

Feeding and oviposition preferences of the diamondback moth
***Plutella xylostella* (Lepidoptera: Plutellidae)**
on six Brassicaceae host plant species

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Abstract

Plutella xylostella (diamondback moth, DBM) is a globally distributed Lepidopteran that feeds and oviposits almost exclusively on plants in the Brassicaceae family. DBM disperses from the southern United States and Mexico into Canada in the spring and summer. Establishment of DBM in Ontario is partially dependent upon the quantity and quality of host plants available and the preference of DBM for different hosts. Host plants include many crops such as broccoli, canola and cabbage, as well as landscape ornamentals and wild plants. It has previously been established that DBM are attracted to host plants by chemicals, specifically glucosinolates. I examined the preference of DBM among crop, wild and ornamental host plant species and how preference varies with insect life stage (3rd and 4th instar larvae and adults). Experiments included exposing DBM larvae from five populations coming from different locations in Canada to six Brassicaceae species and evaluating the preferences and weight gain over one hour. Then adult females were exposed to these same plant species and their oviposition preferences were examined. Populations from Alberta, Saskatchewan and Ontario were compared to assess differences in preference associated with geographic region or species of host plant. The ultimate goal of my study was to understand the potential of various Brassicaceae species to act as reservoirs to sustain and promote population growth of DBM, as well as sinks that may decrease DBM abundance. Results showed that garden cress (*Lepidium sativum*) was highly preferred over other species (wintercress, black mustard, aubretia, broccoli and ornamental kale) for both food and oviposition sources. Previous studies report that garden cress contains saponins, chemicals shown to be toxic to developing DBM larvae, however no studies have yet shown a preference for garden cress. These results provide information on a novel host plant with the potential to control DBM population growth. No difference in preferences was found among populations of DBM from various sources in Canada.

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Chapter 1. Introduction

Literature Review and Proposed Project

The mechanisms by which insects establish themselves in novel or seasonal environments are complex and poorly understood. Insects must first be able to move from their original habitat through one way dispersal or return migration. Secondly, insects must find and utilize habitat that meets their nutritional and reproductive requirements, while avoiding enemies, and in some cases, minimizing competition for resources. In managed environments such as agricultural fields, newly arrived insects can become pests and have negative impacts on ecosystems. The diamondback moth (DBM) (*Plutella xylostella*) is found in many parts of the world. In North America, it disperses from the southern United States of America (USA) and Mexico to Canada each spring, but dispersal routes to various Canadian regions have not been established (Dosdall et al. 2001; Bahar et al. 2013). As an agricultural pest, DBM larvae damage crops such as broccoli, canola, mustard, and cabbage. The costs of preventative measures and damage due to DBM have been estimated at between four and five billion dollars (US) worldwide (Zalucki et al. 2012a). Examination of Canadian DBM behaviour as it relates to their establishment in seasonal habitats can provide information on how to best manage this pest.

Nutritional requirements, as well as the ability of DBM to overcome chemical plant defenses, can change with insect age (Begum et al. 1996). It is important to understand how preferences change as DBM larvae develop. In this thesis, my goal is to examine the preference of DBM among wild, ornamental and crop plants and how preference may vary with insect life stage, particularly 3rd and 4th instar larvae and adult females. This study will provide information on Canadian DBM host plant preferences and whether this insect species could utilize alternate hosts when crops are not available.

1.1 Insect Dispersal

Dispersal of insects into novel environments may be a response to resource deficiencies in their native environments or a density-dependent movement to less congested territory (Loxdale and Lushai 1999; Alerstam et al. 2003). It may also be a seasonal movement (e.g. spring migration) into habitats capable of supporting insect populations at only certain times of the year (Dingle 2009). As they disperse or migrate, herbivorous insects must depend on finding reliable and nutritious plant food sources (Campos et al. 2003). Environments into which insects move commonly include a combination of native and exotic wild plants, crops, and planted

ornamentals, especially in urban and rural environments (Pearse et al. 2013). Other factors, such as insect specialization, community dynamics and plant defenses are involved in insect establishment on host plants (Fig. 1.1). Full insect life cycles must be possible if establishment of insects is to persist in a given environment on host plants, although annual cycles may be interrupted by migrations, die-offs or diapause behaviours. Specific host plants may provide refuges for destructive insect populations and, therefore, information on the preferences and fitness consequences of pest insects on potential host plants in these environments is vital for insect management and control.

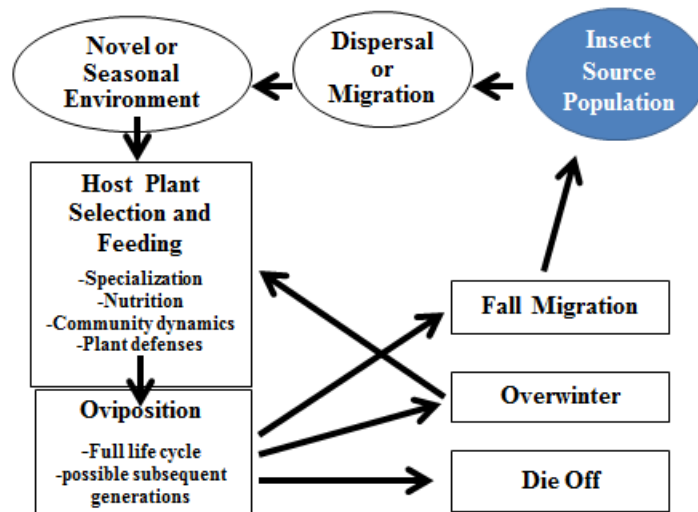


Figure 1.1 Insect establishment in a novel or seasonal environment begins with movement of individuals from a source population. Factors such as insect specialization, host plant nutrition, community dynamics of other species present, and plant defenses, all affect an insect’s preference for host plants and their ability to feed. Oviposition and subsequent development of young must also occur. Some insects have several generations per season. At season’s end, insects will die off, overwinter, or migrate back to their source population.

Though insect movements from tropical and subtropical to temperate latitudes are often due to seasonality, it is predicted that most are one way journeys, culminating in die-offs due to cold temperatures rather than return migrations (Rabb and Stinner 1978). However, some insects do migrate both ways. Silver Y moths (*Autographa gamma*), over several generations, move from their southern Mediterranean Region overwintering grounds into Northern Europe in the spring and summer to mate, then return south again, riding high velocity tailwinds up to 200 metres above ground (Chapman et al. 2012). Likewise, the monarch butterfly (*Danaus plexippus*) relies strongly on northern habitat to provide mating and feeding opportunities that

are not available in its winter habitat, thousands of kilometres to the south (Dingle et al. 2005). Monarchs have the added challenge of being dependent upon only a few species of plants for food, making migration difficult for this “specialist” insect species if their food source is not available. They must find plants in the milkweed family for oviposition and larval food sources, although as adults they are not as limited by their nectar food sources (Vickerman and de Boer 2002; Brower et al. 2006).

A specialist phytophagous insect is one that is restricted to only one or a few species of plants for food, compared with a generalist species that can feed on a variety of host plant species (Bernays 2001). For example, the generalist beet armyworm (*Spodoptera exigua*) is a pest that attacks many species, while the specialist larva of the Granville fritillary butterfly (*Melitaea cinxia*) is restricted to plants in the *Plantago* and *Veronica* genera (Reudler et al. 2011). Although they may have fewer overall plant species to choose from, specialists are more likely to overcome defensive abilities of certain host plants than generalists (Reudler et al. 2011). Thus, although specialist phytophagous insects may have fewer food choices, they are more likely to survive on highly defended (i.e. containing more mechanical or chemical defenses) plant species than generalists, as they may have the mechanisms to overcome those defenses (Reudler et al. 2011).

1.2 Overcoming Plant Defenses

Plants have developed effective mechanisms to avoid, or at least minimize, insect damage. These defenses can be induced (created by the plant when needed) and/or constitutive (present even when not needed) (Ito and Sakai 2009). Most plants maintain a constitutive level of defenses (mechanical and/or chemical), which can increase when induced through injury (Campbell and Kessler 2013; Siemsen and Mitchell-Olds 1998). For example, trichomes (leaf hairs) are present in low concentrations on willow (*Salix cinerea*) leaves, but increase in density after induced through herbivory by adult leaf beetles (*Phratora vulgatissima*) (Björkman et al. 2008).

Trichomes are a mechanical defense that can pierce insects or impede their landing or movement and may also secrete toxic chemicals (Arimura et al. 2005; Handley et al. 2005). Mechanically, plants can deter herbivory through other surface structures as well. The presence of tough structural lignin acts as a barrier to herbivory (Freeman and Beattie 2008). In conifers, for example, lignin blocks phloem-seeking beetles from entering the tree bark (Franceschi et al. 2005). Likewise, sclereids (stone cells) are difficult to chew and increase resistance to weevil

(*Pissodes strobe*) in Sitka spruce (*Picea sitchensis*), for example (Moreira et al. 2012).

Crystalliferous cells, like calcium oxalate and silica, cut insects during chewing and discourage feeding (Freeman and Beattie 2008). Coffee and sugarcane utilize this strategy through high silicate contents in their plant tissues (Kvederes et al. 2007, Amaral et al. 2008). Epicuticular wax crystals and lubricating fluid films produced by many plants create sticky or slippery surfaces that impede insect mobility (Whitney and Federle 2013). Macaranga ant-plants use this type of mechanical defense, enabling them to deter herbivorous insects with waxy stems, while allowing access to specialized ant mutualists (Federle et al. 1997; Whitney and Federle 2013).

Plant chemicals are categorized as either primary (directly involved in development) or secondary (often used in defense) metabolites (Seigler 1998). Secondary metabolites are divided into three groups: terpenoids, phenolics, and alkaloids (Freeman and Beattie 2008). Among the terpenoids, pyrethrins act as neurotoxins to insects (Freeman and Beattie 2008). Many aromatic plants, including spearmint (*Mentha* spp.) and cinnamon (*Cinnamomum* spp.), contain monoterpenoids. Medicarpin, a flavonoid found in alfalfa (*Medicago sativa*), increases upon exposure to pathogens such as *Colletotrichum trifolii*, protecting the plant from further infection (Saunders and O'Neill 2004). Latex, as found in milkweeds (*Asclepias*), can contain mixtures of terpenoids, alkaloids and phenolics (Agrawal et al. 2008). The larvae of monarch butterflies (*Danaus plexippus*) and milkweed tussock moths (*Euchaetes egle*) are two of the few species able to feed on milkweed despite these chemical defenses (Bingham and Agrawal 2010; Zalucki et al. 2012b). Mechanical and chemical defenses serve to protect plants from destruction due to insect herbivory, but their defenses are not always impenetrable.

An insect's adaptive specialization (degress of specialist behaviour) to particular plants allows it to bypass defense strategies evolved by the plant to protect itself from damage (Janz 2011; Reudler et al. 2011). Specialization in insects has led to further adaptation by plants in response to such protective measures. This "arms race" between the plant and the insect is the main force of coevolution, a process by which the interaction of species leads to evolutionary changes in both plant and insect species (Janz 2011). Plants and herbivores coevolve to maximize their respective survival and fitness (Ehrlich and Raven 1964; Janz 2011). While a plant species may develop new ways (e.g. toxic chemicals) to deter an insect species, this same insect species may respond by adapting to these new chemicals and thus overcome these defenses (e.g. digestive enzymes to neutralize the toxins) (Yamazaki et al. 2011). There is evidence that insect herbivores repeatedly adapt to evolving plant traits in order to continue to

utilize them as food, oviposition, and shelter sources (Futuyma and Agrawal 2009; Muola et al. 2010). This coevolution may create escalating specialization of pairs of insect/plant species. For example each individual *Blepharida* beetle species has a monophagous relationship with a single *Bursera* plant host species and has the ability to bypass defenses unique to that plant species (Becerra 2006). The DBM is also thought to have coevolved with its host plants. Brassicaceae plant species, also known as cruciferous plants, have a “mustard oil bomb” defense, initiated when leaves are damaged. Chemicals (myrosinase and glucosinolates) within the leaf are separated until damage causes them to mix, resulting in a combination that is like a toxic “bomb” to many insects (Ratzka et al. 2002). DBM can neutralize glucosinolates using gut enzymes before they react with myrosinase, thereby defusing the “bomb” (Ratzka et al. 2002, Xia et al. 2013). Thus, specialization serves to provide insects with the means to access nutrients from heavily defended plant species, and may create preferences in insects for certain species of plants.

1.3 Host Plant Preference

When offered choices, herbivorous insects may choose a host plant upon which to feed or oviposit (or they may make no choice). Their specialization, as mentioned above, will dictate, to a certain degree, which plants are immediately acceptable. Within the range of acceptable plants, nutritional content, oviposition sites, competition, or shelter potential from enemies may vary between plants. Therefore, an insect’s ability to assess and choose the best plant for its current needs will benefit that insect (Whitham 1978).

Most insects optimize their diet by choosing plants that are high in protein and carbohydrates (Lee 2007). Nutritional contents, including protein and carbohydrates can vary between different plant species, within plants of the same species under different environmental conditions, and even within parts of the same plant, such as old and young leaves (Han et al. 2011; Sarfraz et al. 2009). For example, adult females of *Pemphigus* aphids prefer larger leaves over smaller ones for oviposition because the leaf size is positively correlated with increased nutrients (Whitham 1978). Choosing different host plants with varying nutritional contents can result in different physical and behavioural traits in insects. The African cotton leafworm (*Spodoptera littoralis*) exhibits reduced body size and delayed maturation when given low quality protein foods (Lee 2007). Life history traits, including physical traits such as body size (which is often positively correlated with survival and reproduction), are often used as indicators of “fitness”, a term that describes the long-term ability of an individual or species to reproduce and pass on genes to subsequent generations (McGraw and Caswell 1996).

