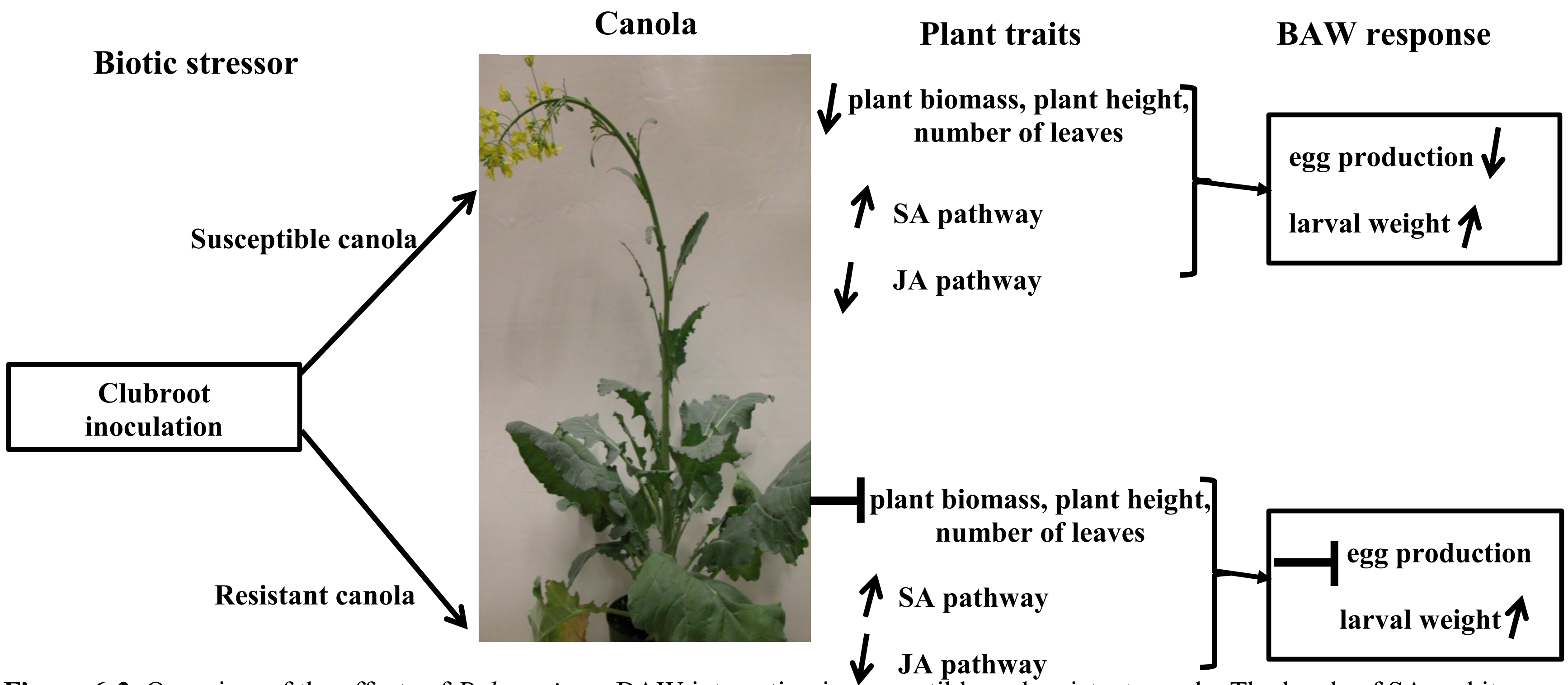


Figure 6-3. Overview of the effects of *P. brassicae* -BAW interaction in susceptible and resistant canola. The levels of SA and its conjugates are higher in *P. brassicae* inoculated plants of both the resistant and susceptible varieties compared to non-inoculated plants. Inoculated, susceptible plants have fewer leaves and smaller biomass and receive fewer BAW eggs, however, larvae reared on susceptible canola plants inoculated with *P. brassicae* were heavier than larvae reared on uninoculated plants. Plant growth and oviposition of BAW is not influenced following *P. brassicae* inoculation in resistant plants, however, heavier larvae resulted when larvae were reared on inoculated resistant plants. ⊢—— Indicates no influence by the treatment

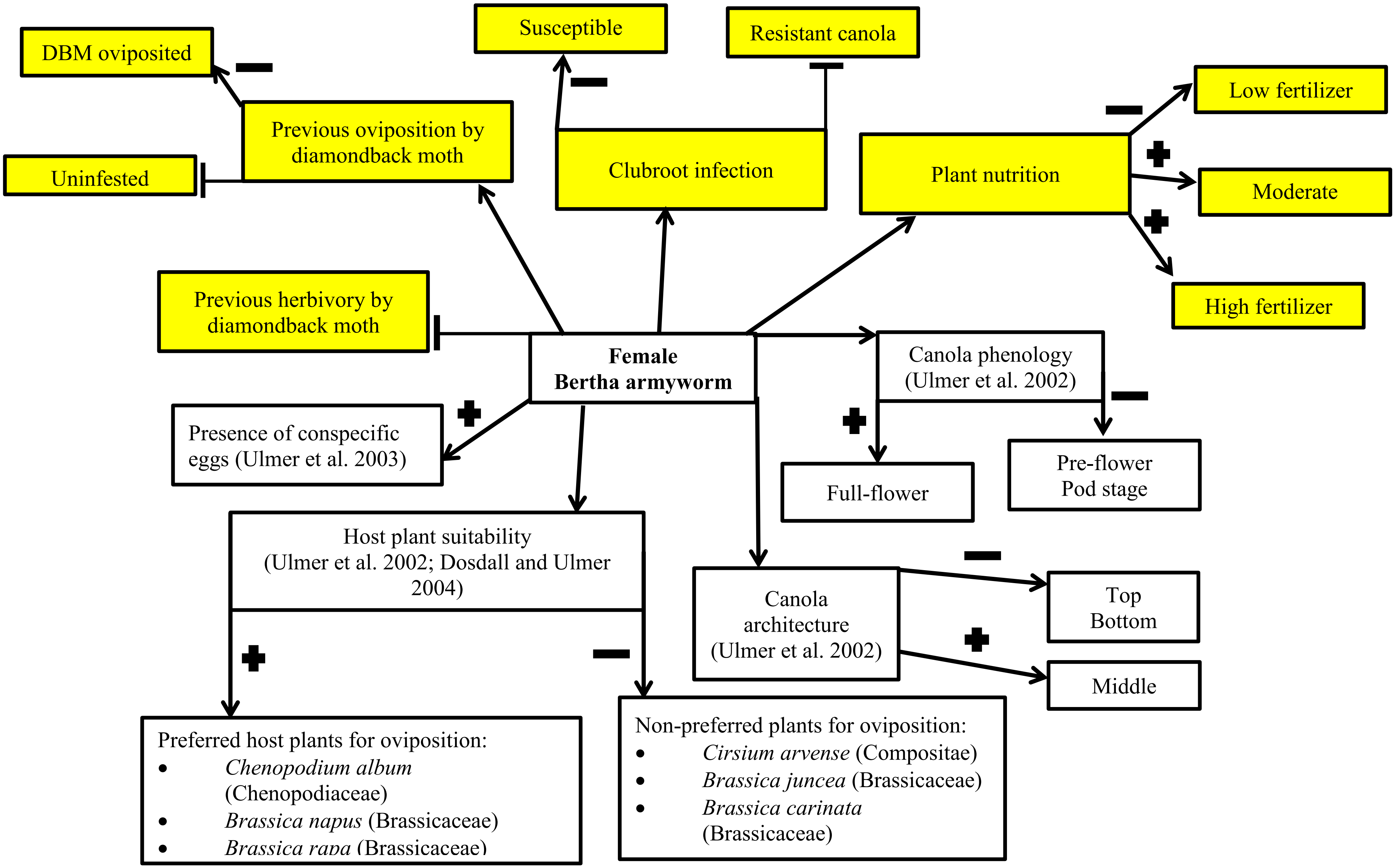


Figure 6-4. Factors influencing oviposition of bertha armyworm female moths in white boxes (Evenden et al., 2017). Newly added interactions from the current study are highlighted in yellow. ├── Indicates no influence by the treatment.

Literature Cited

Agrawal, A. A. 2000. Specificity of induced resistance in wild radish: causes and consequences for two specialist and two generalist caterpillars. Oikos. 89: 493–500.

Amtmann, A., S. Troufflard, and P. Armengaud. **2008**. The effect of potassium nutrition on pest and disease resistance in plants. Physiol. Plant. 133: 682–691.

Bjorkman, C., S. Larsson, and R. Bommarco. **1997**. Oviposition preferences in pine sawflies : A trade-off between larval growth and defence against natural enemies. Oikos. 79: 45–52.

Bodenhausen, N., and P. Reymond. **2007**. Signaling pathways controlling induced resistance to insect herbivores in Arabidopsis. Mol. Plant-Microbe Interact. MPMI. 20: 1406–1420.

Boggs, C. L. 1997. Reproductive allocation from reserves and income in butterfly species with differeing adult diets. Ecology. 78: 181–191.

Boggs, C. L. ., and C. L. . Ross. **1993**. The effect of adult food limitation on life history traits in *Speyeria mormonia* (Lepidoptera : Nymphalidae). Ecology. 74: 433–441.

Bostock, R. M. 2005. Signal crosstalk and induced resistance: straddling the line between cost and benefit. Annu. Rev. Phytopathol. 43: 545–580.

Chen, Y., J. R. Ruberson, and D. M. Olson. **2008**. Nitrogen fertilization rate affects feeding, larval performance, and oviposition preference of the beet armyworm, *Spodoptera exigua*, on cotton. Entomol. Exp. Appl. 126: 244–255.

Dosdall, L. M., and B. J. Ulmer. **2004**. Feeding, development, and oviposition of bertha armyworm (Lepidoptera : Noctuidae) on different host plant species. Environ. Entomol. 33: 756–764.

Evenden, M. L., R. E. Batallas, and C. Weeraddana. **2017**. Biology and management of the generalist herbivore, the bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), on canola in western Canada., pp. 114–129. *In* Reddy, G.V.P. (ed.), Integr. Manag. Insect Pests Canola Other Brassica Oilseed Crop. CABI, Boston, MA.

Fernandez-Conradi, P., H. Jactel, C. Robin, A. J. M. Tack, and B. Castagneyrol. **2018**. Fungi reduce preference and performance of insect herbivores on challenged plants. Ecology. 99: 300–311.

Grant, C. A., D. A. Derksen, D. L. McLaren, and R. B. Irvine. **2011**. Nitrogen fertilizer and urease inhibitor effects on canola seed quality in a one-pass seeding and fertilizing system. F. Crop. Res. 121: 201–208.

Gripenberg, S., P. J. Mayhew, M. Parnell, and T. Roslin. **2010**. A meta-analysis of preference-performance relationships in phytophagous insects. Ecol. Lett. 13: 383–393.

Hahn, P. G., and J. L. Maron. **2018**. Plant water stress and previous herbivore damage affect insect performance. Ecol. Entomol. 43: 47–54.

Hakes, A. S., and J. T. Cronin. **2011**. Environmental heterogeneity and spatiotemporal variability in plant defense traits. Oikos. 120: 452–462.

Hilker, M., and N. E. Fatouros. **2015**. Plant responses to insect egg deposition. Annu. Rev. Entomol. 60: 493–515.

Ibrahim, M. A., A. Stewart-Jones, J. Pulkkinen, G. M. Poppy, and J. K. Holopainen. **2008**. The influence of different nutrient levels on insect-induced plant volatiles in Bt and conventional oilseed rape plants. Plant Biol. 10: 97–107.

Jaenike, J. 1978. On optimal oviposition behavior in phytophagous insects. Theor. Popul. Biol. 14: 350–356.

Jauset, A. M., M. J. Sarasúa, J. Avilla, and R. Albajes. **1998**. The impact of nitrogen fertilization of tomato on feeding site selection and oviposition by *Trialeurodes vaporariorum*. Entomol. Exp. Appl. 86: 175–182.

Koornneef, A., and C. M. J. Pieterse. **2008**. Cross talk in defense signaling. Plant Physiol. 146: 839–844.

Kunkel, B. N., and D. M. Brooks. **2002**. Cross talk between signaling pathways in pathgen defense. Curr. Opin. Plant Biol. 5: 325–331.

Lemarié, S., A. Robert-Seilaniantz, C. Lariagon, J. Lemoine, N. Marnet, M. Jubault, M. J. Manzanares-Dauleux, and A. Gravot. **2015**. Both the jasmonic acid and the salicylic acid pathways contribute to resistance to the biotrophic clubroot agent *Plasmodiophora brassicae* in Arabidopsis. Plant Cell Physiol. 56: 2158–2168.

Li, H., S. H. Futch, J. P. Syvertsen, and C. W. McCoy. **2007**. Time series forecast and soil characteristics-based simple and multivariate linear models for management of *Diaprepes abbreviatus* root weevil in citrus. Soil Biol. Biochem. 39: 2436–2447.

Loader, C., and H. Damman. **1991**. Nitrogen content of food plants and vulnerability of *Pieris rapae* to natural enemies. Ecology. 72: 1586–1590.

Lu, J., J. Li, H. Ju, X. Liu, M. Erb, X. Wang, and Y. Lou. **2014**. Contrasting effects of ethylene biosynthesis on induced plant resistance against a chewing and a piercing-sucking herbivore in rice. Mol. Plant. 7: 1670–1682.

Ludwig-Müller, J., S. Jülke, K. Geiß, F. Richter, A. Mithöfer, I. Šola, G. Rusak, S. Keenan, and S. Bulman. **2015**. A novel methyltransferase from the intracellular pathogen *Plasmodiophora brassicae* methylates salicylic acid. Mol. Plant Pathol. 16: 349–364.

Ludwig-Müller, J., N. Kasperczyk, B. Schubert, and W. Hilgenberg. **1995**. Identification of salicylic acid in Chinese cabbage and its possible role during root infection with *Plasmodiophora brassicae*. Curr. Top. Phytochem. 14: 39–45.

Mayhew, A. P. J., and H. C. J. Godfray. **1997**. Mixed sex allocation strategies in a parasitoid wasp. Oecologia. 110: 218–221.

Mouttet, R., P. Bearez, C. Thomas, and N. Desneux. **2011**. Phytophagous arthropods and a pathogen sharing a host plant: Evidence for indirect plant-mediated interactions. PLoS One. 6.

Mouttet, R., I. Kaplan, P. Bearez, E. Amiens-Desneux, and N. Desneux. **2013**. Spatiotemporal patterns of induced resistance and susceptibility linking diverse plant parasites. Oecologia. 173: 1379–1386.

Mur, L. A. J., P. Kenton, R. Atzorn, O. Miersch, and C. Wasternack. **2005**. The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. Plant Physiol. 140: 249–262.

Peñaflor, M. F. G. V., M. Erb, C. A. M. Robert, L. A. Miranda, A. G. Werneburg, F. C. A. Dossi, T. C. J. Turlings, and J. M. S. Bento. **2011**. Oviposition by a moth suppresses constitutive and herbivore-induced plant volatiles in maize. Planta. 234: 207–215.

Prudic, K. L., J. C. Oliver, and M. D. Bowers. **2005**. Soil nutrient effects on oviposition preference, larval performance, and chemical defense of a specialist insect herbivore. Oecologia. 143: 578–587.

Qiu, B., J. A. Harvey, C. E. Raaijmakers, L. E. M. Vet, and N. M. Van Dam. **2009**. Nonlinear effects of plant root and shoot jasmonic acid application on the performance of *Pieris brassicae* and its parasitoid *Cotesia glomerata*. Funct. Ecol. 23: 496–505.

Robertson, G. W., D. W. Griffiths, W. M. F. Smith, and R. D. Butcher. **1993**. The application of thermal desorption-gas chromatography-mass spectrometry to the analyses of flower volatiles from five varieties of oilseed rape (*Brassica napus* spp. oleifera). Phytochem. Anal. 4: 152–157.

Sarfraz, R. M., L. M. Dosdall, and A. B. Keddie. **2009**. Bottom-up effects of host plant nutritional quality on *Plutella xylostella* (Lepidoptera: Plutellidae) and top-down effects of herbivore attack on plant compensatory ability. Eur. J. Entomol. 106: 583–594.

Sarmento, R. A., F. Lemos, P. M. Bleeker, R. C. Schuurink, A. Pallini, M. G. A. Oliveira, E. R. Lima, M. Kant, M. W. Sabelis, and A. Janssen. **2011**. A herbivore that manipulates plant defence. Ecol. Lett. 14: 229–236.

Schenk, P. M., K. Kazan, I. Wilson, J. P. Anderson, T. Richmond, S. C. Somerville, and J. M. Manners. **2000**. Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. Proc Natl Acad Sci U S A. 97: 11655–11660.

Schweiger, R., A. M. Heise, M. Persicke, and C. Müller. **2014**. Interactions between the jasmonic and salicylic acid pathway modulate the plant metabolome and affect herbivores of different feeding types. Plant, Cell Environ. 37: 1574–1585.

Sterner, R. W., and J. J. Elser. **2002**. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, New Jersey, USA.

Takahashi, H., Y. Kanayama, M. S. Zheng, T. Kusano, S. Hase, M. Ikegami, and J. Shah. **2004**. Antagonistic interactions between the SA and JA signaling pathways in Arabidopsis modulate expression of defense genes and gene-for-gene resistance to cucumber mosaic virus. Plant Cell Physiol. 45: 803–809.

Talsma, J. H. R., A. Biere, and J. A. Harvey. **2008**. Oviposition cues for a specialist butterfly – plant chemistry and size. J Chem Ecol. 34: 1202–1212.

Thompson, J. N. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of off spring in phytophagons insects. Entomol. exp. appl. 47: 3–14.

Ulmer, B., C. Gillott, and M. Erlandson. **2002**. Oviposition preferences of bertha armyworm *Mamestra configurata* Walker (Lepidoptera : Noctuidae) on different crucifer cultivars and growth stages. Environ. Entomol. 31: 1135–1141.

Ulmer, B., C. Gillott, and M. Erlandson. **2003**. Conspecific eggs and bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), oviposition site selection. Environ. Entomol. 32: 529–534.

Ute, W., and B. Meike. **2011**. Glucosinolate breakdown in Arabidopsis: Mechanism, regulation and biological significance. Arab. B. 1–14.

van Wees, S. C., E. a de Swart, J. a van Pelt, L. C. van Loon, and C. M. Pieterse. **2000**. Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in Arabidopsis thaliana. Proc. Natl. Acad. Sci. 97: 8711–8716.

Yi, H. S., J. W. Yang, S. Y. Ghim, and C. M. Ryu. **2011**. A cry for help from leaf to root: Aboveground insect feeding leads to the recruitment of rhizosphere microbes for plant self-protection against subsequent diverse attacks. Plant Signal. Behav. 6: 1192–1194.

Bibliography

Aartsma, Y., F. J. J. A. Bianchi, W. van der Werf, E. H. Poelman, and M. Dicke. **2017**. Herbivore-induced plant volatiles and tritrophic interactions across spatial scales. New Phytol. 216: 1054–1063.

Adutwum, L. A., A. P. de la Mata, H. D. Bean, J. E. Hill, and J. J. Harynuk. **2017**. Estimation of start and stop numbers for cluster resolution feature selection algorithm: an empirical approach using null distribution analysis of Fisher ratios. Anal. Bioanal. Chem. 409: 6699–6708.

Agrawal, A. A. 2000. Specificity of induced resistance in wild radish: causes and consequences for two specialist and two generalist caterpillars. Oikos. 89: 493–500.

Agrawal, A. A., and N. S. Kurashige. **2003**. A role for isothiocyanates in plant resistance against the specialist herbivore *Pieris rapae*. J. Chem. Ecol. 29: 1403–1415.

Agrawal, A. A., and M. F. Sherriffs. **2001**. Induced plant resistance and susceptibility to late-season herbivores of wild radish. Ann. Entomol. Soc. Am. 94: 71–75.

Aist, J. R., and P. H. Williams. **1971**. The cytology and kinetics of cabbage root hair penetration by *Plasmodiophora brassicae*. Can. J. Bot. 49: 2023–2034.

Al-Shehbaz, I. A., M. A. Beilstein, and E. A. Kellogg. **2006**. Systematics and phylogeny of the Brassicaceae (Cruciferae): An overview. Plant Syst. Evol. 259: 89–120.

Alba, J. M., B. C. J. Schimmel, J. J. Glas, L. M. S. Ataide, M. L. Pappas, C. A. Villarroel, R. C. Schuurink, M. W. Sabelis, and M. R. Kant. **2015**. Spider mites suppress tomato defenses downstream of jasmonate and salicylate independently of hormonal crosstalk. New Phytol. 205: 828–840.

Aliferis, K. A., and S. Jabaji. **2012**. FT-ICR/MS and GC-EI/MS metabolomics networking unravels global potato sprout's responses to *Rhizoctonia solani* infection. PLoS One. 7: 1–13.

Amrein, H., and N. Thorne. **2005**. Gustatory perception and behavior in *Drosophila melanogaster*. Curr. Biol. 15: 673–684.

Amtmann, A., S. Troufflard, and P. Armengaud. **2008**. The effect of potassium nutrition on pest and disease resistance in plants. Physiol. Plant. 133: 682–691.

Andersson, J., A. K. Borg-Karlson, and C. Wiklund. **2003**. Antiaphrodisiacs in Pierid butterflies: A theme with variation! J. Chem. Ecol. 29: 1489–1499.

Andersson, J., A. Borg-Karlson, and C. Wiklund. **2000**. Sexual cooperation and conflict in butterflies: a male-transferred anti-aphrodisiac reduces harassment of recently mated females. Proc. R. Soc. London B. 267: 1271–1275.

AOAC. **2000**. Official Methods of Analysis of AOAC International. Assoc. Off. Anal. Chem. Int. Method ce 2-66.

Aplin, R. T., and R. d. Ward. **1975**. Examination of the large white and small white butterflies (*Pieris* spp.) for the presence of mustard oils and mustard oil glycosides. J. Entomol. 50: 73–78.

Awmack, C. S., and S. R. Leather. **2002**. Host plant quality and fecundity in herbivorous insects. Annu. Rev. Entomol. 47: 817–844.

Badenes-Perez, F.R, B. Nault, and A. Shelton. **2005**. Manipulating the attractiveness and suitability of hosts for diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 98: 836–844.

Bailey, C. 1976. A quantitative study of consumption and utilization of various diets in the bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae). Can. Entomol. 108:

1319–1326.

Balbi, V., and A. Devoto. **2008**. Jasmonate signalling network in *Arabidopsis thaliana*: Crucial regulatory nodes and new physiological scenarios. New Phytol. 177: 301–318.

Baldwin, I. T. 1998. Jasmonate-induced responses are costly but benefit plants under attack in native populations. Proc. Natl. Acad. Sci. 95: 8113–8118.

Balmer, D., V. Flors, G. Glauser, and B. Mauch-Mani. **2013**. Metabolomics of cereals under biotic stress: current knowledge and techniques. Front. Plant Sci. 4: 1–12.

Bartelt, R. J., and K. L. Mikolajczak. **1989**. Toxicity of compounds derived from *Limnanthes alba* seed to fall armyworm (Lepidoptera: Noctuidae) and European corn borer (Lepidoptera: Pyralidae) larvae. J. Econ. Entomol. Econ Entomol. v. 82: 1054–1060.

Beckie, H. J., K. N. Harker, L. M. Hall, S. I. Warwick, A. Légère, P. H. Sikkema, G. W. Clayton, A. G. Thomas, J. Y. Leeson, G. Séguin-Swartz, and M. Simard. **2006**. A decade of herbicide-resistant crops in Canada. Can. J. Plant Sci. 86: 1243–1264.

Benrey, B., and R. F. Denno. **1997**. The slow-growth-high-mortality hypothesis: A test using the cabbage butterfly. Ecology. 78: 987–999.

Bjorkman, C., S. Larsson, and R. Bommarco. **1997**. Oviposition preferences in pine sawflies : A trade-off between larval growth and defence against natural enemies. Oikos. 79: 45–52.

Bodenhausen, N., and P. Reymond. **2007**. Signaling pathways controlling induced resistance to insect herbivores in Arabidopsis. Mol. Plant-Microbe Interact. MPMI. 20: 1406–1420.

Boggs, C. L. 1997. Reproductive allocation from reserves and income in butterfly species with differeing adult diets. Ecology. 78: 181–191.

Boggs, C. L. ., and C. L. Ross. **1993**. The effect of adult food limitation on life history traits in *Speyeria Mormonia* (Lepidoptera : Nymphalidae). Ecology. 74: 433–441.

Bos, J. I. B., D. Prince, M. Pitino, M. E. Maffei, J. Win, and S. A. Hogenhout. **2010**. A Functional genomics approach identifies candidate effectors from the aphid species *Myzus persicae* (Green peach aphid). PLoS Genet. 6: e1001216.

Bostock, R. M. 2005. Signal crosstalk and induced resistance: Straddling the line between cost and benefit. Annu. Rev. Phytopathol. 43: 545–580.

Bracken, G. K. 1984. Within plant preferences of larvae of *Mamestra configurata* (Lepidoptera: Noctuidae) feeding on oilseed rape. Can. Entomol. 116: 45–49.

Brennan, R. F., M. G. Mason, and G. H. Walton. **2000**. Effect of nitrogen fertilizer on the concentrations of oil and protein in canola (*Brassica napus*) seed. J. Plant Nutr. 23: 339–348.

Bruessow, F., C. Gouhier-Darimont, A. Buchala, J. P. Metraux, and P. Reymond. **2010**. Insect eggs suppress plant defence against chewing herbivores. Plant J. 62: 876–885.

Bucher, G. E., and G. K. Bracken. **1976**. The bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae). Artificial diet and rearing technique. Can. Entomol. 108: 1327–1338.

Buczacki, S. T., and C. M. Clay. **1984**. Some observations on secondary zoospore development in *Plasmodiophora brassicae*. Trans. Br. Mycol. Soc. 82: 339–342.

Canola council of Canada. 2017. Industry overview. https://www.canolacouncil.org/markets-stats/industry-overview/

Canola Council of Canada. What is canola ? Retrived from https://www.canolacouncil.org/oil-and-meal/what-is-canola/.
(accessed 17 March 2018).

Canola Council of Canada. Insects. Retrived from https://www.canolacouncil.org/canola-encyclopedia/insects/
(accessed on 17 March 2018)

Canola Council of Canada. Diseases. Retrived from https://www.canolacouncil.org/canola-encyclopedia/diseases/
(accessed on 17 March 2018)

Canola digest. Clubroot resistance saves canola in Central Alberta. Retrived from https://canoladigest.ca/science-edition-2017/clubroot-resistance-saves-canola-in-central-alberta/
(accessed on 17 March 2018)

Canola Encyclopedia. 2018. Seeding rate.
https://www.canolacouncil.org/canola-encyclopedia/crop-establishment/seeding-rate/#fnote1

Canolawatch. 2016. How much fertilizer does canola need?
http://www.canolawatch.org/2012/02/09/how-much-fertilizer-does-canola-need/

Chen, X. 2014. Effects of induced plant resistance and potassium fertilization rates on soybean looper (*Chrysodeixis includens*) development in Soybean.

Chen, Y., J. R. Ruberson, and D. M. Olson. **2008**. Nitrogen fertilization rate affects feeding, larval performance, and oviposition preference of the beet armyworm, *Spodoptera exigua*, on cotton. Entomol. Exp. Appl. 126: 244–255.

Chen, Y. Z., L. Lin, C. W. Wang, C. C. Yeh, and S. Y. Hwang. **2004**. Response of two *Pieris* (Lepidoptera: Pieridae) species to fertilization of a host plant. Zool. Stud. 43: 778–786.

Chung, S. H., C. Rosa, E. D. Scully, M. Peiffer, J. F. Tooker, K. Hoover, D. S. Luthe, and G. W. Felton. **2013**. Herbivore exploits orally secreted bacteria to suppress plant defenses. Proc. Natl. Acad. Sci. 110: 15728–15733.

Cipollini, D., S. Enright, M. B. Traw, and J. Bergelson. **2004**. Salicylic acid inhibits jasmonic acid-induced resistance of *Arabidopsis thaliana* to *Spodoptera exigua*. Mol. Ecol. 13: 1643–1653.

Clancy, K., and P. Price. **1987**. Rapid herbivore growth enhances enemy attack: sublethal plant defenses remain a paradox. Ecology. 68: 733–737.

Consales, F., F. Schweizer, M. Erb, C. Gouhier-Darimont, N. Bodenhausen, F. Bruessow, I. Sobhy, and P. Reymond. **2012**. Insect oral secretions suppress wound-induced responses in Arabidopsis. J. Exp. Bot. 63: 727–737.

Cornell, H., and B. A. Hawkins. **2003**. Herbivore responses to plant secondary compounds: a test of phytochemical coevolution theory. Am. Nat. 161: 507–522.

Dangl, J. L., and J. D. G. Jones. **2001**. Plant pathogens and integrated defence responses to infection. Nature. 411: 826–833.

Dempsey, D. A., and D. F. Klessig. **2017**. How does the multifaceted plant hormone salicylic acid combat disease in plants and are similar mechanisms utilized in humans? BMC Biol. 15: 23.

Devos, S., K. Vissenberg, J. Verbelen, and E. Prinsen. **2005**. Infection brassicae on cell wall of leads Chinese to metabolism cabbage a stimulation and by of *Plasmodiophora* plant growth : impacts hormone balance. New Phytol. 166: 241–250.

Diederichsen, E., M. Frauen, E. G. A. Linders, K. Hatakeyama, and M. Hirai. **2009**. Status and perspectives of clubroot resistance breeding in crucifer crops. J. Plant Growth Regul. 28: 265–281.

Diederichsen, E., M. Frauen, and J. Ludwig-Müller. **2014**. Special Issue: Clubroot disease management challenges from a German perspective. Can. J. Plant Pathol. 36: 85–98.

Dixon, G. R. 2006. The biology of *Plasmodiophora brassicae* Wor.- A review of recent advances, pp. 271–282. *In* Acta Hortic. International Society for Horticultural Science (ISHS), Leuven, Belgium.

Dixon, G. R. 2009. The occurrence and economic impact of *Plasmodiophora brassicae* and clubroot disease. J. Plant Growth Regul. 28: 194–202.

Djonovic, S., W. A. Vargas, M. V. Kolomiets, M. Horndeski, A. Wiest, and C. M. Kenerley. **2007**. A Proteinaceous Elicitor Sm1 from the Beneficial Fungus *Trichoderma virens* is required for induced systemic resistance in maize. Plant Physiol. 145: 875–889.

Donald, E. C., G. Jaudzems, and I. J. Porter. **2008**. Pathology of cortical invasion by *Plasmodiophora brassicae* in clubroot resistant and susceptible *Brassica oleracea* hosts. Plant Pathol. 57: 201–209.

Donaldson, J. R., E. L. Kruger, and R. L. Lindroth. **2006**. Competition- and resource-mediated tradeoffs between growth and defensive chemistry in trembling aspen (*Populus tremuloides*). New Phytol. 169: 561–570.

van Den Dool, H., and P. Dec. Kratz. **1963**. A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. J. Chromatogr. A. 11: 463–471.

Dosdall, L. M. 1994. Evidence for successful overwintering of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), in Alberta. Can. Entomol. 126: 183–185.

Dosdall, L. M. 2010. Do Larvae of Bertha armyworm, *Mamestra configurata* (Walker) (Lepidoptera: Noctuidae), express induced feeding responses? J. Appl. Entomol. 134: 531–541.

Dosdall, L. M., J. J. Soroka, and O. Olfert. **2011**. The diamondback moth in canola and mustard: current pest status and future prospects. Prairie Soils Crop. J. 4: 66–76.

Dosdall, L. M., K. N. Harker, J. T. O'Donovan, R. E. Blackshaw, H. R. Kutcher, Y. Gan, and E. N. Johnson. **2012**. Crop sequence effects on root maggot (Diptera: Anthomyiidae: *Delia* spp.) infestations in Canola. J. Econ. Entomol. 105: 1261–1267.

Dosdall, L. M., and B. J. Ulmer. **2004**. Feeding, development, and oviposition of bertha armyworm (Lepidoptera : Noctuidae) on different host plant species. Environ. Entomol. 33: 756–764.

Doss, R. P., J. E. Oliver, W. M. Proebsting, S. W. Potter, S. Kuy, S. L. Clement, R. T. Williamson, J. R. Carney, and E. D. DeVilbiss. **2000**. Bruchins: Insect-derived plant regulators that stimulate neoplasm formation. Proc. Natl. Acad. Sci. 97: 6218–6223.

Drakulic, J., J. Caulfield, C. Woodcock, S. P. T. Jones, R. Linforth, T. J. A. Bruce, and R. V. Ray. **2015**. Sharing a host plant (Wheat [*Triticum aestivum*]) Increases the fitness of Fusarium graminearum and the severity of fusarium head blight but reduces the fitness of grain aphids (*Sitobion avenae*). Appl. Environ. Microbiol. 81: 3492–3501.

Erbout, N., M. De Meyer, C. Vangestel, and L. Lens. **2009**. Host plant toxicity affects developmental rates in a polyphagous fruit fly: Experimental evidence. Biol. J. Linn. Soc. 97: 728–737.

Erlandson, M. A. 2013. Biological Control Programmes in Canada 2001–2012, pp. 228–232. *In* Mason, P.G., Gillespie, D.R. (eds.), Biol. Control Program. Canada 2001–2012. CAB International, Wallingford, UK.

Evenden, M. L., R. E. Batallas, and C. Weeraddana. **2017**. Biology and management of the generalist herbivore, the bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), on canola in western Canada., pp. 114–129. *In* Reddy, G.V.P. (ed.), Integr.

Manag. Insect Pests Canola Other Brassica Oilseed Crop. CABI, Boston, MA.

Farmer, E. E., and C. A. Ryan. **1992**. Octadecanoid Precursors of Jasmonic Acid activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell. 4: 129–134.

Feeny, P., E. Städler, I. Åhman, and M. Carter. **1989**. Effects of plant odor on oviposition by the black swallowtail butterfly, *Papilio polyxenes* (Lepidoptera: Papilionidae). J. Insect Behav. 2: 803–827.

Fernandez-Conradi, P., H. Jactel, C. Robin, A. J. M. Tack, and B. Castagneyrol. **2018**. Fungi reduce preference and performance of insect herbivores on challenged plants. Ecology. 99: 300–311.

Funke, T., H. Han, M. L. Healy-Fried, M. Fischer, and E. Schönbrunn. **2006**. Molecular basis for the herbicide resistance of Roundup Ready crops. Proc. Natl. Acad. Sci. 103: 13010–13015.

Furlong, M. J., D. J. Wright, and L. M. Dosdall. **2013**. Diamondback moth ecology and management: problems, progress, and prospects. Annu. Rev. Entomol. 58: 517–541.

Fürstenberg-hägg, J., M. Zagrobelny, and S. Bak. **2013**. Plant defense against insect herbivores. Int. J. Mol. Sci. 14: 10242–10297.

Geoffrey, M. K., and W. J. Turnock. **1979**. Growth, development and survival of *Mamestra configurata* Walker, (Lepidoptera : Noctuidae) on various reported food plants. Dep. Entomol.

Gerber, G. H., and M. A. Howladert. **1987**. The effects of photoperiod and temperature on calling behaviour and egg development of the bertha armyworm, *Mamestra configurata* (Lepidoptera: Nouctuidae). J. Insect Physiol. 33: 429–436.

Girling, R. D., A. Stewart-Jones, J. Dherbecourt, J. T. Staley, D. J. Wright, and G. M.

Poppy. **2011**. Parasitoids select plants more heavily infested with their caterpillar hosts: a new approach to aid interpretation of plant headspace volatiles. Proceedings. Biol. Sci. 278: 2646–53.

Glazebrook, J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. 43: 205–227.

Golizadeh, A., K. Kamali, Y. Fathipour, and H. Abbasipour. **2007**. Temperature-dependent development of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) on two brassicaceous host plants. Insect Sci. 14: 309–316.

Grainnews. New canola varities for 2017. Retrived from https://www.grainews.ca/2016/11/08/new-canolas-for-2017/

(accessed on March 17, 2018)

Grant, C. A., D. A. Derksen, D. L. McLaren, and R. B. Irvine. **2011**. Nitrogen fertilizer and urease inhibitor effects on canola seed quality in a one-pass seeding and fertilizing system. F. Crop. Res. 121: 201–208.

Greenberg, J. T., A. Guo, D. F. Klessig, and F. M. Ausubel. **1994**. Programmed cell death in plants: A pathogen-triggered response activated coordinately with multiple defense functions. Cell. 77: 551–563.

Gripenberg, S., P. J. Mayhew, M. Parnell, and T. Roslin. **2010**. A meta-analysis of preference-performance relationships in phytophagous insects. Ecol. Lett. 13: 383–393.

Hahn, P. G., and J. L. Maron. **2018**. Plant water stress and previous herbivore damage affect insect performance. Ecol. Entomol. 43: 47–54.

Hakes, A. S., and J. T. Cronin. **2011**. Environmental heterogeneity and spatiotemporal variability in plant defense traits. Oikos. 120: 452–462.

Halkier, B. A., and J. Gershenzon. **2006**. Biology and biochemistry of glucosinolates. Annu. Rev. Plant Biol. 57: 303–333.

Harcourt, D. G. 1954. The biology and ecology of the Diamondback moth, *Plutella maculipennis* Curtis, in Eastern Ontario. Cornell Univ.

Harcourt, D. G. 1957. Biology of the diamondback moth, *Plutella maculipennis* (Curt.), in eastern Ontario. 11. Life-history, behaviour, and host relationships. Can. Entomol. 89: 554–564.

Harvey, J. A., and R. Gols. **2011**. Population-related variation in plant defense more strongly affects survival of an herbivore than its solitary parasitoid wasp. J. Chem. Ecol. 37: 1081–1090.

Hassan, M. N. ul, Z. Zainal, and I. Ismail. **2015**. Green leaf volatiles: Biosynthesis, biological functions and their applications in biotechnology. Plant Biotechnol. J. 13: 727–739.

Heidel, A. J., and I. T. Baldwin. **2004**. Microarray analysis of salicylic acid- and jasmonic acid-signalling in responses of *Nicotiana attenuata* to attack by insects from multiple feeding guilds. Plant, Cell Environ. 27: 1362–1373.

Hennig, J., J. Malamy, G. Grynkiewicz, J. Indulski, and D. Klessig. **1993**. Interconversion of the salicylic acid signal and its glucoside in tobacco. plant J. 4: 593–600.

Hilker, M., and N. E. Fatouros. **2015**. Plant responses to insect egg deposition. Annu. Rev. Entomol. 60: 493–515.