Life history traits are often used to define the fitness of herbivorous insects such as DBM, and will be referred to throughout this paper as indicators of fitness. They include the weight gain of larvae and the number of eggs produced by adult females. Higher weights usually indicate healthier individuals with higher reproductive potential and higher fecundity (reproductive rate) of adults corresponds with increased population survival (Waser et al. 2013). DBM fitness has been shown to vary with the plant species upon which they are reared (Saeed et al. 2010). This fitness variance is due, in large part, to the nutritional composition of host plants (Sarfranz et al. 2009).

The challenge to acquire sufficient nutritional resources for growth and development in animals is termed the nutrient stress hypothesis, and drives animals to choose the most nutritious food sources when they have a choice (Joern and Behmer 1998; Frago and Bauce 2014). Some herbivorous insects, such as the tomato leafminer (*Tuta absoluta*) must eat large amounts of plant tissue to acquire enough nutrients for growth and reproduction. If nutrient concentrations in leaves are low, these insects usually have long development times and low weights (Han et al. 2014). Nutrient concentrations in plants vary throughout the season and may result in suitable food choices for insect herbivores only at certain times, stimulating host plant changes by insects, when possible (Renwick 2002).

Along with changing nutrition as plants age, dietary needs also vary with insect age (Rodrigues and Moreira 2004). As larvae develop and some become more mobile, they may move to different host plants or even different host plant species than the ones their mothers chose for oviposition (Mader et al. 2012). Sex differences may also contribute to host plant preferences. In the mustard leaf beetle (*Phaedon cochleariae*), females respond to leaf colour more than males. Since leaf colour indicates the age and nutritional contents of leaves (yellow is associated with aging, less nutritious leaves), their preference may allow them to locate more nutritious plant tissues. Females of this species also preferred the main volatile organic compound (VOC) from their preferred host plant (*Nasturtium officinale*) more than did males (Kühnle and Müller 2011). Changing host plant (and host plant part) preferences depending on insect age and sex may result from differing nutritional requirements or physiological abilities (Kühnle and Müller 2011).

One of the physiological abilities of insects that has been shown to change with age is the ability to detoxify or tolerate defense chemicals in host plants (Campos et al. 2006; Travers-Martin et al. 2008; Bar-Yam and Morse 2011). The cabbage looper (*Trichoplusia ni*) is

confronted with carcinogenic mycotoxins in many of its food sources, such as corn, peanut and wheat. The ability of cabbage looper to tolerate exposure to mycotoxins increases with age, with 100% mortality of five day old insects compared to 47% mortality of seven day old insects when exposed to 3 ug/g toxins (Zeng et al. 2013). Therefore, as insects acquire the ability to feed on more highly defended plants, they may prefer those plants.

Allelochemicals, chemicals produced by plants as a means of defense against herbivores and other plants, include VOCs that have a high vapour pressure and are therefore, detectable in the air, sometimes far from their source plant (Herrmann 2010). VOCs provide cues to insects concerning the chemical and nutritional content of food plants (Agren et al. 2012; Webster et al. 2012). Herbivorous insects may be subjected to multiple simultaneous VOCs during their search for food, and therefore specific cues are important for them to find the right plants. Most specialist insects are efficient in locating specific plants through this system of chemical cues (Burger et al. 2013). For example, the specialist ant, *Pheidole minutula*, chose treatments with VOCs from its host plant (*Maieta guianensis*) from among treatments with other VOCs (Dáttilo et al. 2009). Therefore, VOC's represent an important aspect of host plant recognition by insects.

In specialist insects, adaptation to VOCs may drive preference, despite the underlying nutritional or chemical components within plants (Cunningham 2011). This “olfactory constraint” is usually beneficial to specialists, as it allows them to locate host plants that are usually good sources of food (Sun et al. 2009). In some cases, however, this constraint leads them to feed or oviposit on host plants that negatively impact their survival (Cunningham 2011). This has been termed an “evolutionary mistake” by Badenes-Perez et al. (2014). It may also illustrate the ongoing arms race of coevolution as plants incorporate new defenses that their herbivorous pests may not yet be able to surpass (Honda and Hayashi 1995; Wei et al. 2013a).

Plant VOCs can change due to damage inflicted upon them by phytophagous insects, as the chewing or piercing process can create reactions in plants at the chemical level (Smith and Beck 2013). These new VOCs may change the preference of insects for that plant, as is seen in the changing preference of the weevil *Ceratapion basicorne* for plants when they are damaged (Smith and Beck 2013). Oviposition can also induce changes in plant VOC composition (Hilker and Meiners 2011). In *Brassica napus* (canola), the pollen-feeding beetle *Meligethes aeneus* induces VOC changes due to bud injury caused by feeding and oviposition (Piesik et al. 2013). VOC changes due to herbivore damage are also used by parasitoids of some herbivores that

signal not only where their hosts are located, but also how many insect hosts are present on the damaged plant (Girling et al. 2011).

Avoidance of parasitoids and other enemies is another element in host plant choice by insects. For adult checkerspot butterflies (*Estigmene acrea*) choosing a host plant, enemy-free space is more important than food quality (Lepidoptera: Arctiidae) (Singer et al. 2004). A study of late goldenrod (*Solidago altissima*) and three of its herbivores shows that food availability and enemy free space were equally important factors in herbivore host plant choice (Abbot and Dwyer 2007). These dynamics influence the preference for a particular host plant in places where several arthropod (or other animal) species are present on the same plant species, even if they are not predators (Millan et al. 2013). In some cases, the most competitive species can trigger changes in its host plant in order to reduce the preference of that plant by another insect species. The mirid bug (*Helopeltis Sulawesii*) induces changes in the chemistry of its host plant, cacao (*Theobroma cacao*), that reduces oviposition of the cacao pest, *Conopomorpha cramerella* (Wielgoss et al. 2012).

Intraspecific competition can also lead to changes in host plant preference. Aphids (*Sitobion avenae*) change their plant food preference for different genotypes of barley (*Hordeum vulgare*) when they are under different levels of competition with individuals of their own species (Zytynska and Preziosi 2013). The goldenrod gallmaker fly (*Eurosta solidaginis*) oviposits fewer eggs on plants with higher numbers of galls compared to plants with fewer galls. This may also lead to preference of ovipositing females on less preferred plant species or even on novel host plants (Hess et al. 1996). Offspring vigour was lower (seen through lower tunneling ability within simulated bark) in *Ips pini* beetles that were raised in high density colonies compared to low densities (Sallé and Raffa 2007). Preference changes due to intraspecific competition allow insects the opportunity to choose plants with more space or nutrients per available surface area.

Yet another factor influencing host plant choice of insects relates to the future food potential of a host plant. Theoretically, insect oviposition preference should be correlated with food preference, since the emerging larvae must first utilize the plant on which they hatch (Cunningham 2011). Then, depending on their level of mobility, they may be able to access other plant species. To optimize offspring success, therefore, a female should choose oviposition sites that are also good food sources (Cunningham 2011). This is termed the mother-knows-best hypothesis (Valladares and Lawton 1991; Vencl et al. 2013), related to the preference-

performance hypothesis (Balagawi et al. 2013). In a review of lepidopteran oviposition preference, Thompson and Pellmyr (1991) show that females exhibit differential preferences towards plants, usually corresponding with most preferred plants being the best larval food sources for their offspring. For example, fern moth (*Herpetogramma theseusalis*) (Lepidoptera: Crambidae) adults prefer marsh fern (*Thelypteris palustris*) over sensitive fern (*Onoclea sensibilis*). Since *T. palustris* is the more nutritious of the two choices for larvae, this choice suggests an oviposition preference that most benefits offspring (Bar-Yam and Morse 2011).

Other studies have shown that sometimes females oviposit on sites that are not always optimal for larval survival (Cunningham 2011). For example, *Cephaloleia* beetle females oviposit on novel host plants despite the fact that offspring fitness is higher on native hosts. Adult female survival is longer on novel hosts, indicating that females may choose host plants that maximize their own survival over their offspring's fitness (García-Robledo and Horvitz 2011). Oviposition preference may be regarded as a strategy to balance food quality and availability, as well as competition, predation, disease and parasitism (Steinbauer and Carroll 2011; Sarfraz et al. 2009). In this case, not only the choice of the plant species is important but also the type of plant tissues where the eggs are laid. For example, adult female cereal leaf beetles (*Oulema melanopus*) prefer to oviposit on young, tender leaves, but larvae feed equally on old and new leaves (Hoffman and Rao 2011). These examples illustrate that adults do not always choose oviposition sites that lead to the best potential offspring nutrition.

However, adults may choose oviposition sites that meet more than just nutritional needs of their offspring. Oviposition of eggs by adult females on older plant leaves could trigger migratory development and behaviour in the subsequent generations that are necessary at that time of the year. Chemical cues within different ages of plant tissues can be used by larvae to develop different phenotypes, some of which are associated with better migratory ability (Campos et al. 2003). Intraspecific competition and the nutritional quality of bird cherry (*Prunus padus*) leaves are cues that result in the development of a winged migrant generation of bird cherry-oat aphids (*Rhopalosiphum padi*). This winged generation then migrates to a different food source (Graminaea grasses) for the summer months, returning to feed on *P. padus* again in the winter (Glinwood and Pettersson 2000). In general, the nutrient quality of plant tissues decrease with age and this phenomenon can trigger migration or dispersal of some species, stimulating them to relocate to habitats with better climates and food resources (Campos et al. 2003).

1.4 Population Isolation and Gene Flow

When migratory insects return to source populations, gene flow is higher than in isolated populations (Ruiz-Montoya and Núñez-Farfán 2013). Gene flow refers to the ability of a species to maintain access among individuals for reproductive purposes. In a study of the pear psylla, *Cacopsylla pyricola*, which migrates annually from a known tropical source population, genetic diversity among populations is very low, indicating frequent migration back to source populations (high gene flow) (Kang et al. 2012). Genetic diversity among populations would be greater if populations remained isolated and adapted to local features such as novel plants or environmental conditions and remained separated from their source. When gene flow is high, individuals have better access to one another, thus enabling the sharing of genetic material among the entire population, while low gene flow indicates that there is restricted access to some individuals, reducing the genes being shared (Frankham 1996).

Dispersal affects gene flow between populations by creating geographical distances between individuals of the same species. When populations become isolated the resulting evolutionary consequences of reduced gene flow include the development of populations with individuals having different alleles, and potentially different genetic mutations. These differences can be due to adaptation of the isolated populations to different environmental conditions (i.e. temperature) or simply to genetic changes over time, such as through mutations or genetic drift. Genetic drift is the change in genetic structure of individuals within a population due to the chance inheritance of each generation when genes are passed on (Star and Spencer 2013). Thus, isolated populations have reduced gene flow with source populations and the potential for adaptation to local environmental conditions leading to additional genetic differences (Ruiz-Montoya and Núñez-Farfán 2013).

Population isolation may also lead to a reduction in overall genetic variation, a phenomenon known as the founder effect, mainly due to the smaller size of isolated populations (less initial genetic variation) (Matute 2013). In some cases, isolation of populations and subsequent genetic changes can lead to speciation, the differentiation of groups of individuals from the same source species that are large enough to constitute their division into new species (Singh 2012; Matute 2013). This effect has been well documented, such as through rearing experiments with the thrip predator, *Orius laevigatus* (Castañé et al. 2014). The numbers of founder individuals (1, 10 or 20 couples) were compared to assess biological and behavioural differences of the resulting population over time. It was determined that a bottleneck (a

significant reduction in genetic diversity due to small population size) occurred at the one couple founder level, leading to differences in predation behaviour in that population compared to the original population (Castañé et al. 2014).

Environmental heterogeneity is the diversity of environmental features such as temperature, pH and wind (Heino 2013). This diversity can create microclimates, pockets of habitats with different features than others in close proximity, sometimes on very small (i.e. microscopic) scales. Adaptation of populations to these specific features has also been shown to contribute to adaptive differences and even speciation. An example of this is seen in the divergence (genetic separation) of four stream insect species (three caddisfly and one mayfly) from geographically close locations with high environmental heterogeneity. Adaptive divergence of the four species was attributed to environmental differences including elevation, sunlight, and nitrogen availability (Watanabe et al. 2014).

If a species exhibits high phenotypic plasticity, it may be able to colonize a variety of habitats with differing environmental conditions without genetic changes (Ruiz-Montoya and Núñez-Farfán 2013). Phenotypic plasticity refers to the ability of an individual to change its behaviour or morphology to meet a new environmental condition (Moczek 2010). In this way, populations made up of individuals with high phenotypic plasticity are more successful in novel environments as they can react to change with more immediate responses than changes seen through natural selection of individuals over generations. The aphid, *Brevicoryne brassicae*, displays varying phenotypes on different host plants. High levels of association between populations of this species allow reproduction between individuals with varying phenotypes and results in this phenotypic plasticity being shared amongst all members. This genetic sharing (gene flow) limits natural selection for any genetic variance that may lead to adaptive speciation (Ruiz-Montoya and Núñez-Farfán 2013).

Most genetic differentiation between populations, therefore, occurs under conditions of isolation and reduced gene flow. In the case of geographic isolation, separation of populations by location leads to individuals that can not interbreed due to distance or barriers such as mountains. This can create geographic differentiation of insect populations that have been subjected to unique selective forces and produce phenotypes (observable traits) or genotypes (genetic traits) that are specifically suited to those environmental factors through adaptation (Via 1991). For example, genetic variation was shown to be significantly different in three cotton

flea hopper (*Pseudatomoscelis seriatus*) populations, each associated with environments with distinct precipitation patterns (Barman et al. 2012).