Hilker, M., C. Kobs, M. Varama, and K. Schrank. **2002**. Insect egg deposition induces *Pinus sylvestris* to attract egg parasitoids. J. Exp. Biol. 205: 455–461.

Hilker, M., O. Rohfritsch, and T. Meiners. **2008**. The plant's response towards insect egg deposition, pp. 205–233. *In* Chemoecology Insect Eggs Egg Depos. Blackwell Publishing

Ltd.

Holopainen, J. K., and J. Gershenzon. **2010**. Multiple stress factors and the emission of plant VOCs. Trends Plant Sci. 15: 176–184.

Honěk, A. 1993. Intraspecific variation in body size and fecundity in insects : A general relationship. Oikos. 66: 483–492.

Hopkinson, R. F., and J. J. Soroka. **2010**. Air trajectory model applied to an in-depth diagnosis of potential diamondback moth infestations on the Canadian Prairies. Agric. For. Meteorol. 150: 1–11.

Howe, G. A., and G. Jander. **2008**. Plant immunity to insect herbivores. Annu. Rev. Plant Biol. 59: 41–66.

Howlader, M. A., and G. Gerber. **1986**. Effects of age, egg development, and mating on calling bahaviour of the bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae). Can. Entomol. 118: 1221–1230.

Hsu, Y.-T., T.-C. Shen, and S.-Y. Hwang. **2009**. Soil fertility management and pest responses: a comparison of organic and synthetic fertilization. J. Econ. Entomol. 102: 160–9.

van Hulten, M., M. Pelser, L. C. van Loon, C. M. J. Pieterse, and J. Ton. **2006**. Costs and benefits of priming for defense in Arabidopsis. Proc. Natl. Acad. Sci. 103: 5602–5607.

Hwang, S. F., H. U. Ahmed, S. E. Strelkov, B. D. Gossen, G. D. Turnbull, G. Peng, and R. J. Howard. **2011**. Seedling age and inoculum density affect clubroot severity and seed yield in canola. Can. J. Plant Sci. 91: 183–190.

Hwang, S. F., H. U. Ahmed, Q. Zhou, A. Rashid, S. E. Strelkov, B. D. Gossen, G. Peng, and G. D. Turnbull. **2013**. Effect of susceptible and resistant canola plants on *Plasmodiophora brassicae* resting spore populations in the soil. 404–412.

Hwang, S. F., H. U. Ahmed, Q. Zhou, S. E. Strelkov, B. D. Gossen, G. Peng, and G. D. Turnbull. **2011**. Influence of cultivar resistance and inoculum density on root hair infection of canola (*Brassica napus*) by *Plasmodiophora brassicae*. Plant Pathol. 60: 820–829.

Ibrahim, M. A., A. Stewart-Jones, J. Pulkkinen, G. M. Poppy, and J. K. Holopainen. **2008**. The influence of different nutrient levels on insect-induced plant volatiles in Bt and conventional oilseed rape plants. Plant Biol. 10: 97–107.

Ingram, D. S., and I. C. Tommerup. **1972**. The life history of *Plasmodiophora brassicae* Woron. Proc. R. Soc. London. 180: 103–112.

Ishiguro, S., A. Kawai-Oda, J. Ueda, I. Nishida, and K. Okada. **2001**. The defective in anther dehiscence 1 gene encodes a novel Phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation, anther dehiscence, and flower opening in Arabidopsis. Plant Cell. 13: 2191–2209.

Jaenike, J. 1978. On optimal oviposition behavior in phytophagous insects. Theor. Popul. Biol. 14: 350–356.

Jahn, L., S. Mucha, S. Bergmann, C. Horn, P. Staswick, B. Steffens, J. Siemens, and J. Ludwig-Müller. **2013**. The clubroot pathogen (*Plasmodiophora brassicae*) influences auxin signaling to regulate auxin homeostasis in Arabidopsis. Plants. 2: 726–749.

Jauset, A. M., M. J. Sarasúa, J. Avilla, and R. Albajes. **1998**. The impact of nitrogen fertilization of tomato on feeding site selection and oviposition by *Trialeurodes vaporariorum*. Entomol. Exp. Appl. 86: 175–182.

Jeffries, M. J., and J. H. Lawton. **1984**. Enemy free space and the structure of ecological communities. Biol. J. Linn. Soc. 23: 269–286.

Jervis, M. A., and P. N. Ferns. **2004**. The timing of egg maturation in insects: Ovigeny index and initial egg load as measures of fitness and of resource allocation. Oikos. 107: 449–460.

Jeschke, V., J. Gershenzon, and D. G. Vassão. **2016**. Insect detoxification of glucosinolates and their hydrolysis products, pp. 199–245. *In* Kopriva, S. (ed.), Adv. Bot. Res. Academic Press.

Jeschke, V., E. E. Kearney, K. Schramm, G. Kunert, A. Shekhov, J. Gershenzon, and D. G. Vassão. **2017**. How glucosinolates affect generalist Lepidopteran larvae: growth, development and glucosinolate metabolism. Front. Plant Sci. 8: 1–12.

Kaloshian, I., and L. L. Walling. **2005**. Hemipterans as plant pathogens. Annu. Rev. Phytopathol. 43: 491–521.

Kaplan, I., R. Halitschke, A. Kessler, S. Sardanelli, and R. F. Denno. **2008**. Constitutive and induced defences to herbivory in above- and below plant tissues. Ecology. 89: 392–406.

Karamanos, R. E., T. B. Goh, and D. P. Poisson. **2005**. Nitrogen, phosphorus, and sulfur fertility of hybrid canola. J. Plant Nutr. 28: 1145–1161.

Karban, R. 2011. The ecology and evolution of induced resistance against herbivores. Funct. Ecol. 25: 339–347.

Karban, R., and A. A. Agrawal. **2002**. Herbivore offense. Annu. Rev. Ecol. Syst. 33: 641–664.

Kazimírová, M. 1996. Influence of larval crowding and mating on lifespan and fecundity of *Mamestra brassicae* (Lepidoptera: Noctuidae). Eur. J. Entomol. 93: 45–52.

Kessler, A., and I. T. Baldwin. **2002**. Plant responses to insect herbivory: The emerging molecular analysis. Annu. Rev. Plant Biol. 53: 299–328.

Kim, J., J. F. Tooker, D. S. Luthe, C. M. De Moraes, and G. W. Felton. **2012**. Insect eggs can enhance wound response in plants: a study system of tomato *Solanum lycopersicum* L. and *Helicoverpa zea* Boddie. PLoS One. 7: e37420.

Koo, Y. J., M. A. Kim, E. H. Kim, J. T. Song, C. Jung, J. K. Moon, J. H. Kim, H. S. Seo, S. I. Song, J. K. Kim, J. S. Lee, J. J. Cheong, and Y. Do Choi. **2007**. Overexpression of salicylic acid carboxyl methyltransferase reduces salicylic acid-mediated pathogen resistance in *Arabidopsis thaliana.* Plant Mol. Biol. 64: 1–15.

Koornneef, A., and C. M. J. Pieterse. **2008**. Cross talk in defense signaling. Plant Physiol. 146: 839–844.

Koricheva, J., S. Larsson, and E. Haukioja. **1998**. Insect performance on experimentally stressed woody plants: a meta-analysis. Annu. Rev. Entomol. 43: 195–216.

Koshihara, T., and H. Yamada. **1981**. Female sex pheromone of the diamondback moth , *Plutella xylostella*. Japan Agric. Res. Q. 15: 22–28.

Kuginuki, Y., H. Yoshikawa, and M. Hirai. **1999**. Variation in virulence of *Plasmodiophora brassicae* in Japan tested with clubroot-resistant cultivars of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). Eur. J. Plant Pathol. 105: 327–332.

Kunkel, B. N., and D. M. Brooks. **2002**. Cross talk between signaling pathways in pathgen defense. Curr. Opin. Plant Biol. 5: 325–331.

Landolt, P. J., M. Tóth, R. L. Meagher, and I. Szarukán. **2013**. Interaction of acetic acid and phenylacetaldehyde as attractants for trapping pest species of moths (Lepidoptera: Noctuidae). Pest Manag. Sci. 69: 245–249.

Larsson, S. 1989. Stressful times for the plant stress : Insect performance hypothesis. Oikos. 56: 277–283.

Lemarié, S., A. Robert-Seilaniantz, C. Lariagon, J. Lemoine, N. Marnet, M. Jubault, M. J. Manzanares-Dauleux, and A. Gravot. **2015**. Both the jasmonic acid and the salicylic acid pathways contribute to resistance to the biotrophic clubroot agent *Plasmodiophora* *brassicae* in Arabidopsis. Plant Cell Physiol. 56: 2158–2168.

Lemmen-lechelt, J. K., T. J. Wist, and M. L. Evenden. **2018**. State-dependent plasticity in response to host-plant volatiles in a long-lived moth, *Caloptilia fraxinella* (Lepidoptera: Gracillariidae). J. Chem. Ecol. 1–12.

Li, H., S. H. Futch, J. P. Syvertsen, and C. W. McCoy. **2007**. Time series forecast and soil characteristics-based simple and multivariate linear models for management of *Diaprepes abbreviatus* root weevil in citrus. Soil Biol. Biochem. 39: 2436–2447.

Little, D., C. Gouhier-Darimont, F. Bruessow, and P. Reymond. **2006**. Oviposition by Pierid butterflies triggers defense responses in Arabidopsis. Plant Physiol. 143: 784–800.

Loader, C., and H. Damman. **1991**. Nitrogen content of food plants and vulnerability of *Pieris rapae* to natural enemies. Ecology. 72: 1586–1590.

Long, J. D., R. S. Hamilton, and J. L. Mitchell. **2007**. Asymmetric competition via induced resistance: Specialist herbivores indirectly suppress generalist preference and populations. Ecology. 88: 1232–1240.

Lovelock, D. A., I. Šola, S. Marschollek, C. E. Donald, G. Rusak, K. H. van Pée, J. Ludwig-Müller, and D. M. Cahill. **2016**. Analysis of salicylic acid-dependent pathways in *Arabidopsis thaliana* following infection with *Plasmodiophora brassicae* and the influence of salicylic acid on disease. Mol. Plant Pathol. 17: 1237–1251.

Lu, J., J. Li, H. Ju, X. Liu, M. Erb, X. Wang, and Y. Lou. **2014**. Contrasting effects of ethylene biosynthesis on induced plant resistance against a chewing and a piercing-sucking herbivore in rice. Mol. Plant. 7: 1670–1682.

Ludwig-Mller, J. 2009. Plant defence - What can we learn from clubroots? Australas. Plant Pathol. 38: 318–324.

Ludwig-Müller, A. J., R. N. Bennett, G. Kiddle, S. Ihmig, M. Ruppel, R. N. Bennett, G. Kiddle, and J. W. G.- Universitdt. **1999**. The host range of *Plasmodiophora brassicae* and its relationship to endogenous glucosinolate content. New Phytol. 141: 443–458.

Ludwig-Müller, J., S. Jülke, K. Geiß, F. Richter, A. Mithöfer, I. Šola, G. Rusak, S. Keenan, and S. Bulman. **2015**. A novel methyltransferase from the intracellular pathogen *Plasmodiophora brassicae* methylates salicylic acid. Mol. Plant Pathol. 16: 349–364.

Ludwig-Müller, J., N. Kasperczyk, B. Schubert, and W. Hilgenberg. **1995**. Identification of salicylic acid in Chinese cabbage and its possible role during root infection with *Plasmodiophora brassicae*. Curr. Top. Phytochem. 14: 39–45.

Ludwig-Müller, J., B. Schubert, K. Pieper, S. Ihmig, and W. Hilgenberg. **1997**. Glucosinolate content in susceptible and resistant Chinese cabbage varieties during development of clubroot disease. Phytochemistry. 44: 407–414.

Macfarlane, I. 1970. Germination of resting spores of *Plasmodiophora brassicae*. Trans. Br. Mycol. Soc. 55: 97–112.

Madlung, A., and L. Comai. **2004**. The effect of stress on genome regulation and structure. Ann. Bot. 94: 481–495.

Mag, T. K. 1983. Canola oil processing in Canada. J. Am. Oil Chem. Soc. 60: 380–384.

Mainguet, A. M., A. Louveaux, G. El Sayed, and P. Rollin. **2000**. Ability of a generalist insect, *Schistocerca gregaria*, to overcome thioglucoside defense in desert plants: Tolerance or adaptation? Entomol. Exp. Appl. 94: 309–317.

Mangel, M. 1987. Oviposition site selection and clutch sizes in insects. J. Math. Biol. 25: 1–22.

Martini, X., K. S. Pelz-stelinski, and L. L. Stelinski. **2014**. Plant pathogen-induced volatiles attract parasitoids to increase parasitism of an insect vector. Front. Ecol. Evol. 2: 1–8.

Masante-Roca, I., S. Anton, L. Delbac, M. C. Dufour, and C. Gadenne. **2007**. Attraction of the grapevine moth to host and non-host plant parts in the wind tunnel: effects of plant phenology, sex, and mating status. Entomol. Exp. Appl. 122: 239–245.

Mason, P. G., A. P. Arthur, O. O. Olfert, and M. A. Erlandson. **1998**. The bertha armyworm (*Mamestra configurata*) (Lepidoptera: Noctuidae) in Western Canada. Can. Entomol. 130: 321–336.

Mason, P. G., M. A. Erlandson, O. O. Olfert, and A. P. Arthur. **1998**. The bertha armyworm (*Mamestra configurata*) (Lepidoptera: Noctuidae) in western Canada. Can. Entomol. 130: 321–336.

Mathur, V., T. O. G. Tytgat, R. M. de Graaf, V. Kalia, A. Sankara Reddy, L. E. M. Vet, and N. M. van Dam. **2013**. Dealing with double trouble: Consequences of single and double herbivory in *Brassica juncea*. Chemoecology. 23: 71–82.

Mayhew, A. P. J., and H. C. J. Godfray. **1997**. Mixed sex allocation strategies in a parasitoid wasp. Oecologia. 110: 218–221.

McCallum, E. J., J. P. Cunningham, J. Lucker, M. P. Zalucki, J. J. De Voss, and J. R. Botella. **2011**. Increased plant volatile production affects oviposition, but not larval development, in the moth *Helicoverpa armigera*. J. Exp. Biol. 214: 3672–3677.

McCloskey, C., and M. B. Isman. **1993**. Influence of foliar glucosinolates in oilseed rape and mustard on feeding and growth of the bertha armyworm, *Mamestra configurata* Walker. J. Chem. Ecol. 19: 249–266.

McCloud, E. S., and I. T. Baldwin. **1997**. Herbivory and caterpillar regurgitants amplify the wound-induced increases in jasmonic acid but not nicotine in *Nicotiana sylvestris*. Planta. 203: 430–435.

Mechaber, W. L., C. T. Capaldo, and J. G. Hildebrand. **2002**. Behavioral responses of adult female tobacco hornworms, *Manduca sexta*, to host plant volatiles change with age and mating status. J. Insect Sci. 2: 5.

Médiène, S., &Muriel Valantin-Morison, J.-P. Sarthou, S. de Tourdonnet, M. Gosme, M. Bertrand, J. Roger-Estrade, J.-N. Aubertot, A. Rusch, N. Motisi, C. Pelosi, and T. Doré. **2011**. Agroecosystem management and biotic interactions : a review. Agron. Sustain. Dev. 31: 491–514.

Merritt, S. Z. 1996. Within-plant variation in concentrations of amino acids, sugar, and sinigrin in phloem sap of black mustard,*Brassica nigra* (L.) Koch (Cruciferae). J. Chem. Ecol. 22: 1133–1145.

Mewis, I., H. M. Appel, A. Hom, R. Raina, and J. C. Schultz. **2005**. Major signaling pathways modulate Arabidopsis glucosinolate accumulation and response to both phloem-feeding and chewing insects. Plant Physiol. 138: 1149–1162.

Miller-Pierce, M. R., and E. L. Preisser. **2012**. Asymmetric priority effects influence the success of invasive forest insects. Ecol. Entomol. 37: 350–358.

Miller, W. E. 2005. Extrinsic effects on fecundity-maternal weight relations in capital-breeding lepidoptera. J. Lepid. Soc. 59: 143–160.

Mithöfer, A., and W. Boland. **2012**. Plant defense against herbivores: Chemical aspects. Annu. Rev. Plant Biol. 63: 431–450.

Montezano, D. G., A. Specht, D. R. Sosa-Gómez, V. F. Roque-Specht, T. M. Bortolin, E. Fronza, P. Pezzi, P. C. Luz, and N. M. Barros. **2014**. Biotic potential, fertility and life table of *Spodoptera albula* (Walker) (Lepidoptera: Noctuidae), under controlled conditions. An. Acad. Bras. Cienc. 86: 723–732.

Moreau, J., B. Benrey, and D. Thiéry. **2006**. Grape variety affects larval performance and also female reproductive performance of the European grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae). Bull. Entomol. Res. 96: 205–212.

Mortvedt, J. J. 1985. Micronutrient fertilizers and fertilization practices. Fertil. Res. 7: 221–235.

Mouttet, R., P. Bearez, C. Thomas, and N. Desneux. **2011**. Phytophagous arthropods and a pathogen sharing a host plant: Evidence for indirect plant-mediated interactions. PLoS One. 6.

Mouttet, R., I. Kaplan, P. Bearez, E. Amiens-Desneux, and N. Desneux. **2013**. Spatiotemporal patterns of induced resistance and susceptibility linking diverse plant parasites. Oecologia. 173: 1379–1386.

Müller, R., M. de Vos, J. Y. Sun, I. E. Sønderby, B. A. Halkier, U. Wittstock, and G. Jander. **2010**. Differential effects of indole and aliphatic glucosinolates on lepidopteran herbivores. J. Chem. Ecol. 36: 905–913.

Mur, L. A. J., P. Kenton, R. Atzorn, O. Miersch, and C. Wasternack. **2005**. The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. Plant Physiol. 140: 249–262.

Mur, L. A. J., P. Kenton, A. J. Lloyd, H. Ougham, and E. Prats. **2008**. The hypersensitive response; The centenary is upon us but how much do we know? J. Exp. Bot. 59: 501–520.

Murmu, J., M. Wilton, G. Allard, R. Pandeya, D. Desveaux, J. Singh, and R. Subramaniam. **2014**. Arabidopsis GOLDEN2-LIKE (GLK) transcription factors activate jasmonic acid (JA)-dependent disease susceptibility to the biotrophic pathogen

Hyaloperonospora arabidopsidis, as well as JA-independent plant immunity against the necrotrophic pathogen *Botryti*. Mol. Plant Pathol. 15: 174–184.

Musser, R. O., S. M. Hum-Musser, H. Eichenseer, M. Peiffer, G. Ervin, J. B. Murphy, and G. W. Felton. **2002**. Herbivory: caterpillar saliva beats plant defences. Nature. 416: 599–600.

Myers, J. H. 1985. Effect of physiological condition of the host plant on the ovipositional choice of the cabbage white butterfly, *Pieris rapae*. J. Anim. Ecol. 54: 193–204.

Nakabayashi, R., and K. Saito. **2013**. Metabolomics for unknown plant metabolites. Anal. Bioanal. Chem. 405: 5005–5011.

Pangesti, N., A. Pineda, C. M. J. Pieterse, M. Dicke, and J. J. A. van Loon. **2013**. Two-way plant mediated interactions between root-associated microbes and insects: from ecology to mechanisms. Front. Plant Sci. 4: 1–11.

Park, S.-W., E. Kaimoyo, D. Kumar, S. Mosher, and D. F. Klessig. **2007**. Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. Science (80). 318: 113–116.

Peñaflor, M. F. G. V., M. Erb, C. A. M. Robert, L. A. Miranda, A. G. Werneburg, F. C. A. Dossi, T. C. J. Turlings, and J. M. S. Bento. **2011**. Oviposition by a moth suppresses constitutive and herbivore-induced plant volatiles in maize. Planta. 234: 207–215.

Philip, H., and E. Mengersen. **1989**. Insect pests of the Prairies. University of Alberta Press, Edmonton.

Phillips, P. W. B. 2003. The economic impact of herbicide tolerant canola in Canada, pp. 119–140. *In* Kalaitzandonakes, N. (ed.), Econ. Environ. Impacts Agbiotech a Glob. Perspect. Springer US, Boston, MA.

Piesik, D., G. Lemńczyk, A. Skoczek, R. Lamparski, J. Bocianowski, K. Kotwica, and K. J.

Delaney. **2011**. Fusarium infection in maize: Volatile induction of infected and neighboring uninfected plants has the potential to attract a pest cereal leaf beetle, *Oulema melanopus*. J. Plant Physiol. 168: 1534–1542.

Pivnick, K. A., B. J. Jarvis, C. Gillott, G. P. Slater, and E. W. Underhill. **1990**. Daily patterns of reproductive activity and the influence of adult density and exposure to host plants on reproduction in the diamondback moth (Lepidoptera: Plutellidae). Environ. Entomol. 19: 587–593.

Van Poecke, R. M. P., M. A. Posthumus, and M. Dicke. **2001**. Herbivore-induced volatile production by *Arabidopsis thaliana* leads to attraction of the parasitoid *Cotesia rubecula*: Chemical, behavioral, and gene-expression analysis. J. Chem. Ecol. 27: 1911–1928.

Poelman, E. H., C. Broekgaarden, J. J. A. Van Loon, and M. Dicke. **2008**. Early season herbivore differentially affects plant defence responses to subsequently colonizing herbivores and their abundance in the field. Mol. Ecol. 17: 3352–3365.

Ponzio, C., R. Gols, C. M. J. Pieterse, and M. Dicke. **2013**. Ecological and phytohormonal aspects of plant volatile emission in response to single and dual infestations with herbivores and phytopathogens. Funct. Ecol. 27: 587–598.

Proffit, M., M. A. Khallaf, D. Carrasco, M. C. Larsson, and P. Anderson. **2015**. "Do you remember the first time?" Host plant preference in a moth is modulated by experiences during larval feeding and adult mating. Ecol. Lett. 18: 365–374.

Prudic, K. L., J. C. Oliver, and M. D. Bowers. **2005**. Soil nutrient effects on oviposition preference, larval performance, and chemical defense of a specialist insect herbivore. Oecologia. 143: 578–587.

Qiu, B., J. A. Harvey, C. E. Raaijmakers, L. E. M. Vet, and N. M. Van Dam. **2009**.

Nonlinear effects of plant root and shoot jasmonic acid application on the performance of *Pieris brassicae* and its parasitoid *Cotesia glomerata*. Funct. Ecol. 23: 496–505.

Rakow, G. 2000. Downey designer of canola. Plant Breed. Rev. 18: 1–12.

Rashid, M. M., M. Jahan, and K. S. Islam. **2016**. Impact of nitrogen, phosphorus and potassium on brown planthopper and tolerance of its host rice plants. Rice Sci. 23: 119–131.

Ratzka, A., H. Vogel, D. J. Kliebenstein, T. Mitchell-Olds, and J. Kroymann. **2002**. Disarming the mustard oil bomb. Proc. Natl. Acad. Sci. U. S. A. 99: 11223–11228.

Redovnikovic, I. R., T. Glivetic, K. Delonga, and J. Vorkapic-Furac. **2008**. Glucosinolates and their potential role in plant. Period. Biol. 110: 297–309.

Reed, D. W., K. A. Pivnick, and E. W. Underhill. **1989**. Identification of chemical imposition stimulants for the diamondback moth, Plutella xylostella, present in three species of Brassicaceae. Entomol. Exp. Appl. 53: 277–286.

Reisenman, C. E., J. A. Riffell, E. A. Bernays, and J. G. Hildebrand. **2010**. Antagonistic effects of floral scent in an insect-plant interaction. Proc. R. Soc. B Biol. Sci. 277: 2371–2379.

Renwick, J. A. A., M. Haribal, S. Gouinguené, and E. Städler. **2006**. Isothiocyanates stimulating oviposition by the diamondback moth, *Plutella xylostella*. J. Chem. Ecol. 32: 755–766.

Riffell, J. A, H. Lei, and J. G. Hildebrand. **2009**. Neural correlates of behavior in the moth Manduca sexta in response to complex odors. Proc. Natl. Acad. Sci. 106: 19219–26.

Robert, C. A. M., M. Erb, M. Duployer, C. Zwahlen, G. R. Doyen, and T. C. J. Turlings. **2012**. Herbivore-induced plant volatiles mediate host selection by a root herbivore. New Phytol. 194: 1061–1069.

Robertson, G. W., D. W. Griffiths, W. M. F. Smith, and R. D. Butcher. **1993**. The application of thermal desorption-gas chromatography-mass spectrometry to the analyses of flower volatiles from five varieties of oilseed rape (*Brassica napus* spp. *oleifera*). Phytochem. Anal. 4: 152–157.

Röder, G., M. Rahier, and R. E. Naisbit. **2011**. Do induced responses mediate the ecological interactions between the specialist herbivores and phytopathogens of an alpine plant? PLoS One. 6: 1–7.

Rojas, J. C. 1999. Electrophysiological and behavioral responses of the cabbage moth to plant volatiles. J. Chem. Ecol. 25: 1867–1883.

Rojas, J. C., and A. Virgen. **2003**. Chemical and tactile cues influencing oviposition of a generalist moth, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Environ. Entomol. 32: 1386–1392.

Rojas, J. C., A. Virgen, and L. Cruz-López. **2003**. Chemical and tactile cues influencing oviposition of a generalist moth, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Environ. Entomol. 32: 1386–1392.

Rostás, M., J. Ton, B. Mauch-Mani, and T. C. J. Turlings. **2006**. Fungal infection reduces herbivore-induced plant volatiles of maize but does not affect naïve parasitoids. J. Chem. Ecol. 32: 1897–1909.

Sarfraz, M., L. M. Dosdall, and B. A. Keddie. **2007**. Resistance of some cultivated Brassicaceae to infestations by *Plutella xylostella* (Lepidoptera: Plutellidae). J. Econ. Entomol. 100: 215–224.

Sarfraz, R. M., L. M. Dosdall, and A. B. Keddie. **2009**. Bottom-up effects of host plant nutritional quality on *Plutella xylostella* (Lepidoptera: Plutellidae) and top-down effects of herbivore attack on plant compensatory ability. Eur. J. Entomol. 106: 583–594.

Sarmento, R. A., F. Lemos, P. M. Bleeker, R. C. Schuurink, A. Pallini, M. G. A. Oliveira, E. R. Lima, M. Kant, M. W. Sabelis, and A. Janssen. **2011**. A herbivore that manipulates plant defence. Ecol. Lett. 14: 229–236.

Schenk, P. M., K. Kazan, I. Wilson, J. P. Anderson, T. Richmond, S. C. Somerville, and J. M. Manners. **2000**. Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. Proc Natl Acad Sci. 97: 11655–11660.

Schmelz, E. a, M. J. Carroll, S. LeClere, S. M. Phipps, J. Meredith, P. S. Chourey, H. T. Alborn, and P. E. a Teal. **2006**. Fragments of ATP synthase mediate plant perception of insect attack. Proc. Natl. Acad. Sci. 103: 8894–8899.

Schweiger, R., A. M. Heise, M. Persicke, and C. Muller. **2014**. Interactions between the jasmonic and salicylic acid pathway modulate the plant metabolome and affect herbivores of different feeding types. Plant, Cell Environ. 37: 1574–1585.

Seino, Y., Y. Suzuki, and K. Sogawa. **1996**. An ovicidal substance produced by rice plants in response to oviposition by the Whitebacked planthopper, *Sogatella furcifera* (Horvath) (Homoptera: Delphacidae). Appl. Entomol. Zool. 31: 467–473.

Seo, H. S., J. T. Song, J. J. Cheong, Y. H. Lee, Y. W. Lee, I. Hwang, J. S. Lee, and Y. D. Choi. **2001**. Jasmonic acid carboxyl methyltransferase: a key enzyme for jasmonate-regulated plant responses. Proc. Natl. Acad. Sci. 98: 4788–93.

Shah, J. 2003. The salicylic acid loop in plant defense. Curr. Opin. Plant Biol. 6: 365–371.

Shapiro, A. M., and J. E. DeVay. **1987**. Hypersensitivity reaction of *Brassica nigra* L. (Cruciferae) kills eggs of Pieris butterflies (Lepidoptera: Pieridae). Oecologia. 71: 631–632.

Shields, V. D. C., and B. K. Mitchell. **1995**. Sinigrin as a feeding deterrent in two crucifer-feeding, polyphagous Lepidopterous species and the effects of feeding stimulant mixtures on deterrency. Philos. Trans. R. Soc. B Biol. Sci. 347: 439–446.

Shroff, R., F. Vergara, A. Muck, A. Svatos, and J. Gershenzon. **2008**. Nonuniform distribution of glucosinolates in *Arabidopsis thaliana* leaves has important consequences for plant defense. Proc. Natl. Acad. Sci. 105: 6196–6201.

Siemens, J., M. C. González, S. Wolf, C. Hofmann, S. Greiner, Y. Du, T. Rausch, T. Roitsch, and J. Ludwig-Müller. **2011**. Extracellular invertase is involved in the regulation of clubroot disease in *Arabidopsis thaliana*. Mol. Plant Pathol. 12: 247–262.

Siemens, J., I. Keller, J. Sarx, S. Kunz, A. Schuller, W. Nagel, T. Schmülling, M. Parniske, and J. Ludwig-Müller. **2006**. Transcriptome analysis of Arabidopsis clubroots indicate a key role for cytokinins in disease development. Mol. Plant-Microbe Interact. 19: 480–494.

Silva, R., and M. J. Furlong. **2012**. Diamondback moth oviposition: Effects of host plant and herbivory. Entomol. Exp. Appl. 143: 218–230.

Sinkov, N. A., and J. J. Harynuk. **2011**. Cluster resolution: A metric for automated, objective and optimized feature selection in chemometric modeling. Talanta. 83: 1079–1087.

Sinkov, N. A., P. M. L. Sandercock, and J. J. Harynuk. **2014**. Chemometric classification of casework arson samples based on gasoline content. Forensic Sci. Int. 235: 24–31.

Skoog, D. A., F. J. Holler, and S. R. Crouch. **2007**. Principles of Instrumental Analysis, 6th ed, Thomson Brooks/Cole, Belmont, CA.

Smith, J. L., C. M. De Moraes, and M. C. Mescher. **2009**. Jasmonate- and salicylate-mediated plant defense responses to insect herbivores, pathogens and parasitic plants. Pest Manag. Sci. 65: 497–503.

Smith, E. G., B. M. Upadhyay, M. L. Favret, and R. E. Karamanos. **2010**. Fertilizer response for hybrid and open-pollinated canola and economic optimal nutrient levels. Can. J. Plant Sci. 90: 305–310.

Soler, R., F. R. Badenes-Pérez, C. Broekgaarden, S. J. Zheng, A. David, W. Boland, and M. Dicke. **2012**. Plant-mediated facilitation between a leaf-feeding and a phloem-feeding insect in a brassicaceous plant: From insect performance to gene transcription. Funct. Ecol. 26: 156–166.

Song, J. T. 2006. Induction of a salicylic acid glucosyltransferase, AtSGT1, is an early disease response in *Arabidopsis thaliana*. Mol Cells. 22: 233–238.

Spitzer, K., M. Rejmfinek, and T. Soldfin. **1984**. The fecundity and long-term variability in abundance of noctuid moths (Lepidoptera: Noctuidae). Oecologia. 62: 91–93.

Spoel, S. H., J. S. Johnson, and X. Dong. **2007**. Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. Proc. Natl. Acad. Sci. 104: 18842–18847.

Stam, J. M., L. ChrÉtien, M. Dicke, and E. H. Poelman. **2017**. Response of *Brassica oleracea* to temporal variation in attack by two herbivores affects preference and performance of a third herbivore. Ecol. Entomol. 42: 803–815.

Stam, J. M., A. Kroes, Y. Li, R. Gols, J. J. A. van Loon, E. H. Poelman, and M. Dicke. **2014**. Plant interactions with multiple insect herbivores: from community to genes. Annu. Rev. Plant Biol. 65: 689–713.

Stam, J. M., A. Kroes, Y. Li, R. Gols, J. J. A. Van Loon, E. H. Poelman, and M. Dicke. **2014**. Plant interactions with multiple insect herbivores : from community to genes. Annu. Rev. Plant Biol. 65: 689–713.

Stamp, N. 2003. Out of the quagmire of plant defense hypotheses. Q. Rev. Biol. 78: 23–55.

Staswick, P. E., and I. Tiryaki. **2004**. The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in Arabidopsis. Plant Cell. 16: 2117–2127.

Sterner, R. W., and J. J. Elser. **2002**. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, New Jersey, USA.

Stevens, V. M., A. Trochet, H. Van Dyck, J. Clobert, and M. Baguette. **2012**. How is dispersal integrated in life histories: A quantitative analysis using butterflies. Ecol. Lett. 15: 74–86.

Stotz, H. U., B. R. Pittendrigh, J. Kroymann, K. Weniger, J. Fritsche, A. Bauke, and T. Mitchell-Olds. **2000**. Induced plant defense responses against chewing insects. Ethylene signaling reduces resistance of Arabidopsis against Egyptian cotton worm but not diamondback moth. Plant Physiol. 124: 1007.

Stout, M. J., a L. Fidantsef, S. S. Duffey, and R. M. Bostock. **1999**. Signal interactions in pathogen and insect attack: systemic plant-mediated interactions between pathogens and herbivores of the tomato, *Lycopersicon esculentum*. Physiol. Mol. Plant Pathol. 54: 115–130.

Strelkov, S. E., and G. R. Dixon. **2014**. Clubroot (*Plasmodiophora brassicae*) on canola and other Brassica species – disease development, epidemiology and management. Can. J. Plant Pathol. 36: 1–4.

Strelkov, S. E., and S. F. Hwang. **2014**. Clubroot in the Canadian canola crop: 10 years into the outbreak. Can. J. Plant Pathol. 36: 27–36.

Strelkov, S. E., V. P. Manolii, T. Cao, S. Xue, and S. F. Hwang. **2007**. Pathotype classification of *Plasmodiophora brassicae* and its occurrence in *Brassica napus* in Alberta, Canada. J. Phytopathol. 155: 706–712.

Strelkov, S. E., J. P. Tewari, and E. Smith-Degenhardt. **2006**. Characterization of

Plasmodiophora brassicae populations from Alberta , Canada. Can. J. Plant Pathol. 28: 467–474.

Stringam, G. R., D. F. Degenhardt, M. R. Thiagarajah, and V. K. Bansal. **1999**. Q2 summer rape. Can. J. Plant Sci. 79: 597–598.

Takahashi, H., Y. Kanayama, M. S. Zheng, T. Kusano, S. Hase, M. Ikegami, and J. Shah. **2004**. Antagonistic interactions between the SA and JA signaling pathways in Arabidopsis modulate expression of defense genes and gene-for-gene resistance to cucumber mosaic virus. Plant Cell Physiol. 45: 803–809.

Talekar, N. S., and A. M. Shelton. **1993**. Biology, ecology, and management of the diamonback moth. Annu. Rev. Entomol. 38: 275–301.

Talsma, J. H. R., A. Biere, and J. A. Harvey. **2008**. Oviposition cues for a specialist butterfly – plant chemistry and size. J Chem Ecol. 34: 1202–1212.

Tan, S., R. R. Evans, M. L. Dahmer, B. K. Singh, and D. L. Shaner. **2005**. Imidazolinone-tolerant crops: history, current status and future. Pest Manag. Sci. 61: 246–257.

Tasin, M., A. C. Bäckman, M. Coracini, D. Casado, C. Ioriatti, and P. Witzgall. **2007**. Synergism and redundancy in a plant volatile blend attracting grapevine moth females. Phytochemistry. 68: 203–209.

Tewari, J. P., S. E. Strelkov, D. Orchard, M. Hartman, R. M. Lange, and T. K. Turkington. **2005**. Identification of clubroot of crucifers on canola (*Brassica napus)* in Alberta. Can. J. Plant Pathol. 27: 143–144.

The Royal Society. How does GM differ from conventional plant breeding ? https://royalsociety.org/topics-policy/projects/gm-plants/how-does-gm-differ-from-conventional-plant-breeding/

(accessed on May 6, 2018)

Thöming, G., and H. R. Norli. **2015**. Olfactory cues from different plant species in host selection by female pea moths. J. Agric. Food Chem. 63: 2127–2136.

Thompson, G. A., and F. L. Goggin. **2006**. Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. J. Exp. Bot. 57: 755–766.

Thompson, J. N. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of off spring in phytophagons insects. Entomol. exp. appl. 47: 3–14.

Tommerup, I. C., and D. S. Ingram. **1971**. The life cycle of Plsmodiphora brassicae Woron. in tissue cultures and in intact roots. New Phytol.

Tooker, J. F., and C. M. De Moraes. **2005**. Jasmonate in lepidopteran eggs and neonates. J. Chem. Ecol. 31: 2753–2759.

Tooker, J. F., and C. M. De Moraes. **2007**. Jasmonate, salicylate, and benzoate in insect eggs. J. Chem. Ecol. 33: 331–343.

Toome, M., P. Randjärv, L. Copolovici, Ü. Niinemets, K. Heinsoo, A. Luik, and S. M. Noe. **2010**. Leaf rust induced volatile organic compounds signalling in willow during the infection. Planta. 232: 235–243.

Traw, M. B. 2002. Is induction response negatively correlated with constitutive resistance in black mustard? Evolution. 56: 2196–2205.

Tuomi, J., T. Fagerstrom, and P. Niemela. 1991. Carbon allocation, phenotypic plasticity, and induced defenses. In: Phytochemical induction by herbivores (eds D.W. Tallamy and M.J. Raupp). John Wiley & Sons, New York, NY, U.S.A., pp. 85-104.

Turlings, T. C. J., J. H. Tumlinson, and W. J. Lewis. **1990**. Exploitation of herbivore-induced Plant odors by host-seeking parasitic wasps. Adv. Sci. 250: 1251–1253.

Turner, J. G., C. Ellis, and A. Devoto. **2002**. The jasmonate signal pathway. Plant Cell. 14 Suppl: S153–S164.

Turnock, W. J. 1985. Developmental, survival, and reproductive parameters of bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae) on four plant species. Can. Ent. 117: 1267–1271.

Turnock, W. J. 1987. Predicting larval abundance of the bertha armyworm, *Mamestra configurata* Wlk., in Manitoba from catches of male moths in sex attractant traps. Can. Entomol. 119: 167–178.

Ulland, S., E. Ian, R. Mozuraitis, A. K. Borg-Karlson, R. Meadow, and H. Mustaparta. **2008**. Methyl salicylate, identified as primary odorant of a specific receptor neuron type, inhibits oviposition by the moth *Mamestra brassicae* L. (Lepidoptera, Noctuidae). Chem. Senses. 33: 35–46.