In addition to geographic differentiation, populations can be isolated locally through behaviour. Within the same geographic region, preference behaviour for different host plants may create isolation among populations of herbivorous insects. This behaviour may create host-plant specialization, also called host-associated differentiation (HAD) that may lead to genotype variation between populations (Guttman et al. 1981, Turrill 2006). Host plant preference differences in two races of ladybird beetle, *Henosepilachna diekei* resulted in specialization on different host plants. This isolation of behaviour and morphology created low gene flow between populations, which in turn led to genetic divergence resulting in the speciation of this beetle (Matsubayashi et al. 2011). HAD is also illustrated when insects are isolated due to their preference for wild host plants instead of crop host plants. A study of the bean beetle, *Acanthoscelides obvelatus*, shows more genetic diversity within beetle populations associated with wild host plants than in beetle populations on cultivated host plants. One reason for this genetic diversity in wild-plant associated beetles is that large crop fields allow mixing of bean beetle individuals, maintaining gene flow, while wild plants are restricted to patches, increasing isolation (Alvarez et al. 2007).

1.5 Study Species- Diamondback Moth (DBM) (*Plutella xylostella*)

DBM is a small, grayish Lepidopteran that feeds almost exclusively on Brassicaceae plants (De Bortoli et al. 2013; Sarfraz et al. 2010a). DBM probably originated in South Africa or the Mediterranean Region, based on the fact that there are over 140 wild Brassicaceae species and 22 species of DBM parasitoids found in this region (Kfir 1998). The coevolution of DBM and Brassicaceae plants likely began in these regions between 54 and 90 million years ago (Kfir 1998). Recently, the entire genome of DBM has been sequenced, and extensive collections are currently underway to establish the specific genetic origin and global spread of DBM (You et al. 2013).

DBM is capable of rapidly developing resistance to every currently used insecticide class, including DDT and the biological agent, *Bt* (*Bacillus thuringiensis*) (Pichon et al. 2006; Saeed et al. 2010). This is a major problem because many Brassicaceae plants, including canola, cabbage, radish, broccoli, cauliflower, mustard, and bok choy are crops grown in most parts of the world, providing a widespread and abundant food source for DBM (De Bortoli et al. 2013). Its

resistance to traditional control methods has resulted in heavy economic and environmental tolls (Zalucki et al. 2012a).

DBM is present year-round in tropical and subtropical climates and occurs seasonally in temperate and polar regions, when temperatures are favorable, between approximately 6 and 31°C (Pichon et al. 2006; Gu 2009; Marchioro and Foerster 2012) (Fig. 1.2). DBM does not seem to overwinter in areas with below freezing temperatures, suggesting that populations in these regions must be recolonized on an annual basis (Gu 2009). However, contradictions to this temperature limit have been demonstrated in that DBM of various life stages are still capable of surviving at -5°C for up to 20 days (Gu 2009) and the thermal tolerance minimum (for 25% survival) for this species was established to be -15.2°C (Nguyen et al. 2014). In regions where temperatures are higher than approximately 35°C, populations cease to be found, but occur seasonally, when temperature drop again (Kohno et al. 2004).

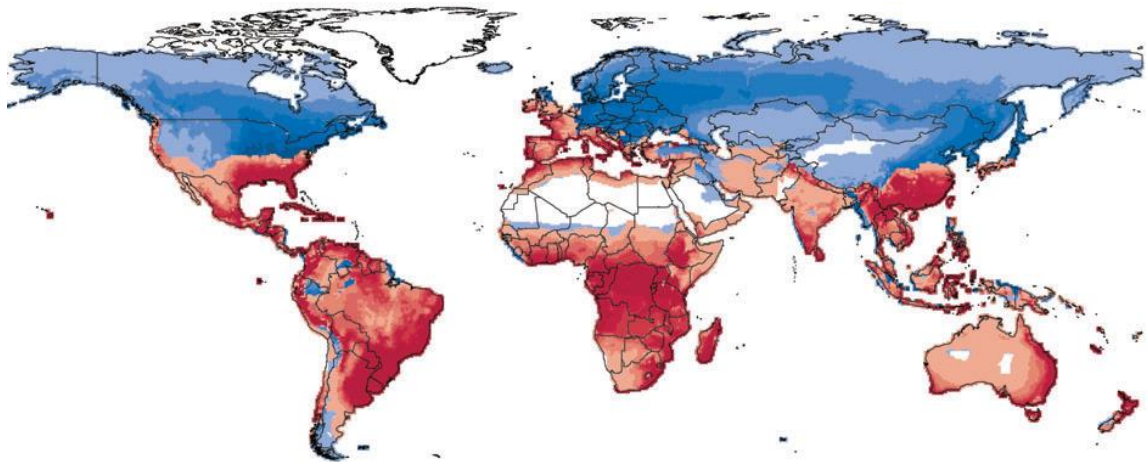


Figure 1.2 Predicted worldwide distribution of diamondback moth (DBM) based on a validated bioclimatic model. Areas shaded in red show regions of the world where the Ecoclimatic Index (EI) is positive and DBM can persist year-round; red shading demarcates the core distribution of DBM (regions where $EI \leq 15-20$ are marginal for year-round DBM survival). Areas shaded in blue show regions of the world where the EI is zero but where the annual growth index (GI) is positive; in these regions DBM cannot persist year-round but it can become a seasonal pest following influxes of moths from elsewhere (Furlong et al. 2013, with permission).

In North America, DBM is widespread, damaging crops throughout Mexico, the United States of America (USA) and Canada, and prompting a cooperative international project in 1988 to determine insecticide resistance levels in many different DBM populations (Shelton et al. 1993). In 1997, a major outbreak in California broccoli fields was linked to a warmer winter and lower rainfall the previous year as well as resistance to additional insecticides (Shelton et al.

2000). The Saskatchewan and Alberta populations are usually seasonal and are thought to originate from these southern populations found in western USA or Mexico (Doddall et al. 2001; Bahar et al. 2013). Hopkinson and Soroka (2010) have reported that DBM found in Saskatchewan come from Texas, but are unable to confirm the route of Alberta DBM, indicating that more information is needed on these two populations. Adults can readily disperse up to 2000 km to new locations or to return to seasonal habitats in temperate climates (Doddall et al. 2001; De Bortoli et al. 2013). Doddall (1994) reports that a mild 1991-1992 winter in Alberta may have allowed DBM to overwinter in canola fields. Hagerty et al. (2008) report the first occurrence of DBM in interior Alaska, which they believe to be the result of either long-range dispersal on wind currents or transfer by plant seedlings. New Brunswick DBM are found on cabbage, cauliflower and broccoli in varying population levels (Maltais et al. 1998). In Ontario, DBM are regularly seen on agricultural plants such as broccoli, cabbage, and cauliflower (OMAFRA 2013), but little evidence regarding their dispersal routes to Ontario is available. The DBM has also been discovered on the wild plant, garlic mustard (*Alliaria petiolata*) in Ontario, indicating its probable presence on additional Brassicaceae species that have not yet been assessed (Yates and Murphy 2008).

1.6 DBM Life History

The life cycle of the diamondback moth takes between 18 and 51 days, depending on temperature, humidity and food availability (De Bortoli et al. 2013, Harcourt 1986). There are four life stages in the DBM life cycle: egg, larva, pupa, and adult. Within the larval stage there are four instars. After hatching, the first instar larvae tunnel within leaf parenchyma (De Bortoli et al. 2013). Second instar larvae move to the lower external leaf surface and continue feeding in inner and lower surfaces, creating windows in upper surfaces (De Bortoli et al. 2013) (Fig. 1.3a). Third instar larvae feed in a similar fashion (De Bortoli et al. 2013). Fourth instar larvae eat all parts of the leaf, as well as other plant parts (De Bortoli et al. 2013) (Fig. 1.3b). A single adult female can lay up to 180 eggs, with the majority of egg laying occurring within the first few days (Harcourt 1986) (Fig. 1.3c and 1.3d). In Southern Ontario, five to six generations per year have been reported (Harcourt 1986). Flight takes place during the evening, overnight and in the morning, with males having longer flight times (approximately 19 hours) than females (approximately 16 hours) (Goodwin and Danthanarayana 1984; Harcourt 1986). Some research indicates that dispersing or migrating generations may have different life spans and nutritional requirements than non-migrating generations (Begum et al. 1996).

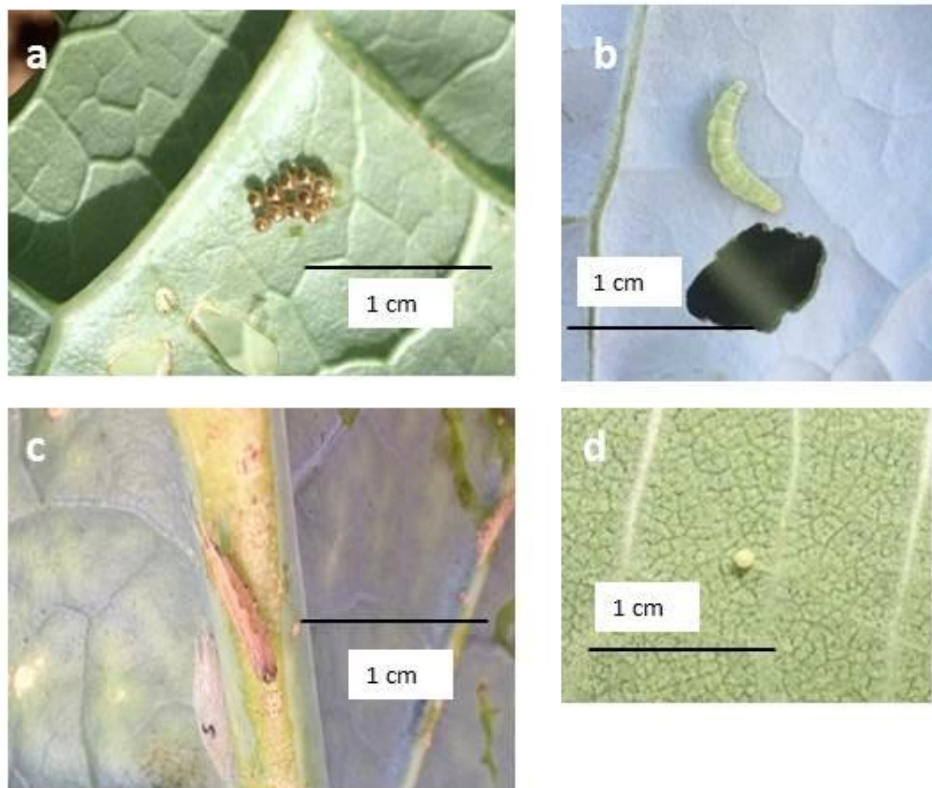


Figure 1.3. a. Window created in leaf by 2nd instar DBM larva b. 4th instar DBM larva feeding on a broccoli leaf c. Adult DBM beside its pupal case on an ornamental kale leaf d. DBM egg (source: Kiera Newman).

1.7 DBM Host Plant Preferences

DBM is considered a specialist, dependent (with very few exceptions) upon plants of the Brassicaceae family for all stages of its life cycle (Mau and Kessing 2007; Sarfraz et al. 2010a). There are approximately 300 genera in the family Brassicaceae throughout the world, with between 2500 and 3700 species (Marzouka et al. 2010). Considering the global range and world-wide availability of Brassicaceae species, both wild and cultivated, the DBM could be considered as much of a generalist feeder as a specialist (Pichon et al. 2006; Furlong et al. 2013). In Brassicaceae family species, glucosinolates and saponins are produced as defense compounds, and are toxic to many insect species (Li et al. 2000; Renwick 2002; Himanen et al. 2009). The DBM, however, is capable of overcoming some of the plant defenses of Brassicaceae (Ratzka et al. 2002).

DBM are attracted to plants by glucosinolates (Sun et al. 2009). Interestingly, though they attract DBM, glucosinolates are also correlated with lower growth rates and weights of

these moths at various life stages (Li et al. 2000). Saponins are also important plant chemicals that affect DBM fitness (Badenes-Perez et al. 2014). Wintercress (*Barbarea vulgaris*), a member of the Brassicaceae family, attracts DBM adults through the presence of glucosinolates. However, triterpenoid saponins in at least one genotype of this species act as feeding deterrents to larvae, leading to larval death (Shinoda et al. 2002). Saponins have also been found in garden cress (*Lepidium sativum*) (Hussein et al. 2011), though no studies have yet confirmed this species as a host plant for DBM.

Brassicaceae species have different types of defenses against herbivory including mechanical and chemical defense traits (Karowe and Grubb 2011; Travers-Martin et al. 2008). Black mustard (*Brassica nigra*), for example, has larger trichomes and higher glucosinolate concentrations in plant parts with greater tissue value (to herbivores) indicating that these plant parts are more heavily defended than less valuable parts (Traw and Feeny 2008). *Brassica oleracea*, which includes the wild variety of cabbage as well as the cultivated varieties broccoli, cauliflower, cabbage, Brussels sprouts, and ornamental kale (Yu et al. 2013), responds to herbivory through the induction of defenses (such as glucosinolate production) that are controlled by jasmonic acid production (Tytgat et al. 2013). Some mustard species in the Brassicaceae family, such as *Arabidopsis thaliana* and closely related *L. sativum*, possess a thick cuticle that protects them from pests as well as serving as a medium for chemical defense transmission (Reina-Pinto and Yephremov 2009; Endo et al. 2012).