Ulmer, B., and C. Gillott. **2001**. Feeding preferences , growth , and development of *Mamestra configurata* (Lepidoptera: Noctuidae) on Brassicaceae. Can. Entomol. 133: 509–519.

Ulmer, B., C. Gillott, and M. Erlandson. **2002**. Oviposition preferences of bertha armyworm *Mamestra configurata* Walker (Lepidoptera: Noctuidae) on different crucifer cultivars and growth stages. Environ. Entomol. 31: 1135–1141.

Ulmer, B., C. Gillott, and M. Erlandson. **2003**. Conspecific eggs and bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), oviposition site selection. Environ. Entomol. 32: 529–534.

Ulmer, B. J. 2002. Crucifer host plant suitability for bertha armyworm (*Mamestra configurata*) and diamondback moth (*Plutella xylostella*).

Underwood, N. 2012. When herbivores come back: Effects of repeated damage on induced resistance. Funct. Ecol. 26: 1441–1449.

Ute, W., and B. Meike. **2011**. Glucosinolate breakdown in Arabidopsis: Mechanism, regulation and biological significance. Arab. B. 1–14.

Utsumi, S., Y. Ando, and T. Ohgushi. **2011**. Evolutionary consequence of indirect interactions among insect herbivores through herbivore-induced plant regrowth. J. Plant Interact. 6: 171–172.

Velasco, P., P. Soengas, M. Vilar, M. E. Cartea, and M. Del Rio. **2008**. Comparison of glucosinolate profiles in leaf and seed tissues of different *Brassica napu*s crops. J. Am. Soc. Hortic. Sci. 133: 551–558.

Veromann, E., M. Toome, A. Kannaste, R. Kaasik, L. Copolovici, J. Flink, K. Gabriella, L. Narits, A. Luik, and N. Ülo. **2013**. Effects of nitrogen fertilization on insect pests, their parasitoids, plant diseases and volatile organic compounds in *Brassica napus.* Crop Prot. 43: 79–88.

Viswanathan, D. V., O. A. Lifchits, and J. S. Thaler. **2007**. Consequences of sequential attack for resistance to herbivores when plants have specific induced responses. Oikos. 116: 1389–1399.

Viswanathan, D. V., A. J. T. Narwani, and J. S. Thaler. **2005**. Specificity in induced plant responses shapes patterns of herbivore occurrence on *Solanum dulcamara*. Ecology. 86: 886–896.

Vlot, A. C., D. A. Dempsey, and D. F. Klessig. **2009**. Salicylic Acid, a multifaceted hormone to combat disease. Annu. Rev. Phytopathol. 47: 177–206.

de Vos, M., K. L. Kriksunov, and G. Jander. **2008**. Indole-3-acetonitrile production from indole glucosinolates deters oviposition by *Pieris rapae*. Plant Physiol. 146: 916–926.

De Vos, R. C. H., S. Moco, A. Lommen, J. J. B. Keurentjes, R. J. Bino, and R. D. Hall. **2007**. Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. Nat. Protoc. 2: 778–791.

Wäckers, T. M., BezemerR., W. M. van Dam, W. H. van Der Putten, and L. F. Wäckers. **2004**. Above- and below-ground terpenoid aldehyde induction in cotton, *Gossypium herbaceum*, following root and leaf injury. J. Chem. Ecol. 30: 53–67.

Wallenhammar, A. C. 1996. Prevalence of *Plasmodiophora brassicae* in a spring oilseed rape growing area in central Sweden and factors influencing soil infestation levels. Plant Pathol. 45: 710–719.

Walling, L. L. 2000. The myriad plant responses to herbivores. J. Plant Growth Regul. 19: 195–216.

Walling, L. L. 2008. Avoiding effective defenses: strategies employed by phloem-feeding insects. Plant Physiol. 146: 859–866.

Wang, J. J., J. H. Tsai, and T. K. Broschat. **2006**. Effect of nitrogen fertilization of corn on the development, survivorship, fecundity and body weight of *Peregrinus maidis* (Hom., Delphacidae). J. Appl. Entomol. 130: 20–25.

War, A. R., M. G. Paulraj, T. Ahmad, A. A. Buhroo, B. Hussain, S. Ignacimuthu, and H. C. Sharma. **2012**. Mechanisms of plant defense against insect herbivores. Plant Signal. Behav. 7: 1306–1320.

Waring, G., and N. Cobb. **1992**. The impact of plant stress on herbivore population dynamics, pp. 167–225. *In* EA, B. (ed.), Insect-Plant Interact. CRC, Boca Raton.

Wasternack, C. 2007. Jasmonates: An update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann. Bot. 100: 681–697.

Wee, S. 2016. Effects of conspecific herbivory and mating status on host searching and oviposition behavior of *Plutella xylostella* (Lepidoptera : Plutellidae) in relation to its host , *Brassica oleracea* (brassicales: Brassicaceae). Florida Entomol. 99: 159–165.

van Wees, S. C., E. a de Swart, J. a van Pelt, L. C. van Loon, and C. M. Pieterse. **2000**. Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. U. S. A. 97: 8711–8716.

White, T. C. R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. Oecologia. 63: 90–105.

Williams, P. 1966. A system for the determination of races of *Plasmodiophora brassicae* that infect cabbage and rutabaga. Phytopathology. 56: 624–626.

Wittstock, U., N. Agerbirk, E. J. Stauber, C. E. Olsen, M. Hippler, T. Mitchell-Olds, J. Gershenzon, and H. Vogel. **2004**. Successful herbivore attack due to metabolic diversion of a plant chemical defense. Proc. Natl. Acad. Sci. 101: 4859–4864.

Wright, G. A., B. D. Skinner, and B. H. Smith. **2002**. Ability of honeybee, Apis mellifera, to detect and discriminate odors of varieties of canola (*Brassica rapa* and *Brassica napus*) and snapdragon flowers (*Antirrhinum majus*). J. Chem. Ecol. 28: 721–740.

Wyckhuys, K. A. G., I. Graziosi, D. D. Burra, and A. J. Walter. **2017**. Phytoplasma infection of a tropical root crop triggers bottom-up cascades by favoring generalist over specialist herbivores. 1–18.

Wylie, H. G., and G. E. Bucher. **1977**. The bertha armyworm, *Mamestra configurata* (Lepidotera: Noctuidae). Mortality of immature stages on the rape crop. Can. Entomol. 109: 823–837.

Xue, S., T. Cao, R. J. Howard, S. F. Hwang, and S. E. Strelkov. **2008**. Isolation and variation in virulence of single-spore isolates of *Plasmodiophora brassicae* from Canada. Plant Dis. 92: 456–462.

Ye, M., S. M. Luo, J. F. Xie, Y. F. Li, T. Xu, Y. Liu, Y. Y. Song, K. Zhu-Salzman, and R. Sen Zeng. **2012**. Silencing COI1 in rice increases susceptibility to chewing insects and impairs inducible defense. PLoS One. 7: 1–11.

Yi, H. S., J. W. Yang, S. Y. Ghim, and C. M. Ryu. **2011**. A cry for help from leaf to root: Aboveground insect feeding leads to the recruitment of rhizosphere microbes for plant self-protection against subsequent diverse attacks. Plant Signal. Behav. 6: 1192–1194.

Zebelo, S., J. Disi, R. Balusu, B. Reeves, and H. Fadamiro. **2017**. *Spodoptera exigua* modulates gossypol biosynthesis in cotton *Gossypium hirsutum*. J. Plant Interact. 12: 121–127.

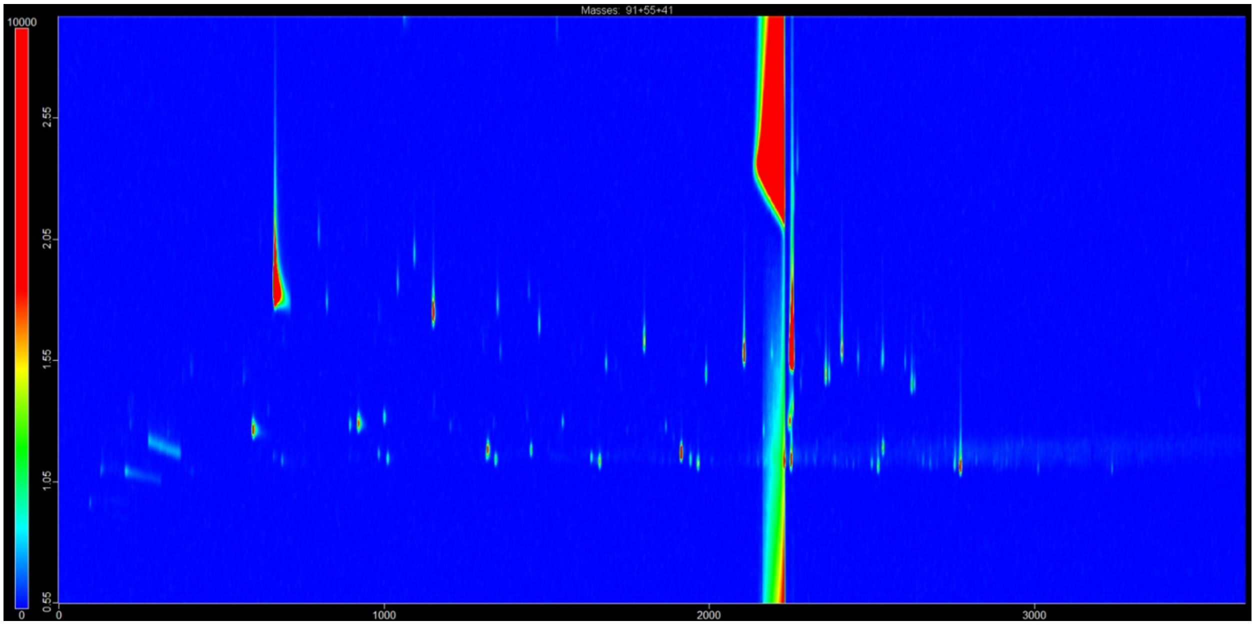
Zhang, P., G. Ma, C. Wang, H. Lu, S. Li, Y. Xie, D. Ma, Y. Zhu, and T. Guo. **2017**. Effect of irrigation and nitrogen application on grain amino acid composition and protein quality in winter wheat. PLoS One. 12: 1–15.

Zou, X., Z. Xu, H. Zou, J. Liu, S. Chen, Q. Feng, and S. Zheng. **2016**. Glutathione S-transferase SlGSTE1 in *Spodoptera litura* may be associated with feeding adaptation of host plants. Insect Biochem. Mol. Biol. 70: 32–43.

Züst, T., and A. A. Agrawal. **2017**. Trade-offs between plant growth and defense against insect herbivory: An emerging mechanistic synthesis. Annu. Rev. Plant Biol. 68: 513–534.

Appendix A

GC×GC-TOFMS contour plot of a susceptible canola sample. Mass channels 91+55+41



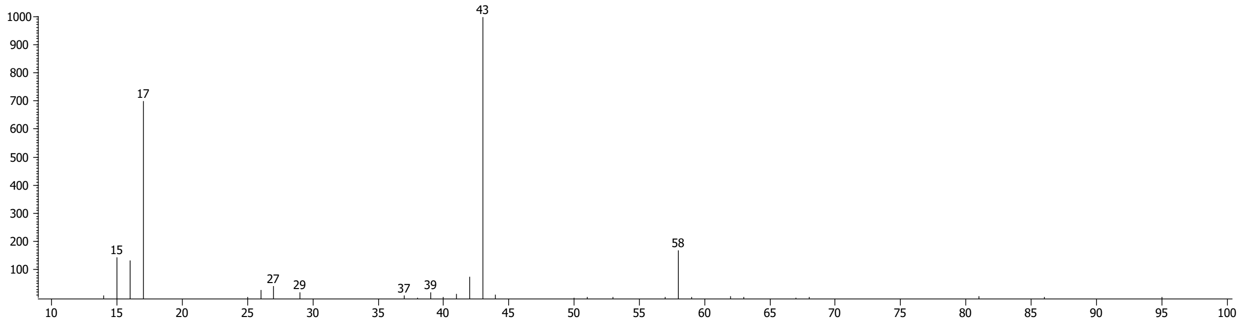
Appendix B. Analytes selected clubroot-guided BE/FS algorithm to distinguish between 3 different types of treatment combinations peaks which could be tentatively identified by mass spectral search and retention index are identified by tentative ID. Selected peaks which could not be assigned a chemical identity are identified by a unique feature ID number.

Compound	**Infected vs non-infected**	**Susceptible vs resistant**	**Bertha armyworm preference vs non-preference**	**Similarity mass spec**	**Exp. Ri**	**Lib. Ri**
2-propanone	X			646	547	479
Analyte 221	X					
1,3,5-cycloheptatriene	X			573	753	756
Analyte 104	X					
Pentadecane	X			549	1394	1413
Analyte 325	X					
Analyte 343	X					
Benzene	X			531	636	648
Bicyclo[3.2.1]oct-2-ene, 3-methyl-4-methylene-	X			632	1177	
Analyte 167	X		X			
Oxazole,4,5-dihydro-2-methyl-4-(1-methylethyl)-, (s)-	X			718	1295	
Furan, 2-methyl-	X			386	607	646
Benzene, 1,2-dimethyl-	X	X		645	867	883
Analyte 413	X					
Nonanal	X			517	1101	1112
Analyte 368	X					
Analyte 207	X		X			
Analyte 226	X					
[1,1-bicyclopentyl]-2-one	X			426	1274	1273
Analyte 166	X	X				
Analyte 59	X		X			
Analyte 410	X					
Benzene, 1,2,3-trimethyl-	X			555	994	986
Analyte 49	X					
Analyte 111	X					
B-ocimene, (e)-		X		551	1046	1048
Analyte 103		X		642	553	

Compound	**Infected vs non-infected**	**Susceptible vs resistant**	**Bertha armyworm preference vs non-preference**	**Similarity mass spec**	**Exp. Ri**	**Lib. Ri**
2-cyclohexen-1-one, 2-(2-methyl-2-propenyl)-		X		695	1353	1228
Benzene, methoxy-		X		701	916	920
Cycloheptanone, 2-(3-buten-1-yl)-		X		589	1435	1360
Undecane, 2-methyl-		X		629	1693	1164
Analyte 97		X				
1-dodecene		X		669	1487	1188
Analyte 332		X				
Analyte 58		X				
Analyte 439		X				
Analyte 419		X				
Analyte 202		X				
Analyte 64		X				
Analyte 326		X				
Analyte 384		X				
Tridecane				777	1296	1300
Analyte 557						
Analyte 43						
Nonane				383	895	900
Acetaldehyde				546	543	381
Analyte 1						
B-myrcene				844	988	979
Analyte 537						
Limonene				817	1030	1014
Analyte 328						
Acetic acid				513	591	594
Analyte 54						
Analyte 557						
Analyte 165						
Analyte 217						
Analyte 248						
Analyte 119						
Analyte 188						

Appendix C. Mass spectra for tentatively identified compounds

2-PROPANONE



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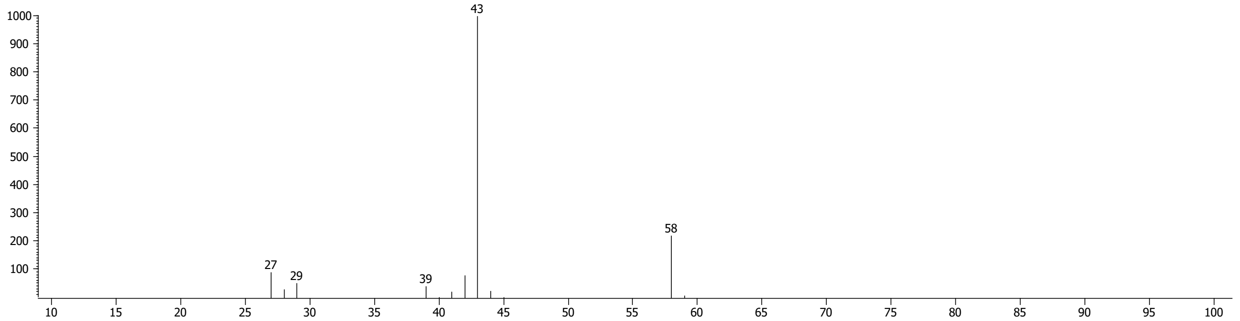
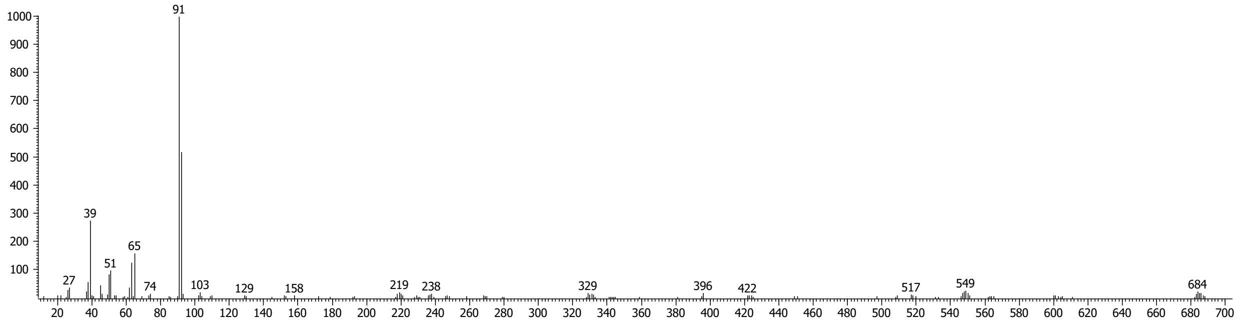


1,3,5-CYCLOHEPTATRIENE



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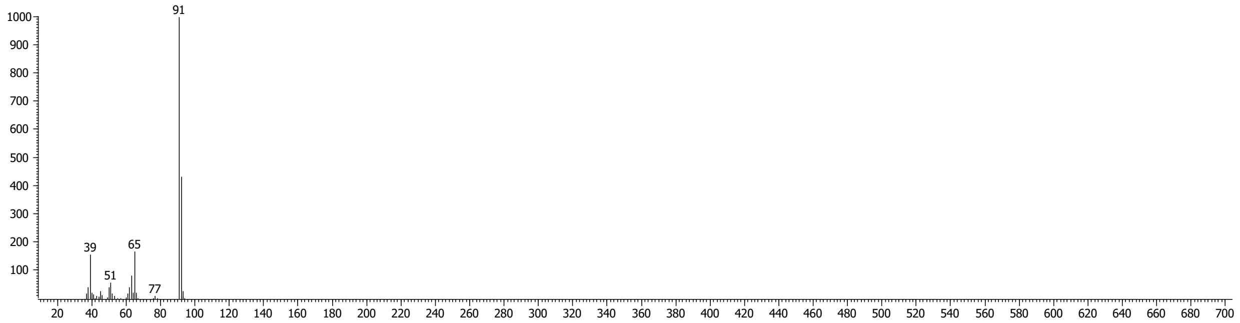
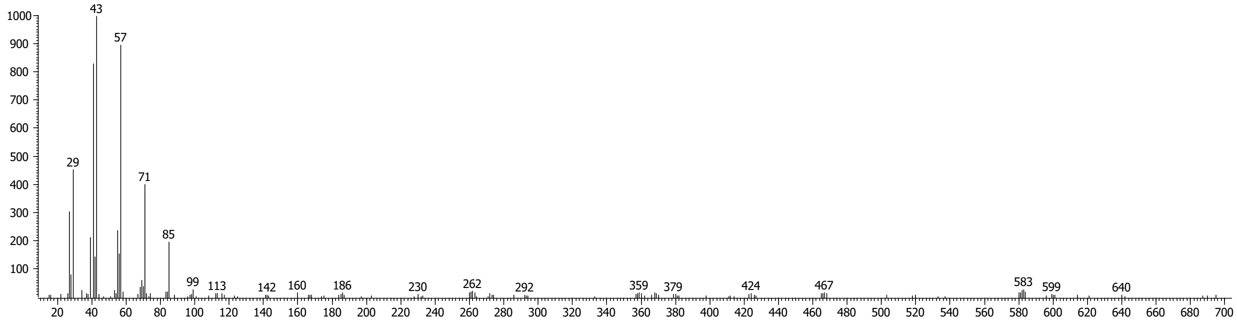
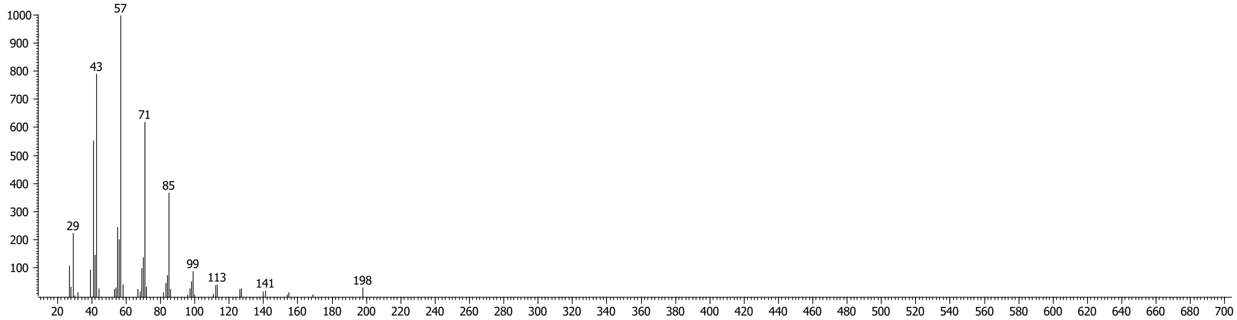