Some studies have been published on the fitness of DBM reared on different plant species. In most of these studies, however, DBM are exposed to only one host plant at a time (i.e. there is “no choice” involved) (De Bortoli et al. 2013; Saeed et al. 2010). In these no-choice tests, the study species (i.e. DBM) is only given one plant species and specific growth and development characteristics (i.e. weight) are recorded and compared to other plant species. DBM has shorter developmental time and higher survival on canola (*Brassica napus* var. *Canola*) and mustard (*Brassica compestris*) compared to cauliflower (*Brassica oleracea* *Botrytis*), radish (*Raphanus sativus*) and turnip (*Brassica rapa*) (Saeed et al. 2010). Research on no-choice effects of rearing DBM on canola (*Brassica napus* L. cv. *Thunder* TT), Chinese cabbage (*B. rapa* L. *Pekinensis*, cv. *Wombok*), and cabbage (*B. oleracea* L. *Capitata*, cv. *sugarloaf*) showed that 90% more DBM complete development on leaf six (which corresponded with higher nutrients and lower glucosinolates) of each plant species compared to leaf one (Silva and Furlong 2012). Though these experiments establish a fitness difference among DBM reared

on various plant species or plant parts, they do not examine preferences of DBM offered multiple plant choices. While no-choice tests may provide valuable information in crop situations involving large swathes of monoculture plants, preference tests would be useful in environments with crops bordered by wild plants or in nursery settings where both crops and ornamentals are grown.

In some cases, DBM have been offered multiple crop plant choices and their preferences assessed. When offered cabbage, broccoli, cauliflower and collard greens, DBM larvae preferred collard greens while adult females preferred to oviposit on cauliflower (De Bortoli et al. 2013). Oviposition testing led to no significant difference in eggs laid on broccoli or cauliflower, but more eggs were laid on cabbage (Hamilton et al. 2005). Conflicting results could be due to variability in crop cultivars, as well as differences in chemistry within the same species. For example, Sarfraz et al. (2010b) show that DBMs prefer canola leaves high in phosphorus, nitrogen and sulfur over canola leaves in the same field with lower concentrations of these nutrients.

Wild host plants can serve as refuges that help pests, such as DBM, quickly reestablish infestations in crop fields when they become available again (Furlong et al. 2013; Harcourt 1986). Comparisons of the fecundity and lifespan of DBM reared on the wild Brassicaceae species *Rorippa indica* and *Lepidium virginicum* versus cabbage (*Brassica oleracea* var. *Capitata*) show that adult females live but were less fecund on wild species (Begum et al. 1996). A recent study by Sarfraz et al. (2011) shows that DBM prefer wild mustard (*Sinapis arvensis*) over two other wild choices, *Erysimum cheiranthoides* (L.) and *Capsella bursa-pastoris* (L.). This preference resulted in higher survival, rate of development and number of eggs on *S. arvensis* compared with the other two species (Sarfraz et al. 2011). The quantity and quality of nutrients in these different host plants probably influences the number of eggs produced by females (Begum et al. 1996). Muhamad et al. (1994) have determined that among *L. virginicum*, *Cardamine flexuosa*, *C. bursa-pastoris*, *R. indica*, and *R. islandica*, only the latter was a better host plant than cabbage. As with crop testing, wild host plants show variability in both preference by, and resulting fitness of DBMs. However, most tests confirm that DBM prefer wild over crop species despite lower fitness consequences on wild species (Begum et al. 1996; Charleston and Kfir 2000). It has also been established that most wild Brassicaceae plants contain higher levels of glucosinolates than cultivated species (Gols and Harvey 2009).

In addition to crop and wild host plants, landscape ornamentals are potential host plants that may maintain populations or even promote infestations. Ornamental kale and cabbage are popular landscape plants (Whipker 2013). Ornamental kale has weaker glucosinolate concentrations than ornamental cabbage (Kushad et al. 2004), yet, both sustain significant damage by DBM in greenhouse settings (Cloyd 2013). No documented reports of DBM on purple rock cress (*Aubretia*) (*Aubretia deltoidea*), another popular landscape plant in the Brassicaceae family (Pilon 2009), can be found.

It has been suggested that the DBM is constrained by its olfactory sense, promoting the choice of Brassicaceae species for oviposition that may not be optimal for larval fitness (Cunningham 2011). The strong preference of DBM for certain Brassicaceae species over others provides valuable trap cropping opportunities (Serizawa et al. 2001). Trap crops act as a sink to agricultural pest herbivores by providing them with a more attractive food or oviposition source relative to the crop that is cultivated for market (Holden et al. 2012). In order for trap crops to be successful, they must both attract and retain pests in sufficient numbers to allow cash crops to flourish (Holden et al. 2012). A dead-end trap crop is one that attracts female insects for oviposition, but suppresses the hatching and/or development of eggs and larvae (Shelton and Nault 2003). This “evolutionary mistake” made by insects when they are attracted to lethal plants may be due to their olfactory constraint to plants that trigger preference behaviour, but have underlying toxic components (Badenes-Perez et al. 2014). George et al. (2009) determine that white mustard (*Sinapis alba* L.) may be an effective trap crop of DBM in cauliflower (*Brassica oleracea* (L.) var. *Lateman*) fields. Studies with wintercress (*Barbarea vulgaris* (R.Br.)) have shown that it attracts ovipositing females and prevents development of young, thereby providing a dead-end trapping opportunity (Cunningham 2011). However, a *B. vulgaris* genotype susceptible to DBM has also been documented (Agerbirk et al. 2003). In addition, fertilizing *B. vulgaris* with sulfur increased the efficacy of this trap crop against DBM infestation (Badenes-Perez et al. 2010). A study by Musser et al. (2005) shows significantly more eggs were oviposited on the trap crop glossy collard (*Brassica oleracea* L. var. *Acephala*) than on cabbage (*Brassica oleracea* L. var. *Capitata*) in 2002, but not in 2003. Indian mustard (*Brassica juncea*) may also be effective in attracting more ovipositing females than crops such as broccoli (*Brassica oleracea* var. *Italica* L.), cauliflower (*Brassica oleracea* var. *Botrylis* L.), and Chinese cabbage (*Brassica pekinensis* (Lour.) (Charleston and Kfir 2000).

In addition to trap cropping, other methods of DBM control include exposure to extracts from plants such as *Vitex negundo* L., which significantly reduces DBM larval survival and adult oviposition (Yuan et al. 2006). Extracts from *Muntingia calabura* fruits and flowers also show high toxicity to larvae, pupae and adult DBM, suggesting effective use of these compounds as insecticides for the control of DBMs (Neto Bandeira et al. 2013). DBM may also be controlled by other integrated pest management methods. For example, hymenopteran parasitoids (about 60 species) have been used with limited success to control DBM numbers (De Bortoli et al. 2013; Furlong et al. 2013) (Fig. 1.4).



Figure 1.4 Parasitoid (*Diadegma* sp.) of a DBM larva (source: Kiera Newman)

Proposed Study and Questions

Understanding DBM and its host plant preferences remains very limited despite the importance to control the species. Information on DBM that breed in Ontario, Canada is also scarce. Considering the literature review and what is currently known about DBM, the following questions can be asked, and the following hypotheses and predictions formulated:

1. Do larvae and adults of DBM show a food or oviposition preference when exposed to different host plant choices?

Hypothesis: If DBM attraction to host plants is positively correlated to glucosinolate concentration, then more individuals will prefer plant species with higher glucosinolate contents.

Prediction: Larvae and adults will prefer garden cress and wintercress because, among the six plant species tested, they are the ones reported to contain the most glucosinolates, followed by black mustard, as its glucosinolate concentration is also reportedly high. The remaining plants will be equally preferred as they are either reported to have similar glucosinolate concentrations or no data could be found.

2. Do 3rd instar DBM larvae display different host preferences than 4th instars?

Hypothesis: Since digestive abilities have been shown to develop with age in DBM larvae, they may prefer plant species that are more difficult to digest as they mature.

Prediction: Fourth instars will more highly prefer those plants reported to have higher glucosinolates, such as garden cress, wintercress and black mustard, than other choices compared to 3rd instars. Fourth instars will prefer more black mustard than 3rd instars, as the mechanical defenses in this plant species may pose ingestion and digestion difficulties that may be better overcome in latter life stages.

3. Does host plant choice affect ingestion rate of diamondback moth larvae over one hour of feeding?

Hypothesis: Since plant species with reportedly higher chemical and mechanical defenses compared to other plants are more difficult to ingest, lower weight gain will occur on these plant species compared to less heavily defended plant species.

Prediction: After one hour of feeding, DBMs will gain more weight on broccoli, ornamental kale and aubretia (as they are generally less defended) compared to garden cress, wintercress and black mustard.

4. Does the origin of the DBM population (Alberta, Saskatchewan, Ontario Crop, Ontario Ornamental, Ontario Wild) affect the choice of plant or weight gain over one hour of feeding?

Hypothesis: If populations of DBM have established ecotypes based on geographic or host-associated differentiation, then differences in preferences or weight gain may result among populations.

Prediction: If geographic isolation has caused differentiation, then individuals within populations will prefer the same plant choices, but between populations there will be preference differences. Specifically, Alberta, Saskatchewan and all Ontario populations together will vary in their preferences. If host-adapted specialization has created ecotypes, then Alberta, Saskatchewan and Ontario Crop populations will prefer broccoli, aubretia and ornamental kale, as these plant species are cultivated and their reduced glucosinolate profiles may seem familiar to these insect populations. Ontario Wild larvae will prefer wintercress and black mustard as they are wild plants, as well as garden cress, as it is cultivated for its chemical attributes, and therefore may seem more like a wild plant to larvae. Ontario Ornamental larvae will prefer aubretia and

ornamental kale plants as they would seem more familiar to larvae with a predisposition for nursery-raised plant species. Likewise, weight gain differences over one hour between populations may indicate ecotype formation.

Chapter 2.

This chapter is formatted as an article as it will be submitted for publication.

Are wild or ornamental Brassicaceae plants more attractive than crops to *Plutella xylostella* larvae?

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2.1 Abstract

Host plant feeding preferences of insect pests are critical information when forming strategies for pest management. Alternate host plants available to phytophagous insects in agricultural settings can provide positive or negative developmental opportunities, as they may act as either reservoirs to support pest populations or sinks to reduce them. Laboratory tests of multiple simultaneous food choices were conducted to assess preferences of the diamondback moth (DBM) (*Plutella xylostella*) on crop, wild, and ornamental host plants. DBM preferred garden cress (*Lepidium sativum*) over all other plant choices: wintercress (*Barbarea vulgaris*), black mustard (*Brassica nigra*), aubretia (*Aubretia deltoidea*), ornamental kale (*B. oleracea* var. *Acephala*) and broccoli (*B. oleracea* var. *Lieutenant*). Weight gain during a one hour food preference test was lowest in larvae which fed on black mustard, and highest on aubretia, broccoli and ornamental kale. There were no feeding differences among populations (Alberta, Saskatchewan, Ontario Crop, Ontario Ornamental and Ontario Wild). Garden cress is shown to be a highly attractive food plant species for DBM third and fourth instar larvae. It is assumed from previous literature that levels of saponins within garden cress make this host plant a candidate for dead-end trap cropping. Results indicate that wintercress and garden cress may support later life stages of DBM larvae, although full life cycles are improbable on this plant. *Aubretia deltoidea* is also shown for the first time to be a potential host plant species for DBM. These results also support evidence that alternate host plants near crops or in greenhouse environments may act both as reservoirs and sinks to DBM populations.

2.2 Introduction

Insect host plant preference is affected by a variety of factors, such as chemical and visual cues, enemy avoidance, changes in plant chemistry and nutritional requirements of insect life stages (Lee 2007; Webster et al. 2012). How an insect weighs these factors when choosing a plant has been the focus of many studies (Allmann et al. 2013; Gamberale-Stille et al. 2013; Abed-Vieillard et al. 2014). Unlike generalists that eat a variety of plant species, specialist phytophagous insects are confined to one or few host plant species (Becerra 2006; Reudler et al. 2011; Ali and Agrawal 2012). Specialization on certain plants can give these insects an advantage, i.e. the ability to access nutrients that generalists cannot, due to mechanical and/or chemical plant defenses (Kojima et al. 2010). This advantage may come with a price, such as reduced fitness, as additional energy is needed to bypass defenses (Camara 1997).

In natural communities, insects are exposed to a variety of potential host plants, while in managed agricultural systems, especially in monocultures, the diversity of plants is reduced. Insects attracted to these monocultures have sufficient supply of that single host plant type during a certain time of the year. Before planting or after harvest, however, insects must find other host plants in order to survive. During a growing season, nutritional contents in plants can vary, also driving insects to search for alternate hosts (Badenes-Perez et al. 2005). Plants in close proximity to crops, such as intercrops, weeds, or landscape ornamentals may act as potential hosts for adults and mobile larvae (Kim et al. 2011; Nair et al. 2012; Mcfadyen and Muller 2013).

Flying adults may sense several potential hosts simultaneously, and make decisions based on preference factors including chemical attraction or plant type and size (Potter et al. 2012). This is also true for some species at their larval stages such as *Manduca sexta* and *Polygonia c-album* (Potter et al. 2012; Gamberale-Stille et al. 2013). In the case of insect pests, identifying preferred and alternate host plants can help predict infestations and understand mechanisms related to food preference (Kalaitzaki et al. 2013; Macfadyen and Muller 2013). By knowing variation in insect population dynamics depending on host plant type, pest management can be improved (Bianchi et al. 2008; Srinivasin et al. 2013).

The diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Plutellidae), is a global specialist pest of Brassicaceae (cruciferous) species, including many agricultural crops such as

canola, cabbage, mustard, and broccoli (De Bortoli et al. 2013). The extensive cultivation of Brassicaceae crops means that this pest can be found in most parts of the world (Furlong et al. 2013). It is a year-round resident in regions where temperatures vary between 6° and 31°C, and occurs seasonally in regions where temperatures are above or below these values at times during the year (Pichon et al. 2006; Gu 2009; Marchioro and Foerster 2012). In addition to crops, many wild and ornamental host plants are available for, and used by DBM (Muhamed et al. 1994).

In this study, we examined food preferences of the 3rd and 4th instar DBM larvae from different populations when offered multiple simultaneous plant choices. Until now, most studies have investigated fitness parameters such as growth, survival and egg quantities of DBM under no-choice conditions (De Bortoli et al. 2013; Saeed et al. 2010). Considering this species can adapt to various environments and that, in general, different types of Brassicaceae species (e.g. weeds, crops, and ornamentals) can be readily available in a given area, we aimed to determine whether 3rd and 4th instar larvae from different populations would generally prefer the same species, regardless of their origin when offered six plant choices.

The Brassicaceae family is comprised of plants exhibiting different types of plant defenses. For example, *Brassica rapa* (canola) has trichomes and secretes chemicals when damaged (Handley et al. 2005; Travers-Martin et al. 2008; Karowe and Grubb 2011). *Brassica nigra* (black mustard), has been shown to induce leaf trichome density increases in response to DBM feeding (Traw and Feeny 2008). A thick cuticle protects *Lepidium sativum* (garden cress) plant tissues from herbivores (Endo et al. 2012). Although they contain a range of physical defenses, plants in this family are most notable for their chemical defense ability.

All Brassicaceae species produce glucosinolates, chemicals that attract and stimulate feeding in DBM (Freeman and Beattie 2008). These chemicals may also act as cues to some insects, particularly those that have coevolved with these plants, such as DBM (van Leur et al. 2008; Mathur et al. 2011). *Barbarea vulgaris* (wintercress), attracts DBM, but at least one phenotype of this species has been shown to contain saponins. Saponins are chemicals toxic to the development of DBM larvae (Agerbirk et al. 2003; Wei et al. 2013a), and as such this species can be used as a dead-end trap crop (Badenes-Perez et al. 2014). *Lepidium sativum* is another similar species that contains high levels of glucosinolates, specifically glucotropaeolin, as well as saponins (Gerendaś et al. 2008; Prajapati et al. 2014). It has yet to be tested regarding its attractiveness to DBM and its deterrent effect on DBM feeding.

The ability of DBM larvae to digest toxins varies with life stage, as phenotypic differences in the activity of enzymes responsible for breaking down food and insecticide toxins have been found in 3rd and 4th instars. Studies have shown an increased ability of 4th instar larvae to neutralize glucosinolates (Liang et al. 2003; Gong et al. 2013). Other studies have confirmed the changing abilities of larvae of similar phytophagous insect species to digest toxins as they mature (Saastamoinen et al. 2007, Yu and Hsu 1993). It is likely that 3rd and 4th instar DBM larvae can move among plants to optimize nutritional requirements (George et al. 2009). We therefore predicted that 4th instar larvae would prefer plants with higher chemical and mechanical defenses and feed more on these plants than 3rd instar larvae.

In Canada, DBM is seasonally present, arriving in late April or May and migrating or dying off around October. Few data exist regarding the source of Canadian populations, specifically those in Eastern Canada. Hopkinson and Soroka (2010) suggest that Alberta and Saskatchewan populations come from different southern source populations. Gene flow among these populations may be limited thus potentially leading to ecotypes or genetically different populations. Considering the rapid life cycle of the species (between 18 and 51 days) (Harcourt 1986), and potential genetic isolation of populations in various environments for at least several months of the year (potentially longer in populations that overwinter in greenhouses), we hypothesized that populations from different regions would have developed preference differences over time and would thus prefer different experimental host plants. We expected that larvae from different environmental types (crop, wild, ornamental) would choose plant species more related to the host plants from their source environments compared to other plants when exposed to a variety of different plant species. We also expected that if Alberta, Saskatchewan and Ontario DBM came from different source populations or were isolated due to overwintering, then they would show different food preferences, as well.

It is important to note that in Ontario, and especially in the Niagara Region, where agriculture and greenhouse industries are prevalent, no previous survey of DBM has been conducted to determine its occurrence and abundance. This information could establish the presence of DBM in microclimates that may allow overwintering. So, prior to the food preference experiment, a field survey was completed to assess distribution of DBMs in various locations.

2.3 Materials and Methods

Insect Populations

For this study, five populations of DBMs were acquired during July and August of 2013. The Alberta population was collected from a canola field in Lethbridge, Alberta. The Saskatchewan population came from an Agriculture Canada canola field in Saskatoon, Saskatchewan. In the Niagara Region, Ontario, 15 sites were surveyed. At each site, 50 plants were randomly chosen and searched, between July 15 and August 15, for larvae, pupae and adult DBMs. Five sites were agricultural fields growing broccoli, kale, and cabbage; five were natural sites containing patches of black mustard; five were garden nurseries growing ornamental cabbage and kale. The populations used for the food preference experiment, therefore, came from a conventional broccoli field (called Ontario Crop), a meadow with black mustard, *Brassica nigra* (Ontario Wild), and a landscape nursery growing ornamental kale and cabbage (Ontario Ornamental).

DBM Rearing Conditions

Each population was reared at 20 - 25°C, 50 - 70% relative humidity, 16 h light/8 h dark (Sarfranz et al. 2009). Four to six week old radish plants (*Raphanus sativus*) were used as the only food source for the larvae. Pupae were collected every two days from leaves and transferred to a 500 ml plastic bottle (suspended 5-10 cm above plants) to develop as adults, and for mating and oviposition, with no more than 50 pupae per bottle at a given time. Once the eggs hatched, the first instar larvae descended on their own threads onto the radish leaves below through small holes on the bottom of the bottle. Adults were fed 10% honey solution on cotton balls within the suspended plastic bottles. Radish plants were changed at approximately 75% defoliation. At least three generations of each DBM population were reared before experimentation.

Plants

Two crop species: broccoli (*Brassica oleracea* var. *Lieutenant*) and garden cress (*Lepidium sativum*), two wild species: black mustard (*Brassica nigra*) and wintercress (*Barbarea vulgaris*), and two ornamental nursery species: ornamental kale (*B. oleracea* var. *Acephala*) and aubretia (*Aubretia deltoidea*) were used in this experiment. All of these species are common in Ontario and have the potential to support DBM populations. Seeds were planted in Sunshine Mix No. 3 soil and resulting plants were grown in the greenhouse (25°C day/18°C night, 70% relative humidity, 16:8 h light: dark cycle) until 4-6 weeks old (at which point the phenological age of all

plant species was similar). Plants were watered daily and not fertilized. When a plant had leaf material removed for experimentation, that plant was not used in subsequent experiments.

Experimental Methods

Larval Food Preference Trials

All DBM experiments were carried out in controlled laboratory conditions as described above. Leaves were taken from the same location on each plant for experimentation (second leaf from the top). For each trial, six leaf discs (one of each test plant species) of 2 cm diameter were placed in a 9 cm diameter petri dish lined with moistened filter paper (similar to Bar-Yam and Morse 2011). The discs were weighed before starting the trial and placed in random order around the edge of each petri dish. Discs were separated by aluminum partitions to reduce the mixing of volatile organic chemicals from the various plants. Sterilized petri dishes and partitions were used for each trial. Experiments were performed between 0900h and 1300h (as per George et al. 2009). Larvae were starved for 15 hours prior to the experiments. One 3rd or 4th instar larva was then weighed and placed (using a small paintbrush) into a stainless steel ring in the middle of the petri dish to acclimate the individual to the environment. When the larvae began movement out of the ring, timing was started. The larva was watched for 60 minutes to determine which (if any) leaf disc(s) it chose and the weight of leaf material it ingested in that time. Pretrials were carried out to four hours to determine optimal timing for weight gain. Sixty minutes was chosen as an optimal time, as weight gain levelled off after this time and defecation began shortly thereafter. The leaf discs and larva were weighed again at the end of the trial. Measures of evaporative weight loss of each plant species over 60 minutes were also taken. Since leaf weights before and after experimentation, with or without larval feeding, were minimal and highly variable due to evaporative weight loss amounts, these measurements were not used in statistical analyses. Instead, larval weights before and after experimentation were used to assess short-term weight gain (associated with ingestion) of individuals on each plant species. The experiment was repeated approximately 19 (range of 18 to 21) times for each instar age (3rd instars=94; 4th instars=97) and population (Alberta=41; Saskatchewan=37; Ontario Crop=38; Ontario Ornamental=37; Ontario Wild=38) for a total sample size of 191 individuals. Each trial involved naïve individuals. Plant position in relation to each other, and plant preparation order (cutting into discs) were randomized to reduce error associated with mixing plant volatiles and the leaf cutter was sterilized in between each cutting.

Larval Rearing Experiments

After the second weight was taken, each 3rd instar larva was reared in a separate petri dish with moistened filter paper, and with the host plant it chose (in no case did a larva choose more than one plant). Fourth instars pupated within a day or so after the trials and were not used in rearing tests. Plant leaves were changed daily and filter paper re-moistened. Time to pupation, weight, and gender of pupae were recorded for all reared larvae. Sample sizes of individuals raised to pupation varied depending on the plant species they preferred: garden cress (n=39), wintercress (n=16), black mustard (n=14), aubretia (n=10), broccoli (n=5), and ornamental kale (n=5).

Since 3rd and 4th instar larvae fed on garden cress and wintercress, and some 3rd instars survived to pupation on these species, while literature indicates that the saponin in these plants causes larval death, rearing full life cycles on these plants was attempted. Twelve pupae were placed into rearing boxes following the procedure described above in rearing conditions. One box had garden cress and the other box had wintercress plants (4 – 6 weeks old). Boxes were checked daily for 45 days in order to assess successful completion of full DBM life cycles on these two plant species.

Statistical Analyses

Multiple choice testing has the intrinsic autocorrelation of data in that “preference” requires the dependence between at least two choices and is, therefore, not independent (Roa 1992). As independence of variables is a prerequisite of some statistical tests, this data was better suited to analysis using models without that assumption. Generalized linear models (GLZMs) are a better fit for non-normal and non-independent data, as they retain the power of parametric tests in situations of non-independence (Molina and Trumper 2012). The dependent variable was the percentage of individual larvae that chose each plant species. The probability distribution was normal. The link function was the larval identity number. Model intercepts were plant species, insect source population and instar.

RWG for larvae was calculated as $100 * [(final\ body\ weight - initial\ body\ weight) / initial\ body\ weight]$. SGR was calculated as $100 * ((\ln\ pupal\ weight - \ln\ final\ larval\ weight\ after\ feeding\ trial) / total\ days\ to\ pupation)$ in % growth/day. RPW was calculated as $100 * [(pupal\ weight - final\ body\ weight) / final\ body\ weight]$. A factorial ANOVA was used to compare RWG of all larvae during the 60 minute feeding trial, with population, plant choice, and instar as independent variables. Based on those results, a subset of the data was used in a subsequent factorial

ANOVA using RWG as the dependent variable and plant choice as fixed factor to assess differences due to life stage. A Bonferroni-adjusted significance level of 0.025 was used to assess significant differences in this ANOVA and post-hoc tests results. Exploratory ANOVA tests were done to assess SGR of 3rd instar larvae to pupae and RPW with population set as a fixed factor, however no significant differences were found and sample sizes were small and variable. Data for all ANOVAs were first tested for normality and homogeneity of variance and transformed as necessary (log10RWG, log10SGR, sqrtRPW). Tukey post hoc tests were performed to determine which plant species and populations were significantly different from others. All analyses were performed in IBM SPSS v. 20.

2.4 Results

Population survey results from Niagara showed that DBM were present in 13 out of 15 sites; all sites had 50 plants examined. In the crop sites, 4 out of 5 locations had DBM, with a range of 5 to 36 individuals (mean density=15.6±12.10). Four out of 5 ornamental nursery sites also had DBM, with a range of 6 to 61 individuals (mean density = 20.8±22.51) found on the surveyed plants. DBM was found in all five wild plant locations with between 9 and 16 individuals (mean density=10±5.52).

In all experimental trials, larvae either made no choice or chose and remained on the same plant for the 60 minute time period. Out of 191 individuals tested, only 3% of larvae (n=9) did not make any choice. These individuals were included in statistical tests of preference, but were excluded in further testing of weight gain and exploratory tests. Taking all the populations and both instars together, 43.4% of all larvae (that chose a leaf disc (n=182)) preferred garden cress (n=79), wintercress was chosen by 20.3% of larvae (n=37), black mustard was chosen by 15.9% (n=29), aubretia by 9.3% (n=17) and both broccoli and ornamental kale by 5.5% of larvae (Fig. 2.1).

The results from the GLZM showed that DBM significantly chose garden cress more than any other plant species (Wald $X^2=153.565$; $p<0.001$) (Table 2.1). Pairwise tests showed that wintercress was chosen significantly less than garden cress, and significantly more than all other plant types (except black mustard) (Wald $X^2=16.291$; $p<0.001$). Black mustard was also chosen significantly less than garden cress (Wald $X^2=42.537$; $p<0.001$), but more than all other plant types (except wintercress) (Wald $X^2=7.240$; $p=0.007$). Populations chose test plant species in the same proportions (Wald $X^2=0.111$; $p=0.999$). There was also no significant difference in plant preference between 3rd (n=94) and 4th instars (n=97) (Wald $X^2=0.001$; $p=0.970$).