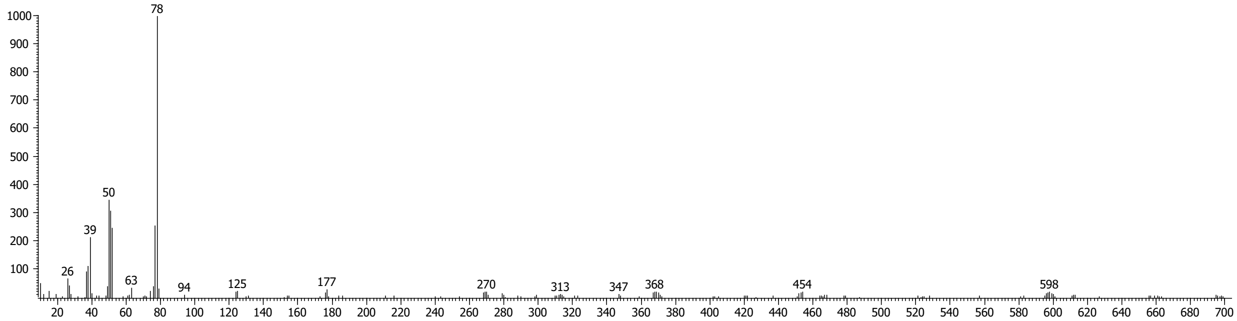
TETRATADECANE



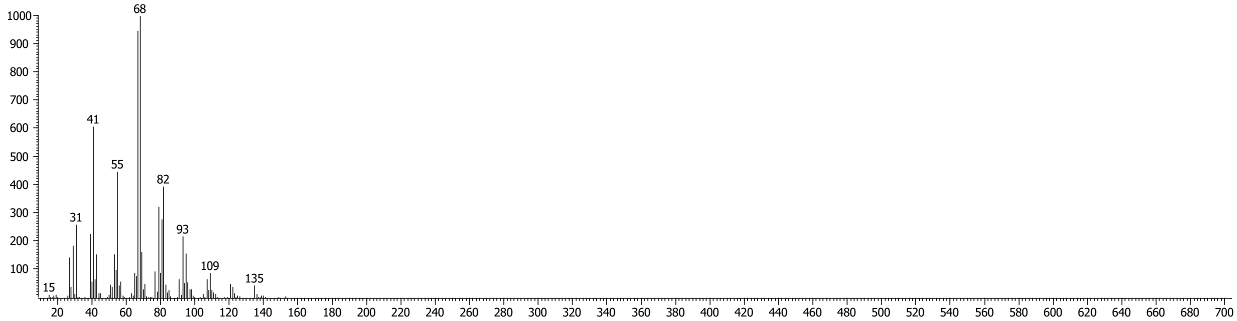
Library Hit - similarity 549, "Tetradecane"



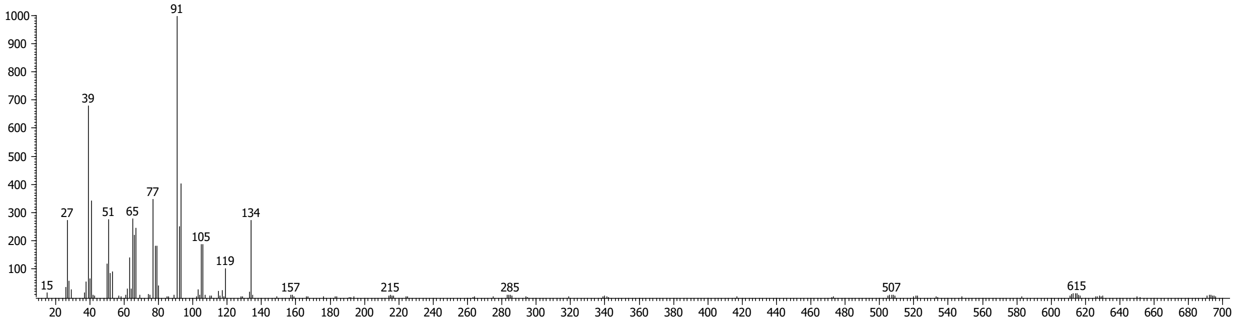
BENZENE



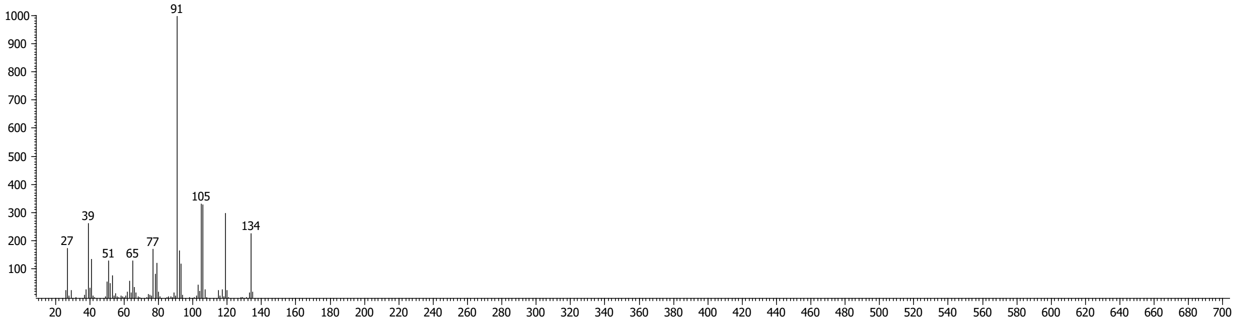
Library Hit - similarity 531, "Benzene"



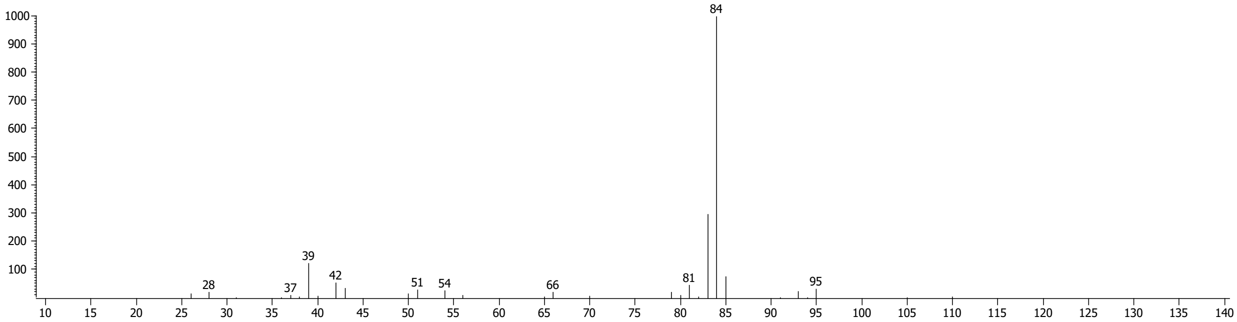
BICYCLO[3.2.1]OCT-2-ENE, 3-METHYL-4-METHYLENE-



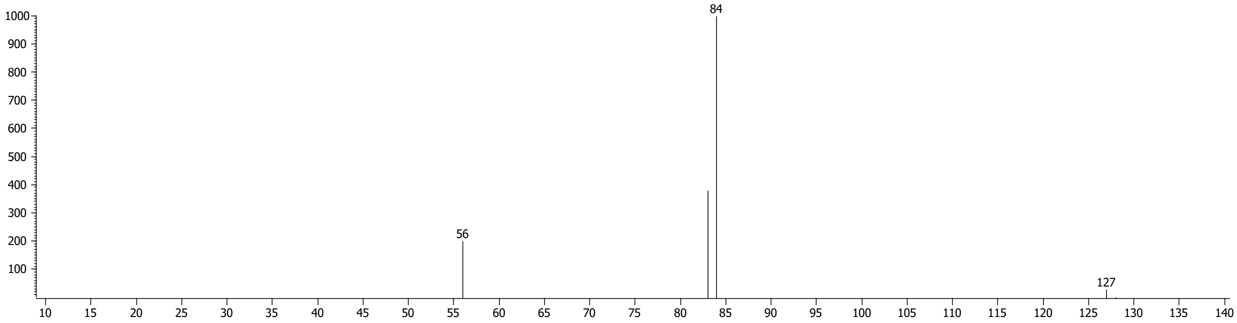
Library Hit - similarity 702, "Bicyclo[3.2.1]oct-2-ene, 3-methyl-4-methylene-"



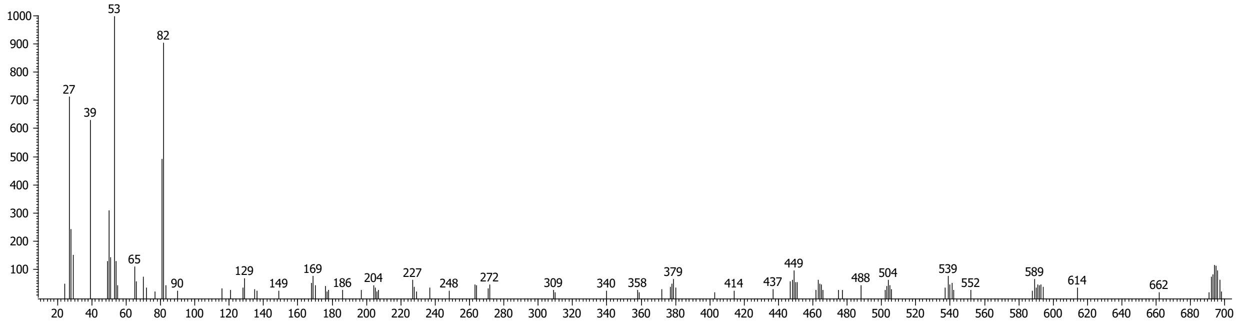
OXAZOLE, 4,5-DIHYDRO-2-METHYL-4-(1-METHYLETHYL)-, (S)-



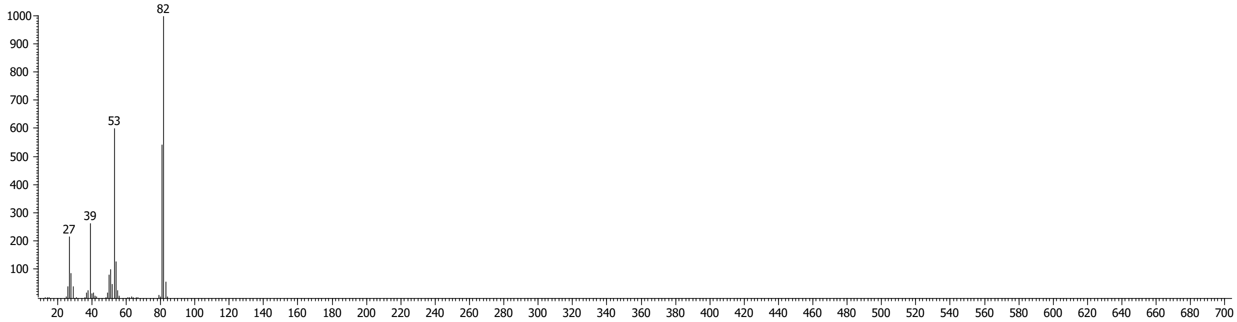
Library Hit - similarity 718, "OXAZOLE, 4,5-DIHYDRO-2-METHYL-4-(1-METHYLETHYL)-, (S)-"



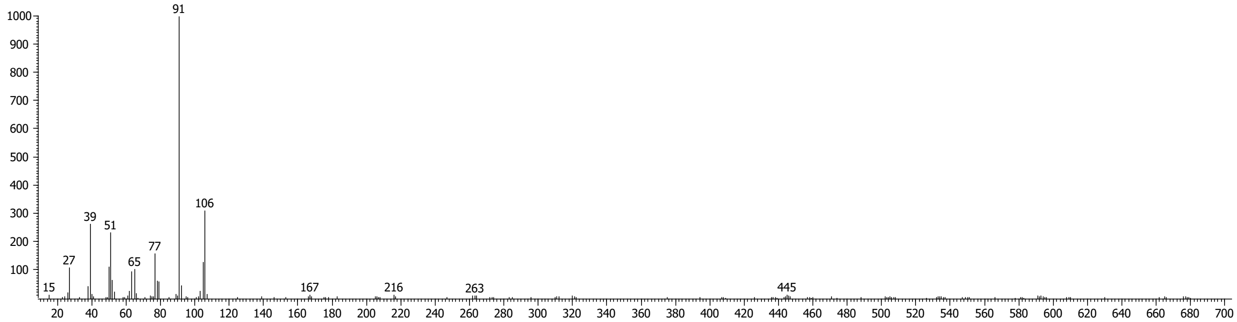
FURAN, 2-METHYL-



Library Hit - similarity 386, "Furan, 3-methyl-"



BENZENE, 1,2-DIMETHYL-



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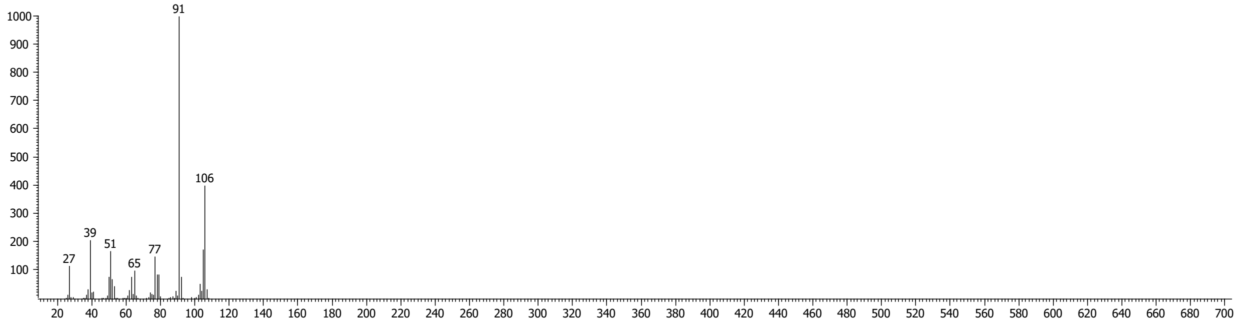
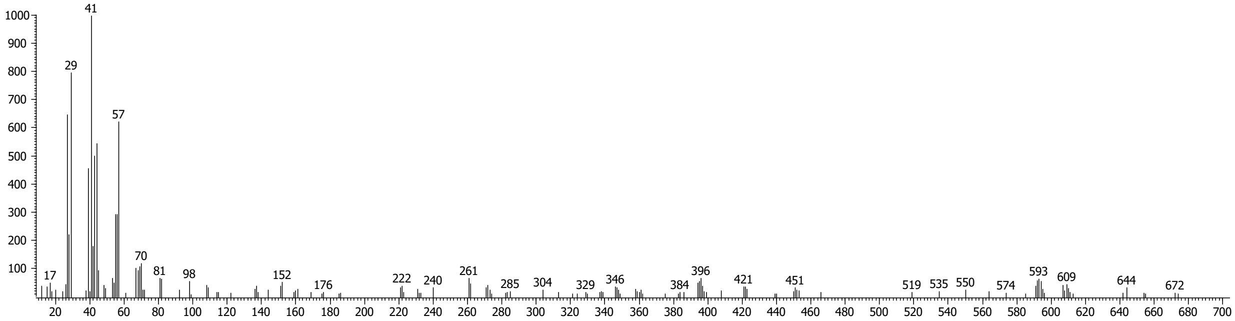


NONANAL



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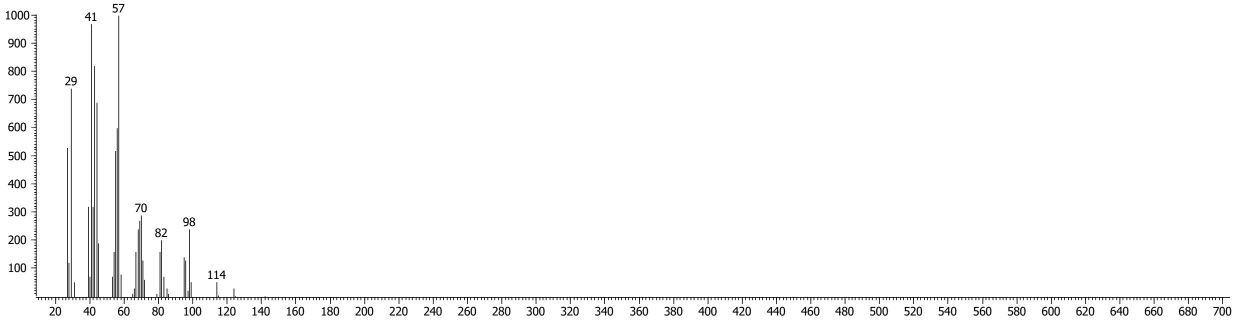
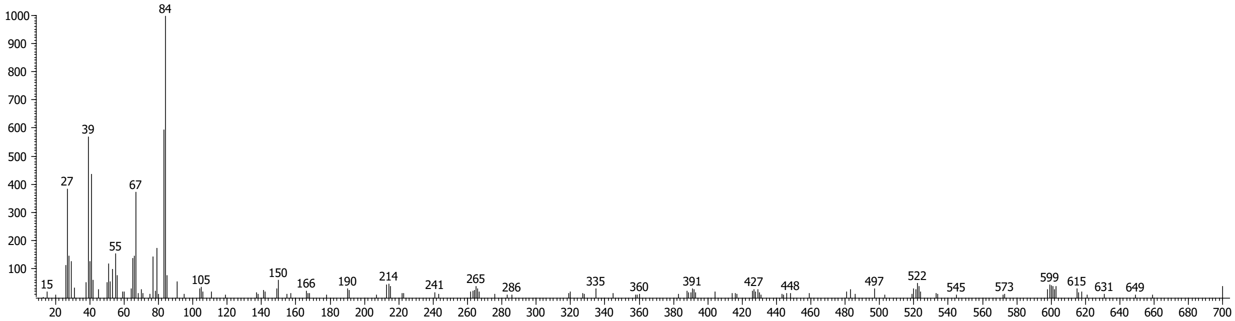
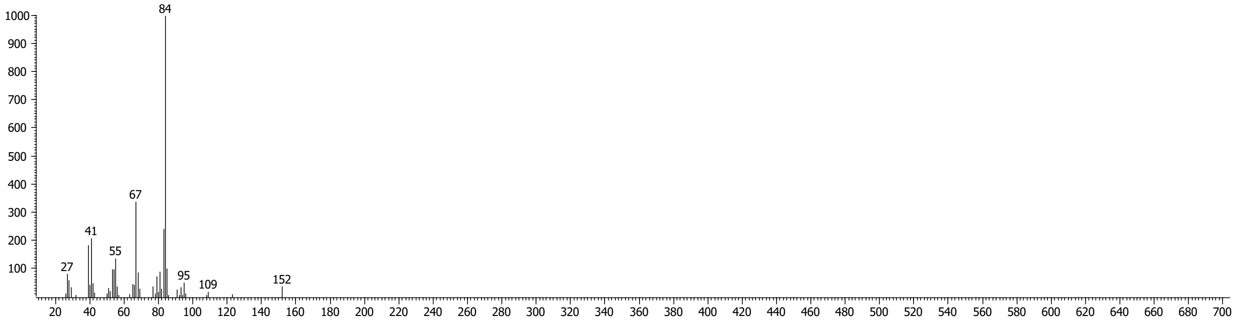