Relative weight gain (RWG) over one hour by all larvae (n=182) together significantly differed among plant choices (ANOVA) ($F=7.509$; $df=5$; $p<0.001$) (Table 2.2). Tukey post hoc tests showed that larvae that chose black mustard showed significantly lower weight gain than those on broccoli ($p<0.001$), aubretia ($p<0.001$), garden cress ($p=0.001$), wintercress ($p=0.021$) and ornamental kale ($p=0.005$). Larvae that chose aubretia gained more weight than those on garden cress ($p<0.001$) and wintercress ($p<0.001$). However, there were no significant differences in weight gain among populations ($F=0.409$; $df=4$; $p=0.802$). There were also no significant interaction effects among populations, instars, and plant choices over one hour of feeding (results not shown).

RWG over one hour was also significantly different between instars (Table 2.2). Overall, 3rd instar larvae gained more weight over one hour of feeding than 4th instars (ANOVA) ($F=50.343$, $p<0.001$). A subsequent ANOVA was performed on data from 3rd and 4th instars separately (Table 2.3; Fig. 2.2). In 3rd instar larvae, weight gain was significantly lower on black mustard (mean \pm SE) (6.13 ± 1.30 mg) compared to garden cress (15.64 ± 1.47 mg) ($p=0.010$), aubretia (28.10 ± 2.96 mg) ($p<0.0001$), broccoli (25.62 ± 4.82 mg) ($p=0.002$), and ornamental kale (21.40 ± 4.26 mg) ($p=0.007$), but not wintercress (17.06 ± 2.04 mg) ($p=0.03$). In 3rd instar larvae, weight gain was also significantly higher on aubretia than garden cress ($p=0.021$). In 4th instar larvae, weight gain differences among plants were less than in 3rd instar larvae and weight gain was less variable among individuals on the same plant. In 4th instar larvae, weight gain was significantly lower on black mustard (3.80 ± 0.69) compared to aubretia (11.32 ± 0.78 mg) ($p<0.001$), broccoli (10.13 ± 1.49 mg) ($p=0.011$) and garden cress (7.37 ± 0.67 mg) ($p=0.007$).

Sixty seven out of 89 (75%) larvae survived to the pupal stage. Numbers of pupae that survived on the various plants were highly variable: garden cress (n=31), wintercress (n=13), black mustard (n=5), aubretia (n=10), broccoli (n=4), ornamental kale (n=4). Sample sizes of larvae reared on various plant species and from the five populations were uneven and some were too small for definitive statistical testing, so exploratory tests (ANOVAs) were performed. Results showed no differences among populations in terms of SGR or RPW.

To investigate the possible negative effect of wintercress and garden cress on DBM growth and survival, larval rearing was attempted on both plant species. Adults were observed to emerge from pupae (12 each for wintercress and garden cress containers), lay eggs and new hatchlings were seen to drop onto test plants. Plants were watered and checked daily for larvae and pupae for 45 days. No larvae (stages I to IV) or pupae were found on either

wintercress or garden cress (indicating that neither plant type could support initial life stages). Leaves were analyzed under a microscope and very little damage was observed. Therefore it is unlikely that larval death occurred at 2nd instar stage or later, as “windows” would have been seen in leaves due to feeding.

2.5 Discussion

In our experiments, DBM larvae strongly preferred garden cress, followed by wintercress, over all other plant choices. This does not fit with the nutrient stress hypothesis, as garden cress and wintercress contain saponins, chemicals known to be unacceptable to feeding DBM larvae. The nutrient stress hypothesis is used to explain why insects choose plants in order to maximize their nutrient intake (Joern and Behmer 1998; Frago and Bauce 2014). In general, DBM are attracted to plants that benefit them nutritionally (Sun et al. 2009). In this case, adaptation to preference of plants with high glucosinolate VOCs by DBM generally result in positive outcomes, as these chemicals signal the proximity of DBM host plants (Brassicaceae) (van Leur et al. 2008). Although a few species of Brassicaceae (i.e. wintercress and garden cress) contain saponins, the majority of DBM host plants are safe for larval development (Badenes-Perez et al. 2014). Thus, olfactory cue recognition and preference usually provides a valuable signal to searching DBM. DBM are constrained by their olfactory attraction to glucosinolates (Cunningham 2011). This olfactory constraint has been described as an “evolutionary mistake” in that it can over-ride the detection of detrimental chemicals, such as saponins, and lead to preference for lethal plants (Badenes-Perez et al. 2014)

Black mustard was also more preferred by DBM larvae than broccoli and ornamental kale. This phenomenon is reported by other researchers with plants high in glucosinolates, such as black mustard compared to a low-glucosinolate species, *B. oleracea* var. *Gemmifera* (Brussels sprout) (Gols and Harvey 2009). One rationale for the lower glucosinolate profiles in some Brassicaceae species is due to artificial selection of traits in crop (such as broccoli) and ornamental (such as kale and aubretia) plants that often reduces the amount of defense chemicals in agricultural plants (Gols and Harvey 2009). For example, canola (*Brassica napus* L.) has been extensively selected to decrease its glucosinolate concentrations (Ma and Li 2007). Garden cress, cultivated for its high chemical profile, acts more like a wild plant in this case, with reportedly higher glucosinolate content than crops artificially selected for lower glucosinolates (Gerendaś et al., 2008). Although glucosinolate concentrations were not measured for the test plants, other papers have shown that garden cress has a concentration of glucotropaeolin (a

parent compound of benzyl isothiocyanate) between 1.79 to 4.57 μmolg^{-1} (Sarıkamış and Yanmaz 2011). Wintercress is reported to have a total glucosinolate concentration of between 1.52 and 3.08 μmolg^{-1} (Badenes-Perez et al. 2014). Using literature values, direct comparison of the total glucosinolate concentrations among the test plants used in these experiments was impossible due to the lack of consistency in specific glucosinolates included in “total” glucosinolates (Kushad et al. 2004; Traw and Feeny, 2008; Hwang and Kim 2013). However, in general, reported glucosinolate concentrations are usually lower in broccoli and ornamental kale than in wintercress, garden cress and black mustard (Hansen et al. 1995; Traw and Dawson 2002; Kushad et al. 2004; Gerendás et al. 2008). No information could be found on glucosinolates in aubretia, suggesting the importance of further examination of this plant species.

Greater weight gain of larvae over an hour of feeding on aubretia and broccoli compared to black mustard could be attributed to the mechanical difficulties (due to trichomes) of feeding on black mustard (Traw and Dawson 2002). Other species of insects, such as *Spodoptera litura* have also been shown to have difficulty moving and lower weight gain on mustard species with trichomes compared to plant species with less mechanical defenses (Mathur et al. 2011). Ansari et al. (2010) report similar results, where DBM weight was lower on *Brassica juncea* (a close relative of black mustard) than on cabbage (*B. oleracea*). Like black mustard, *B. juncea* also has trichomes and high chemical concentrations which affect DBM attraction and growth rates (Mathur et al. 2011).

Lower weight gain of larvae on garden cress compared to aubretia and broccoli could be due to the reported presence of saponins in garden cress (Hussein et al. 2011; Najeeb-ur-Rehman et al. 2011). Due to the attractant nature of garden cress and its saponin content, this plant may also have applications as an effective trap crop. However, it should be noted that in our study both wintercress and garden cress were able to support 3rd and 4th instar DBM larvae through pupation and to adult emergence. Subsequent rearing on each of wintercress and garden cress from 1st instar larvae produced no viable offspring, however, so it is likely that young larvae are more susceptible to saponin. It is also possible that a different phenotype of garden cress, like that shown in wintercress by van Leur et al. (2008) and Wei et al. (2013b), is capable of supporting DBM populations due to a difference in chemical qualities, such as the absence of saponin.

There are fundamental differences between early larval life stages and the one that precedes pupation (Koch 1996; Mason et al. 2003; Shintani et al. 2003). Differences in weight

gain of 3rd and 4th instar DBM larvae on the different test plants could be due to age-related changes in the ability to digest plant material and associated chemicals (Liang et al. 2003). The reduced weight gain in the 4th instar compared to the 3rd instar larvae may be explained by the preparation of 4th instars for pupation (Mason et al. 2003). Other studies have confirmed that feeding in insect stages immediately before pupation is often reduced or unnecessary (Shintani et al. 2003). In many lepidopteran species, 4th instar DBM larvae are generally more voracious than 3rd (Scriber and Slansky 1981). However, the 4th instar life stage is also shorter in duration, lasting only an average of 1.4 days compared to an average of 2.6 days for 3rd instars, when reared on canola (Soufbaf et al. 2010). It is possible, therefore, that in choosing larvae for this study, many were already nearing their time of pupation or were cued by starvation into preparing for pupation early, and were therefore, less voracious. Future studies should include a control for this effect by testing the weight gain and length of time to pupation of 4th instar individuals that were not starved.

While migration tends to increase gene flow and genetic variability when individuals return to source populations, if migratory or dispersal routes are isolated in terms of geography or if host-plant adaptation isolates populations, then, over time, populations can differentiate into different ecotypes (Turrill 2006). Our results indicated that there were no differences in host plant preferences of DBM populations within Alberta, Saskatchewan and various Ontario environments. However, lab populations were established with one hundred individuals or less in each population, and therefore, may not have truly represented the phenotypic or genotypic variability of the entire DBM population within a collection area. Further testing should be done to establish whether ecotypes exist in any of these regions. Establishing that populations differ by province or host plant association in either their behaviour or other life history traits could provide information needed for effective pest management practices, as insecticide resistance and host plant preference may vary by ecotype. This is especially important in the Niagara Region of Ontario due to the prevalence of year-round greenhouse operations for fruit, vegetables, and ornamentals, which can lead to potential resident reservoir populations. These isolated populations may develop more rapid resistance to insecticides used in the greenhouses and/or initiate field populations of DBM earlier in the season than could dispersing DBM from southern areas.

Overall this research shows that specialist insects, like DBM, may be constrained by their evolutionary specialization to prefer host plants that are not good food sources (Cunningham 2011; Badenes-Perez et al. 2014, Marchioro and Foerster 2012). Usually insects optimize their

foraging to promote higher fitness levels by choosing the most beneficial plant species or parts, depending on their current developmental needs (Beckerman et al. 2010; Prado and Frank 2014). However, sometimes insects choose plants that reduce their own or offspring fitness (Srinivasan et al. 2013). This was seen here through DBM larval preference for garden cress, wintercress, and black mustard. Aubretia, a landscape ornamental previously untested for DBM preference, was determined to be a suitable, although not highly preferred, host plant for DBM. Our surveys showed that DBM are present at numerous greenhouses in Niagara, Ontario. This is the first time, to our knowledge, that garden cress has been assessed as a highly preferred, potential host plant for DBM. Due to its saponin contents, future research should determine the ability of garden cress to attract and limit DBM populations in field or greenhouse settings.

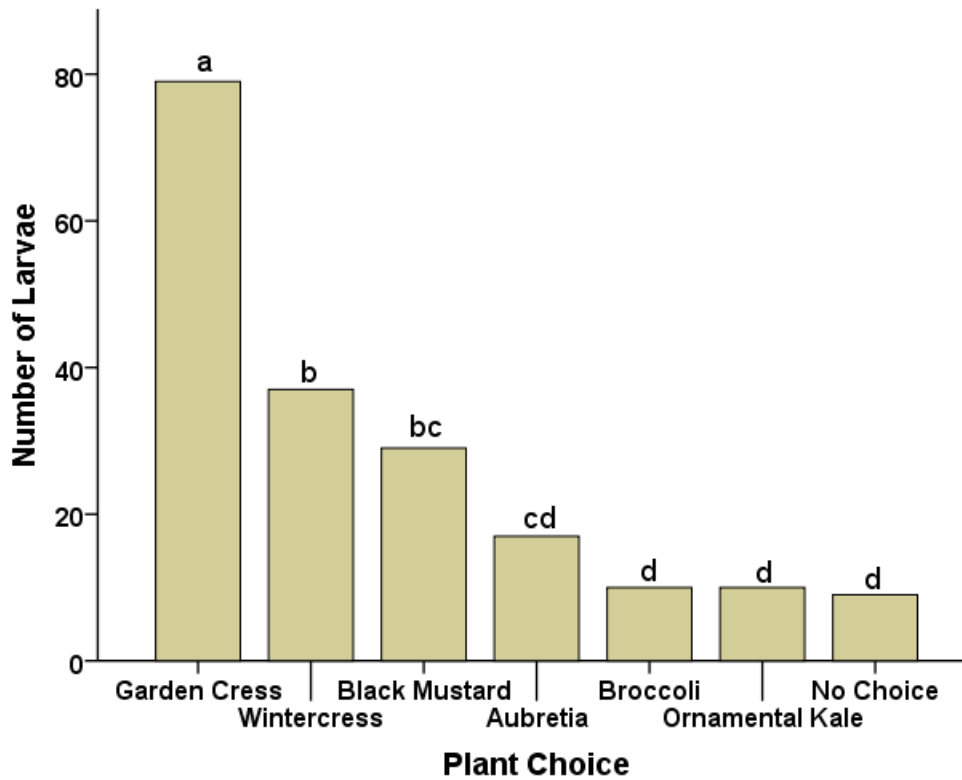


Figure 2.1 Number of DBM larvae (both 3rd and 4th instars combined) from all populations (Alberta, Saskatchewan, Ontario Crop, Ontario Wild and Ontario Ornamental) that chose each plant species or made no choice (n=191). Letters denote significant differences between plant species, as determined by Tukey post hoc tests.