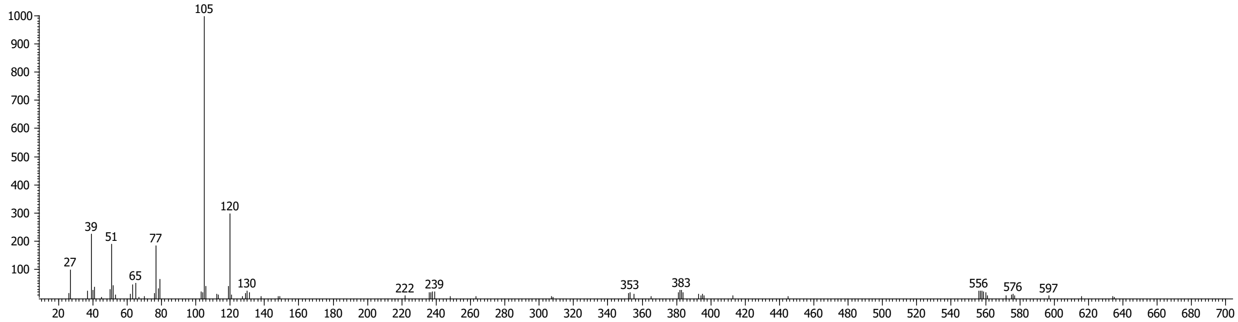
[1,1-BICYCLOPENTYL]-2-ONE



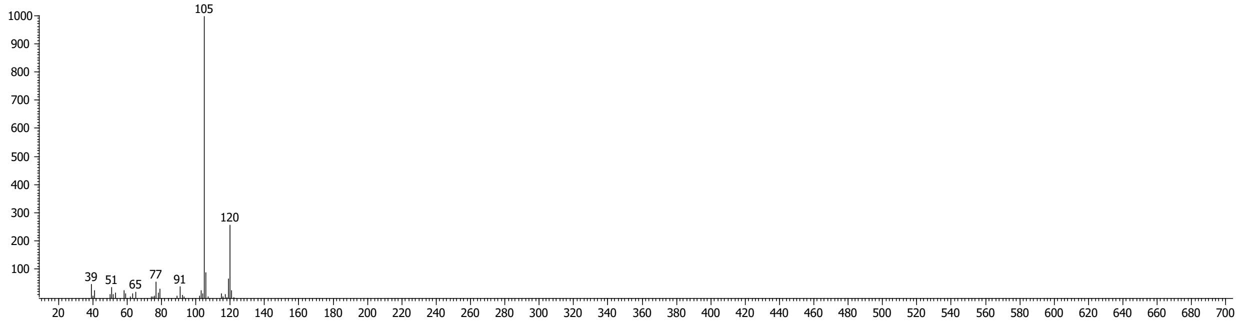
Library Hit - similarity 426, "[1,1'-Bicyclopentyl]-2-one"



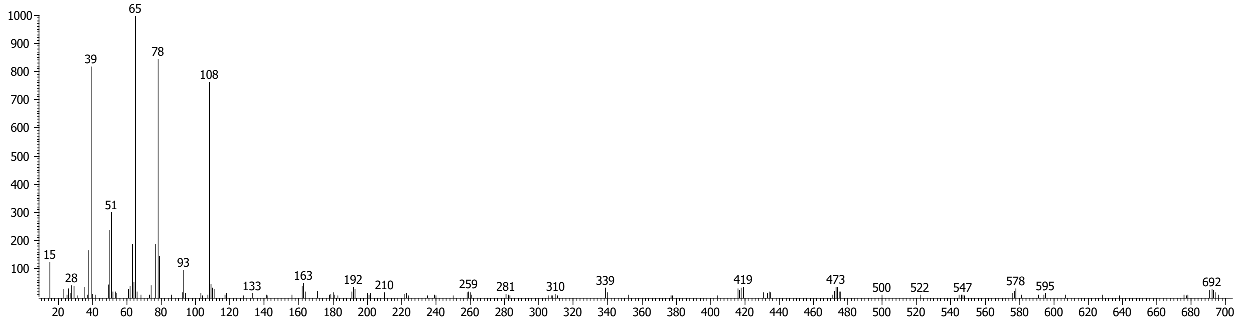
BENZENE, 1,2,3-TRIMETHYL-



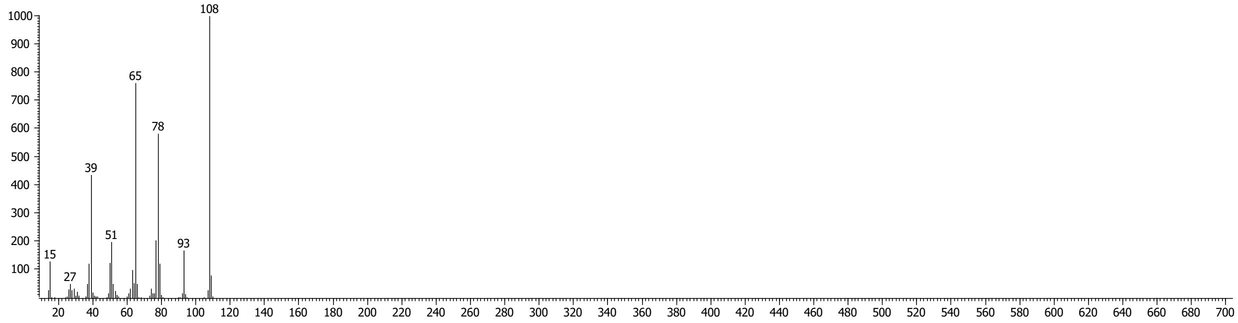
Library Hit - similarity 555, "BENZENE, 1,2,4-TRIMETHYL-"



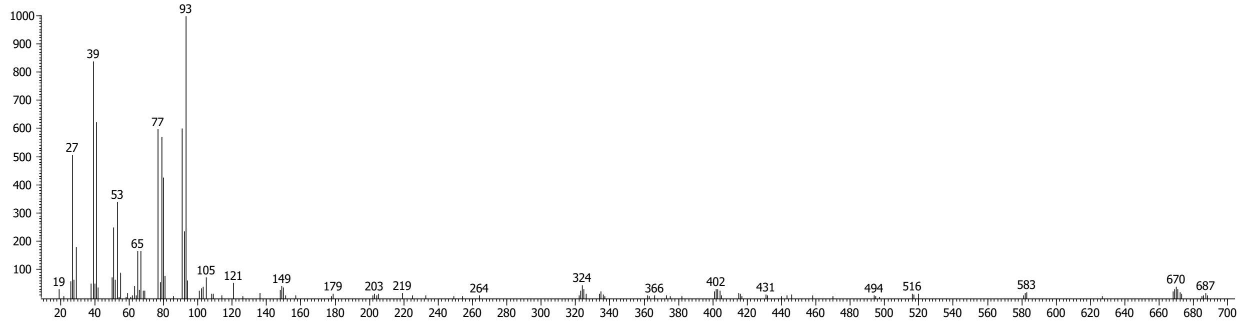
BENZENE, METHOXY-



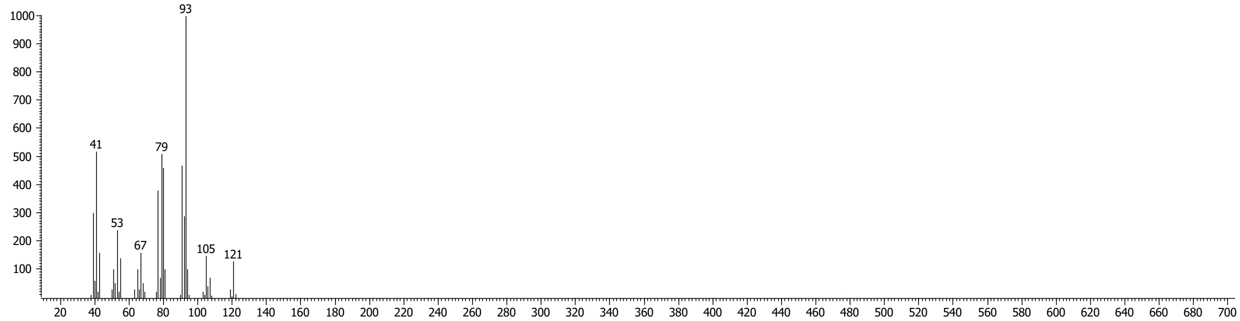
Library Hit - similarity 701, "BENZENE, METHOXY-"



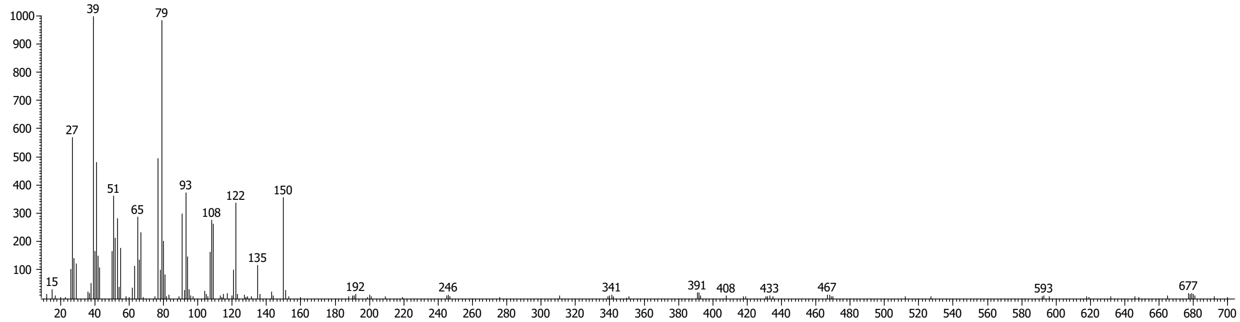
β-OCIMENE, (E)-



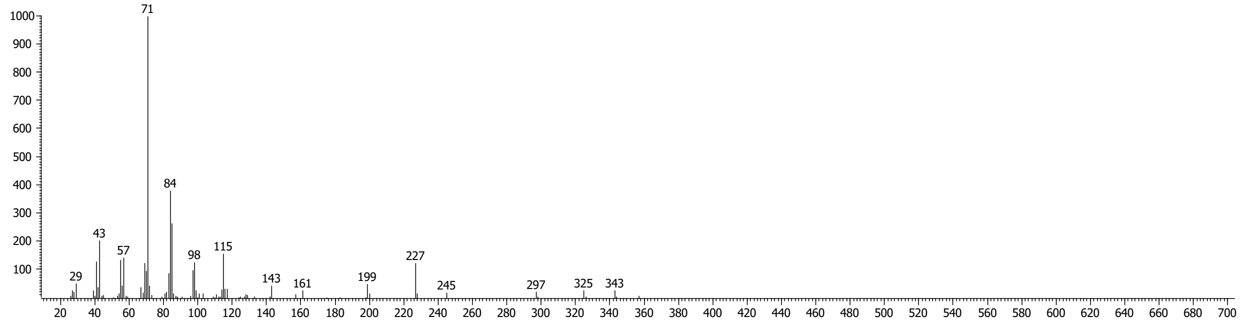
Library Hit - similarity 551, "(E)-BETA-OCIMENE"



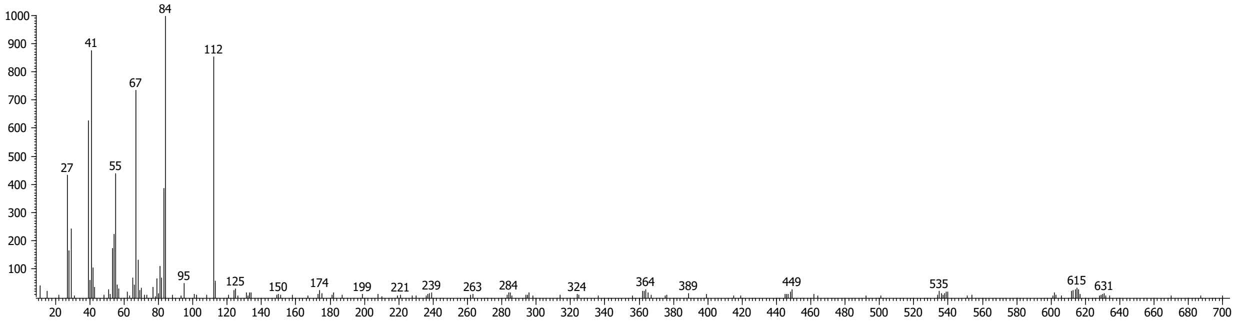
2-CYCLOHEXEN-1-ONE, 2-(2-METHYL-2-PROPENYL)-



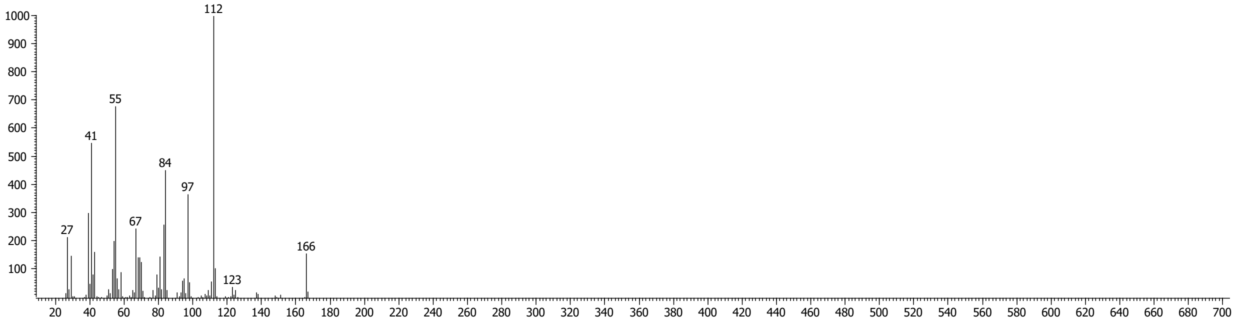
Library Hit - similarity 695, "2-Cyclohexen-1-one, 2-(2-methyl-2-propenyl)-"



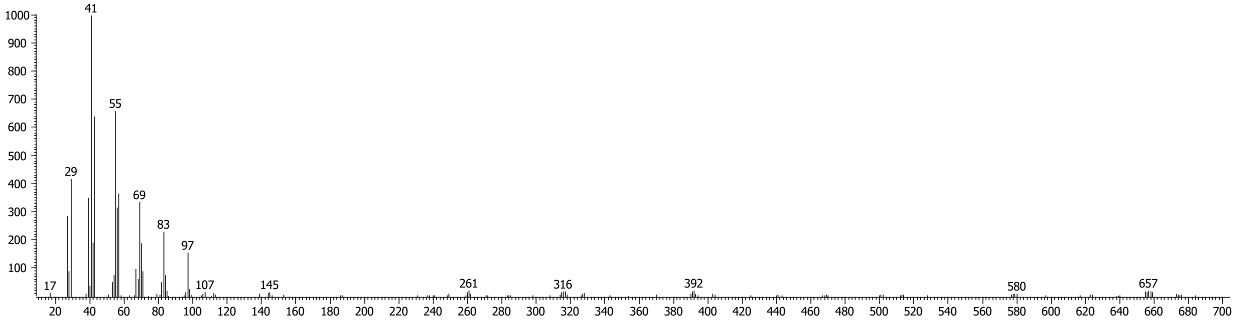
CYCLOHEPTANONE, 2-(3-BUTEN-1-YL)-



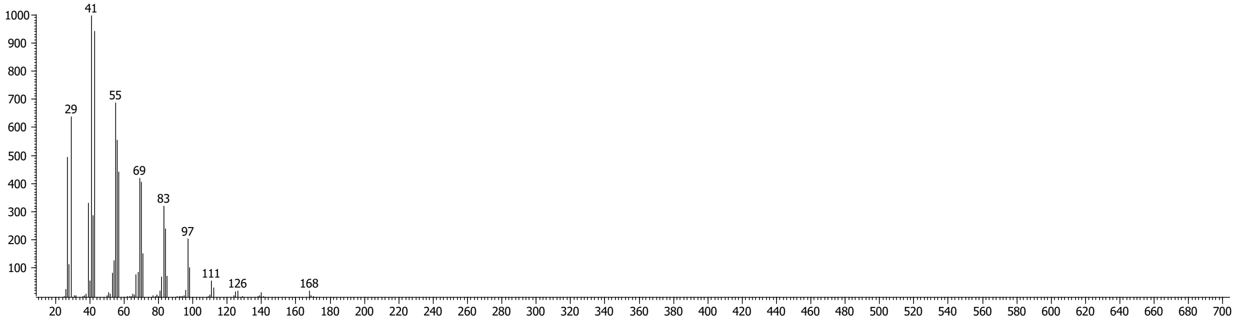
Library Hit - similarity 503, "Cycloheptanone, 2-(3-buten-1-yl)-"