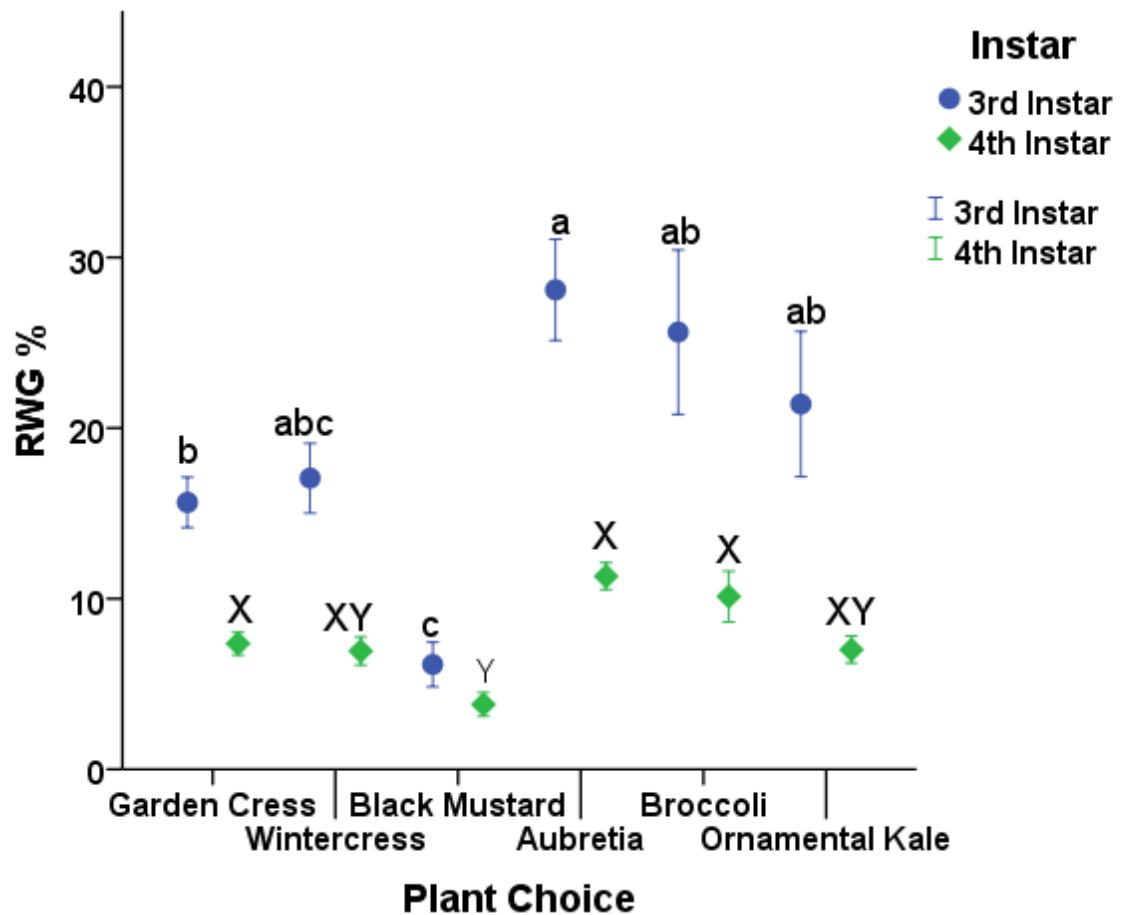


Figure 2.2 Percent mean relative weight gain (RWG) of 3rd and 4th instar DBM larvae after one hour of feeding on different plant species (garden cress, wintercress, black mustard, aubretia, broccoli, and ornamental kale) (n=182) from all populations (Alberta, Saskatchewan, Ontario Crop, Ontario Wild, Ontario Ornamental) combined. Letters denote differences between plant species. Error bars represent one standard error above and below mean.

Table 2.1 Summary statistics from a generalized linear model (GLZM) analysis of DBM plant species preferences (garden cress, wintercress, black mustard, aubretia, broccoli and ornamental kale) (as indicated through percentage of larvae that chose each plant type) between populations (Alberta, Saskatchewan, Ontario Crop, Ontario Wild and Ontario Ornamental), and larval instars (3rd and 4th). Significance levels were set to $\alpha=0.05$; < 0.05*, <0.01**, <0.001***.

Source	Wald Chi-Square	df	P
Population	0.111	4	0.999
Plant Choice	153.565	5	<0.001***
Instar	0.003	1	0.958

Table 2.2 ANOVA summary statistics for DBM weight gain over one hour of feeding by all larvae (n=182) on six different Brassicaceae plant species (garden cress, wintercress, black mustard, aubretia, broccoli and ornamental kale) and from five different populations (Alberta, Saskatchewan, Ontario Crop, Ontario Wild, Ontario Ornamental). Significance levels were set to $\alpha=0.05$; < 0.05*, <0.01**, <0.001***.

Source	Type III Sum of Squares	df	Mean Square	F	P
Population	0.080	4	0.065	0.409	0.802
Plant Choice	1.840	5	0.368	7.509	<0.001***
Instar	2.468	1	2.468	50.343	<0.001***

Table 2.3 ANOVA results for DBM weight gain over one hour of feeding on six different Brassiceae plant species (garden cress, wintercress, black mustard, aubretia, broccoli and ornamental kale) in 3rd (n=94) and 4th (n=97) instar larvae for all populations combined (Alberta, Saskatchewan, Ontario Crop, Ontario Wild and Ontario Ornamental). Significance levels were set to $\alpha=0.025$; < 0.025*, <0.001**, <0.001***.

Instar	Type III Sum of Squares	df	Mean Square	F	P
3 rd	1.611	5	0.322	7.061	<0.001***
4 th	1.393	5	0.279	5.097	<0.001***

Chapter 3.

This chapter is formatted as an article as it will be submitted for publication.

The diamondback moth finds novel host plant attractive: preference of *Plutella xylostella* for *Lepidium sativum*

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3.1 Abstract

The mother-knows-best hypothesis posits a maternal strategy in which an increase in insect larval performance is due to oviposition on certain plants that are associated with higher offspring fitness and survival. However, host plant preferences of phytophagous insects can result in oviposition on plants that are suboptimal or even lethal to developing offspring. The aim of this study was to examine oviposition preferences of the diamondback moth (DBM) (*Plutella xylostella*) when offered six host plant choices. Five populations of DBM were studied, three from Ontario environments (wild, crop and ornamental nursery), one from Alberta, and one from Saskatchewan. Previous research shows that DBM prefer to oviposit on wintercress (*Barbarea vulgaris*), containing saponins that lead to larval death. Our results confirmed the attractiveness of wintercress to DBM, but showed a surprisingly higher preference for garden cress (*Lepidium sativum*), which also contains saponins. This preference result suggested that offspring performance was not the primary criterion of DBM females when choosing oviposition sites. Black mustard (*Brassica nigra*), a plant with leaf trichomes (a mechanical defense), was also more preferred than broccoli (*B. oleracea* var. *Lieutenant*) and ornamental kale (*B. oleracea* var. *Acephala*) for oviposition. Information on oviposition preferences for DBM may be used to develop strategies for ecologically based management such as dead-end trap cropping.

3.2 Introduction

The mother-knows-best hypothesis is a derivation of the preference-performance hypothesis, and states that females will oviposit on plants that provide optimal fitness potential to their offspring (Valladares and Lawton 1991; Vencel et al. 2013). Fitness is described here in terms of number, survival and growth of offspring (McGraw and Caswell 1996). However, in

some insect species, such as *Helicoverpa armigera* and *Pieris macdunnoughii*, females sometimes prefer to oviposit on plants that are substandard, or even toxic, to their offspring (Srinivasan et al. 2013; Nakajima et al. 2013). Why would mothers choose poor development sites for their offspring? The benefits of choosing an optimal larval host plant for oviposition are increased fecundity and higher levels of genes passed on to subsequent generations compared to ovipositing on suboptimal plants (Garcia-Robledo and Horvitz 2012). The cost of choosing suboptimal host plants, therefore, is a reduction in genetic material that is passed on. A meta-analysis of over twenty phytophagous insects that tested their oviposition preference and offspring fitness on both an optimal and suboptimal host plant revealed that in most cases, the mother knows best hypothesis was supported (Gripenberg et al. 2010). The benefits for mothers that choose suboptimal larval food plants include potentially increasing their own survival, if their choice helps them to avoid enemies, reduce search time or competition, or provide better access to their own nutritional resources, such as nectar plants (Scheirs et al. 2000; Gripenberg et al. 2010). Whiteflies (*Bemisia argentifolii*) sometimes reduce oviposition on plants with more predators, even when those plants are correlated with higher fecundity, indicating that enemy avoidance by adult females may supersede offspring fitness in plant choice (Lee et al. 2011). Similarly, intraspecific larval competition causes females to choose oviposition sites that reduce potentially competitive environments for their offspring (Buser et al. 2014). Increasing their own longevity may provide future opportunities for reproduction, thus their immediate survival may be more important than ovipositing on preferred plants (Scheirs et al. 2000). However, in some cases, even longer maternal survival does not explain host plant choice for oviposition on suboptimal plant species (Badenes-Perez et al. 2014).

Attraction via chemical cues, such as volatile organic compounds (VOCs) may drive females to oviposit on plants that are not optimal for offspring development (Honda et al. 1995; Forister et al. 2009). This reliance on VOCs is the driving force behind host plant recognition of most herbivorous insects (Visser 1986; Bruce and Pickett 2011). Specialist insects in particular, utilize chemical cues to discriminate among host plants (Janz and Nylin 1997; Späthe et al. 2013). Many specialist species have evolved mechanisms to detoxify or bypass chemicals in plants that are toxic to other insects (Futuyma and Agrawal 2009). The diamondback moth (DBM) (*Plutella xylostella*) is one such specialist that has been shown to neutralize defense chemicals, mainly glucosinolates, found in its host plants in the Brassicaceae family. Glucosinolates and myrosinase are separated in undamaged leaves, but are highly toxic to a

variety of insects, including DBM, when they interact due to leaf damage (Ratzka et al. 2002). Glucosinolate sulfatase produced in the gut of DBM breaks down glucosinolates before they mix with myrosinase (Yang et al. 2009; You et al. 2013). Glucosinolates remain strong feeding and oviposition stimulants for DBM, attracting them to many Brassicaceae plants, such as crops, ornamental landscape plantings, and weeds (both native and exotic) (Renwick et al. 2006; Zhang et al. 2012). In the past sixty years, the DBM has become resistant to every known type of insecticide, including *Bacillus thuringiensis* (*Bt*) and DDT (Pichon et al. 2006; Saeed et al. 2010; Sarfraz and Keddie 2005). This resistance has been linked to the ability of DBMs to digest glucosinolates, as the same or similar gut enzymes are involved in insecticide detoxification (Ratzka et al. 2002; Xia et al. 2013). Its rapid life cycle (between 18 and 51 days) also enables resistant populations to build quickly (Fathi et al. 2011).

DBM is limited by its preferred temperature range between 5 and 31°C and the availability of its host plants, many of which are cultivated as crops (such as canola, mustard and broccoli) (Pichon et al. 2006). In North America, populations of DBM exist year round in southern areas of the United States of America (USA) and Mexico and seasonally in more northern temperate habitats (Shelton et al. 1993). Increasing temperatures due to climate change, as well as increased plantings of crops suitable for DBM have led to more reports of DBM in agricultural settings, and recently in wild and nursery greenhouse environments (Marchioro and Foerster 2012; Cloyd 2013).

Understanding DBM oviposition preferences is essential if one wishes to develop management strategies to control this pest using certain host plants as trap crops or dead-end trap crops in agricultural settings (Lu et al. 2004; Musser et al. 2005). Trap crops can be planted alongside cash crops, attracting more insect pests and incurring more damage than cash crops. At a high level of infestation, trap crops are removed (along with the pests), leaving the cash crops less damaged (Holden et al. 2012; Landl and Glauning 2013). In the case of DBM, glucosinolates may be the main attractant to plants such as white mustard (*Sinapis alba*), but fitness levels may be low on some high-glucosinolate plants, thus DBM are sometimes trapped on low quality hosts (George et al. 2009). With the presence of saponins, some Brassicaceae host plants may become dead-end trap crops, as larvae cannot develop at all (Badenes-Perez et al. 2014). Saponins are chemicals that have been shown to deter feeding in DBM larvae, leading to death (Wei et al. 2013b). Wintercress (*Barbarea vulgaris*) has been shown to contain saponins, and has been used as a dead-end trap crop to control DBM (Badenes-Perez et al. 2014).

Many crop plants have been artificially selected to reduce the amount of chemicals (such as glucosinolates), often because of their unpalatability to humans (Gols et al. 2009; Blanckaert et al. 2012). Highly domesticated plants, such as canola, have been selected to contain fewer secondary metabolites and also show lower preference by insects such as DBM, compared to phylogenetically similar, but uncultivated plant species (Chavadej et al. 1994). Other Brassicaceae species, such as garden cress, have not likely been selected to have reduced secondary plant metabolites since they are used as herbs for medicinal purposes (Bhasin et al. 2012). Garden cress should be highly preferred by DBM; however no investigation has been done to show this. Although it may attract ovipositing females, garden cress also contains saponins. Shinoda et al. (2002) determined that saponins sprayed on cabbage resulted in 100% 1st instar larval death and reduced feeding in 3rd instar DBM larvae to 11% compared to the controls. Under these circumstances garden cress would not be a suitable plant for larval development, and would have trap-cropping potential, similar to wintercress. Variability in glucosinolate (and other chemical) concentrations during plant development, in different plant part tissues, and among plant species have led to inconsistent results regarding the usefulness of some of the Brassicaceae species as trap crops (Sarfraz et al. 2009; Badenes-Perez et al. 2014). In addition, many Brassicaceae plants that have not been fully researched could have potential for trap cropping (Charleston and Kfir 2000).