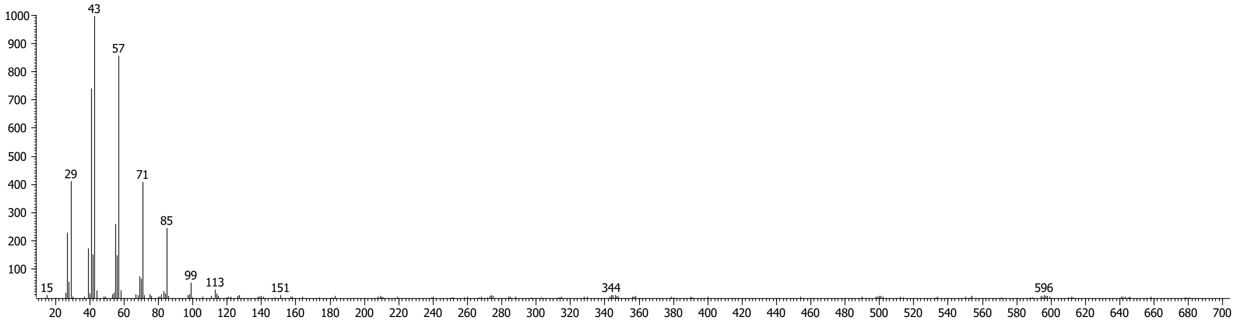
1-DODECENE



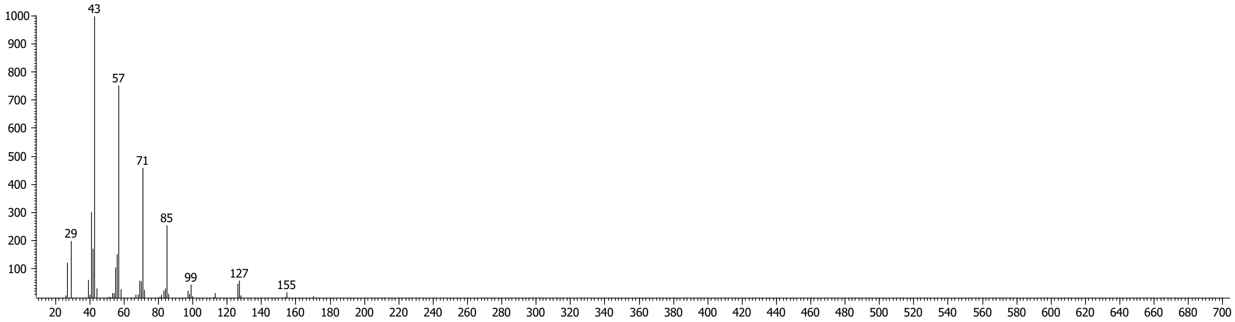
Library Hit - similarity 612, "1-DODECENE"



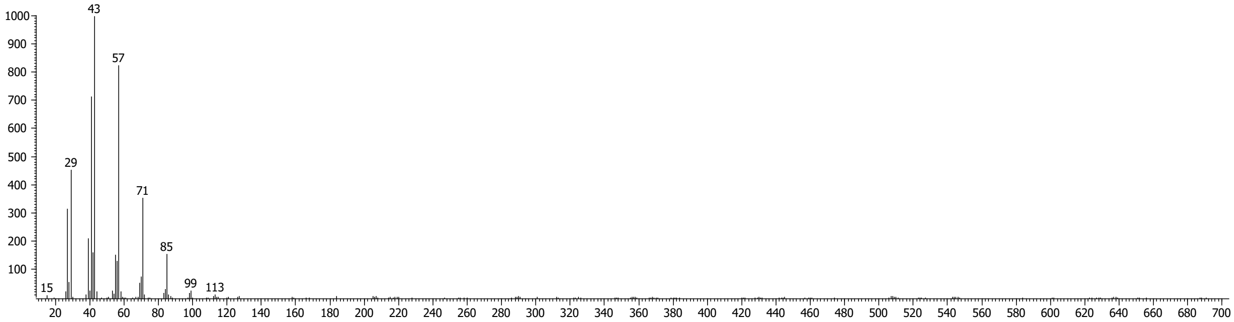
UNDECANE, 2-METHYL-



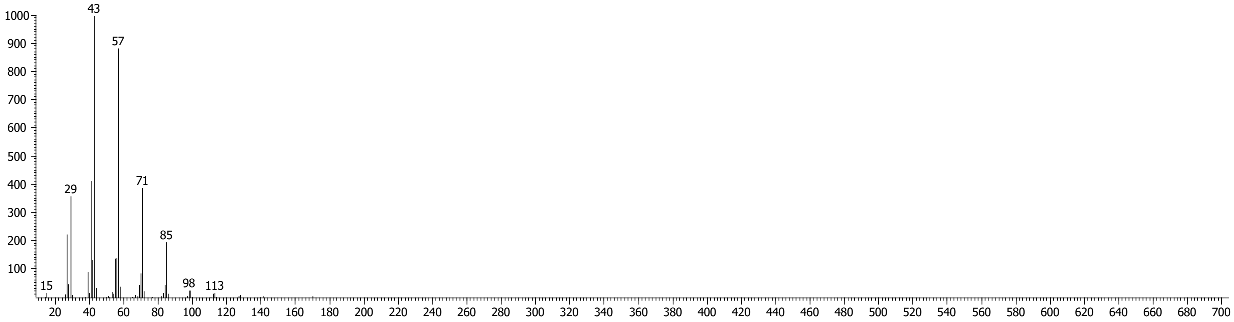
Library Hit - similarity 691, "UNDECANE, 2-METHYL-"



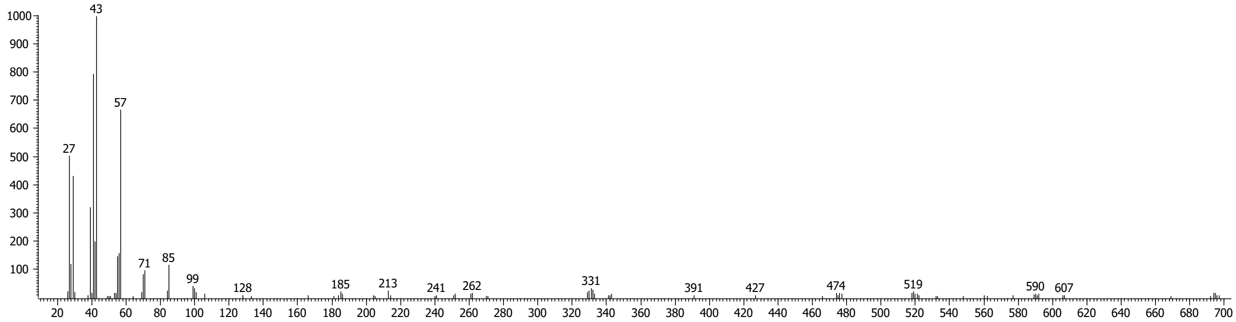
TRIDECANE



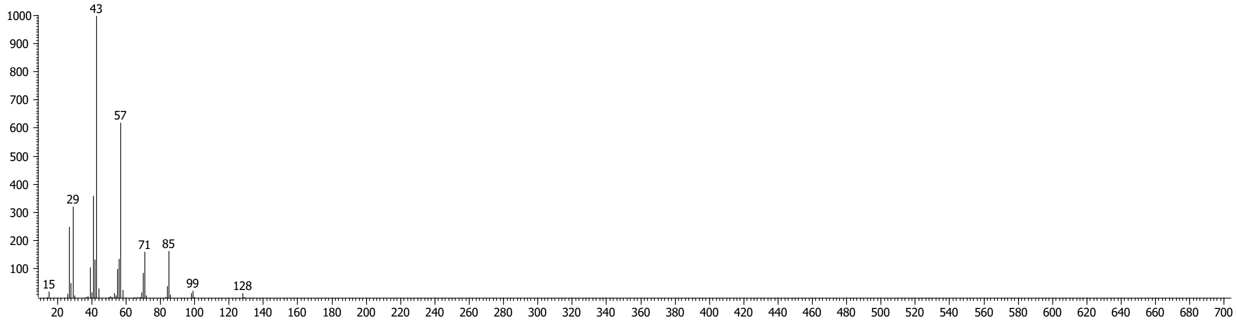
Library Hit - similarity 750, "DODECANE"



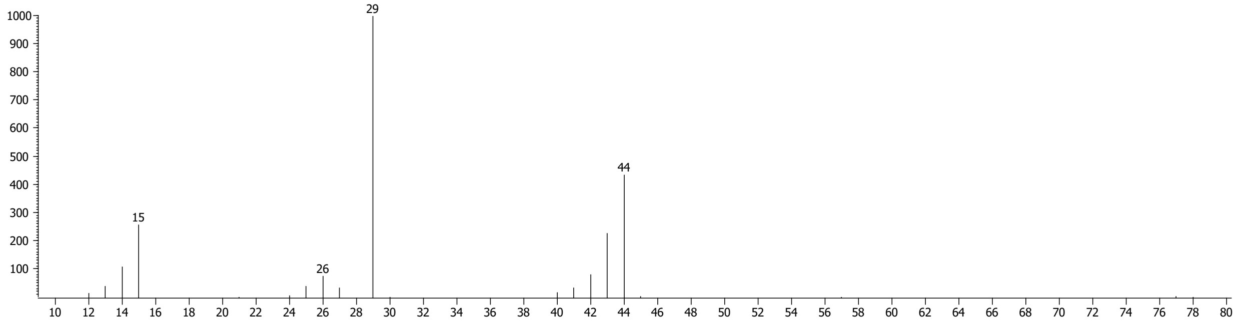
NONANE



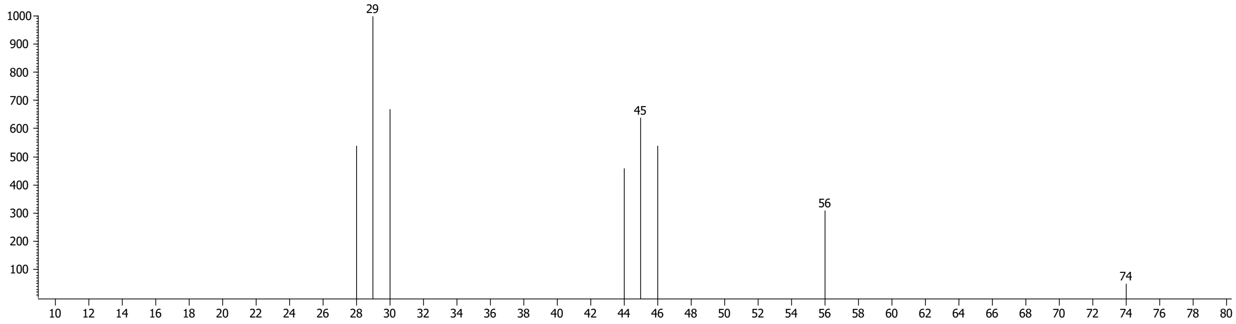
Library Hit - similarity 538, "NONANE"



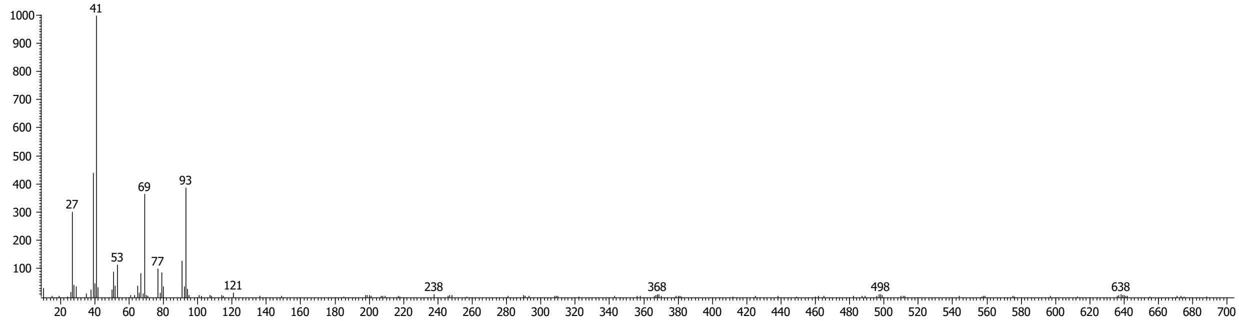
ACETALDEHYDE



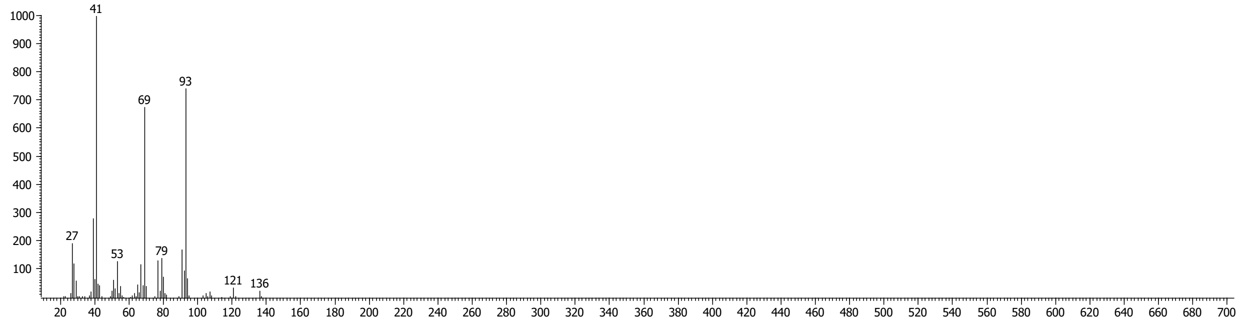
Library Hit - similarity 773, "Acetaldehyde"



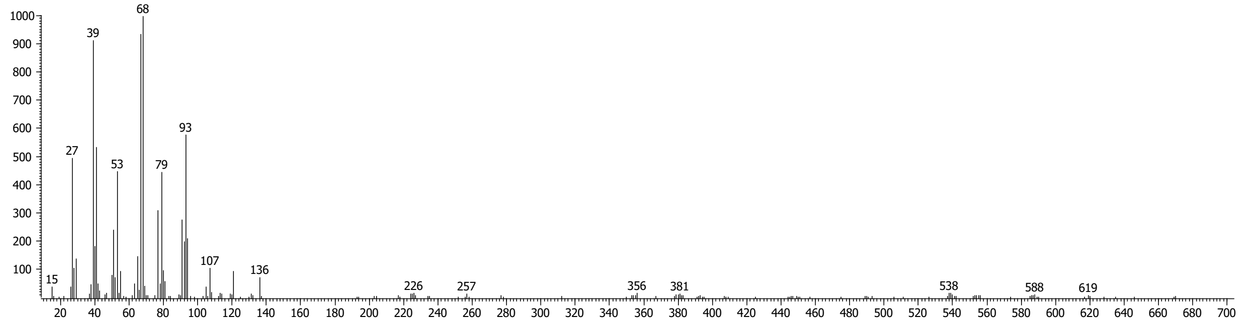
B-MYRCENE



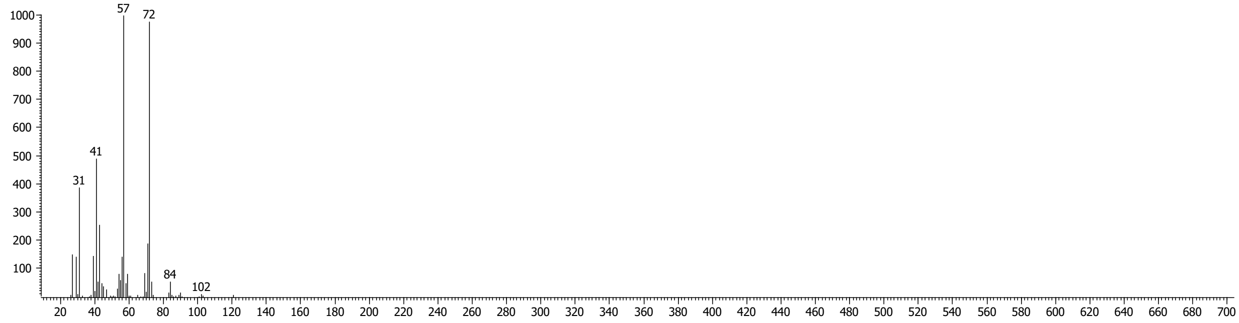
Library Hit - similarity 637, "B-MYRCENE"



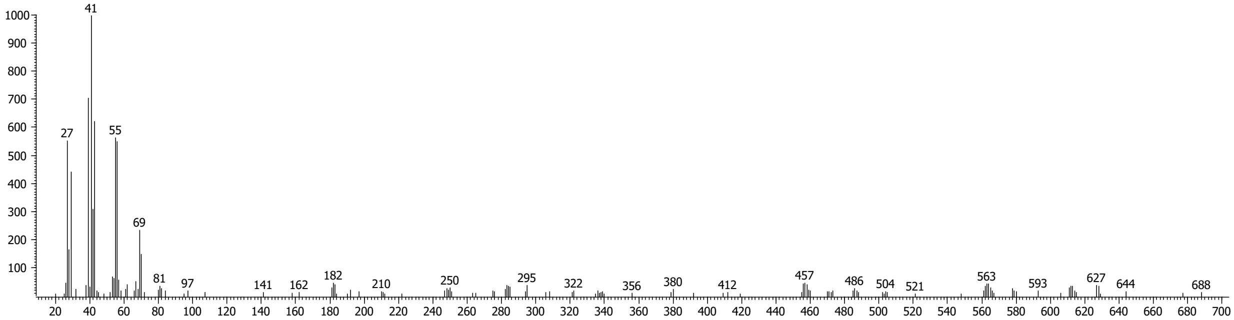
LIMONENE



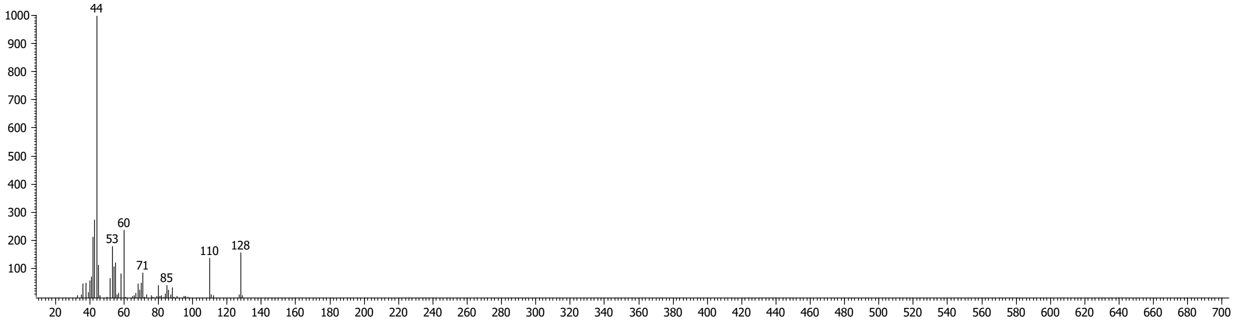
Library Hit - similarity 718, "Limonene"



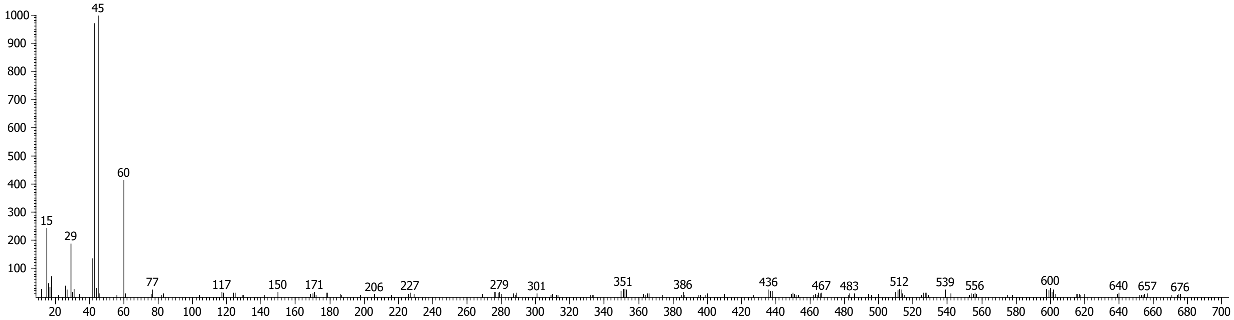
(2E)-2-NONENE



Library Hit - similarity 408, "2-Nonene, (E)-"



ACETIC ACID



Library Hit - similarity 462, "ACETIC ACID"