Preference by certain individuals of the same insect species for different plants or plant parts within the larger spectrum of suitable host plants can lead to further specialization of populations of insects, and is termed host-associated differentiation (HAD) (Guttman et al., 1981). This isolation of populations may lead to genetic differences through natural selection, even without geographic separation, potentially leading to the creation of ecotypes (Turrill 2006). For example, genetic analysis of four monophagous insect species on *Hesperoyucca whipplei* (Agavaceae), were shown to display different phylogeographic histories associated with the plant part on which they fed (Althoff et al. 2007). In these cases, we may expect that the same species coming from different regions may have different preferences and adapt and gradually evolve differently to trap crops due to their resistance and capacity to neutralize the various secondary metabolites of host plants.

The purpose of this study was to analyze the oviposition preference of different DBM populations when offered six Brassicaceae plants: broccoli (*Brassica oleracea* var. *Lieutenant*), garden cress (*Lepidium sativum*), wintercress (*Barbarea vulgaris*), black mustard (*Brassica*

nigra), ornamental cabbage (*B. oleracea* var. *Acephala*) and aubretia (*Aubretia deltoidea*). The mother-knows-best hypothesis was tested to determine whether DBM females prefer plants that have the potential to be optimal food sources for their offspring. It was hypothesized that, considering the current knowledge of the species preferences, DBM would prefer to oviposit on plants shown to be suboptimal or toxic to developing larvae, which would not be consistent with mother-knows-best. We predicted that DBM would prefer to oviposit eggs on wintercress and black mustard, as they are wild plants and therefore may have a greater amount of glucosinolates, as well as garden cress, as it is a cultivated Brassicaceae plant used for its chemical contents. We further predicted that broccoli would not be preferred as an oviposition host, since its lower glucosinolate contents have shown it to be less preferred than other Brassicaceae plants (Reddy et al. 2003). Finally, we hypothesized that populations of DBM from across Canada would show different levels of host plant preferences (although all still preferring plant species high in glucosinolates), indicating isolation among populations and possible geographic specificity or ecotype formation.

3.3 Materials and Methods

DBM Populations and Rearing

For this study, three populations of DBM were collected in the summer of 2013 from Niagara, Ontario, from a broccoli farm (termed Ontario Crop), a field with black mustard (Ontario Wild), and a plant nursery growing ornamental cabbage (Ontario Ornamental). Two other populations were collected from canola fields in both Saskatoon, Saskatchewan (Saskatchewan) and Lethbridge, Alberta (Alberta) through Agriculture and Agrifood Canada. Although the term “population” usually refers to a naturally occurring group of individuals from the same species, we will be using it here to refer to the colonies reared in the lab that were started from individuals from each collected origin (approximately 100 individuals per site).

Populations were reared in cages under laboratory conditions at 20 - 25°C, 50 - 70% relative humidity, 16:8 h light:dark cycle, according to Sarfraz et al. (2009). Larvae were fed only four to six week old radish plants (*Raphanus sativus*). Adults were fed with 10% honey solution on cotton balls. Insects were housed in rearing boxes with a 500 ml plastic bottle suspended above radish from the box lid. The plastic bottle had approximately 100 pin-holes facing the radish plants and was used to house pupae until they emerged as adults, mated, and their hatchlings eclosed from eggs laid on the interior bottle surface. Hatchlings descended on their own threads to the radish below to feed. Pupae were removed every few days and

transferred to the plastic bottle, with never more than 50 pupae per bottle. Radish plants were watered regularly and replaced as needed. At least three generations of moths were reared from the time of field collection until experimentation. For experimentation, pupae were placed into separate test tubes with cotton stoppers until eclosion. When sexed, male/female pairs of adults were placed into a different box containing a tray of test plants. A 10% honey solution-soaked cotton ball was hung from the lid equidistant above all plants and changed daily.

Plants

Broccoli, garden cress, black mustard, wintercress, ornamental kale and aubretia were grown in the greenhouse (25°C day/18°C night, 70% relative humidity, 16:8 h light:dark cycle) until 4-6 weeks old. All are common in Ontario and, as members of the Brassicaceae family, have the potential to support DBM populations. However, wintercress and garden cress are known to contain saponins, chemicals toxic to developing larvae, so further study of these species is of interest. Aubretia has not yet been assessed for its ability to attract DBM for oviposition, and limited information exists pertaining to ornamental kale and DBM. For the trials, an individual of each species was planted in a tray measuring 6 x 10 x 20 cm, such that each tray contained the six plant species. Species were planted in all different combinations to reduce error associated with specific combinations of plants.

Adult Oviposition Preference Trials

To test adult oviposition preference, one DBM female and one male from the same population were placed in a rearing box containing a tray of 4-6 week old plants, one each of broccoli, aubretia, garden cress, wintercress, ornamental kale and black mustard. Adult moths were two to four days old at the time of experimentation, and previously unmated. Each pair was left for four nights in the rearing box in lab conditions to allow enough time for mating and oviposition. Pre-trials established that after four nights the number of eggs significantly decreased. The upper and lower surfaces of the leaves from each plant were then examined by microscope to count the number of eggs. Trials were repeated until eight pairs of DBMs from each of the five populations had been tested.

Statistical Analyses

Generalized linear model analysis (GLZM) with log link was used to fit the number of eggs laid on each plant type during the experiment. GLZM was chosen as the model with best fit

as it does not assume independence of variables. In this case, the plant choices were not independent of another, as the nature of “choice” is dependent upon comparison with other choices. In fitting the GLZM, number of eggs laid was the dependent variable, and plant species and population were factors. The Likelihood ratio chi-square test was used to determine differences in preferences. Wald chi-square test was used to evaluate differences among least-squares means (as per Malagnini et al. 2013). Due to variability in the leaf sizes of each species, standardized leaf surface area was calculated for all plant material using a leaf area machine by dividing the number of eggs per leaf by the total leaf surface area available for each plant species. A chi-square test was used to determine whether there were differences in the number of eggs laid on upper and lower leaf surfaces. Differences in the number of eggs laid on upper and lower leaf surfaces were compared with paired t-tests. All tests were performed using IBM SPSS 20.

Rearing trials on wintercress and garden cress

To test whether full life cycles of the DBM could survive on wintercress and garden cress, twelve pupae were placed into a container with either wintercress or garden cress plants. Adults were seen to emerge and 1st instar larvae were observed dropping onto plants. Plants were checked daily for larvae and pupae and watered, for 45 days (since larvae would have been visible by this time). At that point, each leaf was removed and searched by microscope for signs of damage or the presence of larvae or pupae.

3.4 Results

The number of eggs laid on the different host species varied significantly (Wald $\chi^2=159.925$; $df=5$; $p<0.001$), but no difference was seen among populations (Wald $\chi^2=1.251$; $df=4$; $p=0.870$) (Table 3.1). Both eggs per plant and eggs per cm^2 were calculated and eggs per cm^2 data were used as these give a better representation of available surface area for oviposition (Table 3.2). Pairwise tests showed that DBM significantly oviposited more eggs on garden cress than any other plant species ($p<0.001$). After garden cress, they oviposited more eggs on wintercress than on aubretia ($p=0.021$), broccoli and ornamental kale ($p<0.05$), and more eggs on black mustard than broccoli and ornamental kale ($p=0.001$). Broccoli and ornamental kale were the least preferred, as determined by the number of eggs laid on these species, and not significantly different from one another ($p=0.975$) (Fig. 3.1).

When all plant species were taken together, significantly more eggs were laid on the upper (7.85 ± 8.44) than lower (6.92 ± 7.73) surface of the leaves ($\chi^2 = 277.17$; $n=240$; $p<0.001$) (Table 3.3). Although all plant species, except ornamental kale displayed this trend, significant differences were only found with aubretia ($t=2.29_{(39)}$; $p=0.027$) and black mustard ($t=2.18_{(39)}$; $p=0.036$) (Fig. 3.2).

In rearing trials of DBM from eggs on both wintercress and garden cress, no larvae were found on either plant species during or after 45 days. Dropping showed that 1st instar larvae hatched and descended onto plants via silken threads. Minimal damage to leaves was observed, confirming that larvae dropped onto leaves but did not survive, or moved away and died.

3.5 Discussion

Our results suggest that the mother-knows-best hypothesis should be rejected as female DBM preferred plants like garden cress and wintercress which contains the feeding deterrents, saponins, that kill young larvae (Valladeres and Lawton 1991; Hussein et al. 2011). In our case, DBM from all five populations tested preferred these species rather than other crop, ornamental or wild species.

Why did females oviposit most eggs on suboptimal plant choices? Previous research indicates that glucosinolates are oviposition stimulants for DBM (Renwick et al. 2006; Sun et al. 2009). Wintercress and garden cress are reported to contain high levels of glucosinolates compared to other Brassicaceae species (van Leur et al. 2008; Najeeb-ur-Rehman et al. 2011). It is likely that the adaptation of DBM to be attracted to their host plants through glucosinolates is usually a benefit to this species, as it is a specialist and must locate Brassicaceae plants in order to feed and oviposit. However, this adaptation may be detrimental to DBM when not only glucosinolates, but also saponin, are present in host plants, such as in garden cress and wintercress. In these cases, the overriding attraction to glucosinolates drives DBM females to oviposit on plants that are toxic to developing larvae (Cunningham 2011).

The choice of wintercress as one of the preferred oviposition host plants is consistent with other studies (Shinoda et al. 2002; Badenes-Perez et al. 2014). Shelton and Nault (2003) also found 5.5 times more eggs oviposited on wintercress than broccoli or cabbage. We can hypothesize that garden cress has a similar feeding deterrent consequence for emerging larvae, resulting in low or no survival on this plant. Follow-up experimentation in our lab confirmed that, although adult females oviposit on both garden cress and wintercress, these plants are

unable to support full life-cycles of DBM. Further field testing would determine the suitability of garden cress to be used as a dead-end trap crop, similar to wintercress, although the former species is generally smaller in growth and may only be useful in limited spaces.

Black mustard is high in glucosinolates but was less preferred than garden cress (Table 3.2) probably due to the presence of trichomes that deter DBM females (Traw and Dawson 2002; Mathur et al. 2011). Niu et al. (2014) found that 1st instar DBM larvae take longer to feed on *Orychophragmus violaceus*, a wild Brassicaceae, compared to several other wild hosts, due to the obstruction to movement caused by leaf hairs. Wild mustard plants (black mustard, white mustard and other related species) are prevalent weeds in many areas of North America, so increasing the host plant options in an area, even with choices that produce fewer offspring, can increase an ovipositing female's overall fitness (Johansson et al. 2005). Therefore, wild Brassicaceae such as black mustard may provide alternate host plants to DBM. This is especially important in temperate regions, such as Ontario, where the species is quite abundant and present when crops are not available. When crops are available, the preference for black mustard could reduce infestation on cash crops.

Cultivated plants such as broccoli, ornamental kale and aubretia were less attractive to ovipositing females than wild Brassicaceae and garden cress. The reduction in glucosinolates, through breeding selection, in cultivated crops such as broccoli and ornamental kale, may explain why these plants are not preferred when offered alongside plants with higher chemical concentrations (Gols et al. 2008; Blanckaert et al. 2012). While DBM has been shown here and in other experiments to prefer other types of Brassicaceae over broccoli when given the choice, it has also been established that DBM pupae grow larger on broccoli than other host plants (Niu 2014). In addition, in fields where only broccoli can be found, the species can still cause significant damage (Shelton 2003).

There is a growing concern that landscape ornamentals may become a refuge, leading to greater infestation of this insect (Kim et al. 2011; Latif et al. 2013; Vásquez and de Moreas 2013). To our knowledge, no data could be found on the interactions of DBM with aubretia as a host plant. However Cloyd (2013) reports damage to ornamental cabbage (similar to ornamental kale) due to the DBM and suggests parasitoids as biological control and removal of plant debris to reduce refugia opportunities. Both aubretia and ornamental kale were significantly less preferred than other plants tested for oviposition. These results indicate that they might not necessarily become substantial reservoirs if preferred options are available. However, due to the

prevalence of landscape nurseries in the Niagara Region, populations that do establish may be more successful than those in crop or wild settings. This may be due to limited parasitoids and competition from other herbivorous insects within greenhouse environments. In addition, overwintering in greenhouses may be possible due to the creation of microclimates. Cervantes et al. (2011) determine that, while outside populations of cabbage looper, *Trichoplusia ni* (Lepidoptera: Noctuidae) cannot survive through the winter, some can survive inside the greenhouse, even with heating turned off and the application of *Bacillus thuringiensis* in the spring. Pupae of *Spodoptera litura* also survive in an unheated greenhouse when temperatures outside are below the critical survival range in southernmost regions of the Boso Peninsula in Japan (Maatsura 1997).

It is important to note that in 2012, greenhouse area in the Niagara Region totaled 140.2 million sq. ft. (13.0 million sq. m.) (TOGA 2014), so herbivorous pests such as DBM pose a significant threat to this industry. While it is unclear what proportion of these greenhouses have Brassicaceae plants, even a small percentage means that the species can rapidly colonize nearby fields the next season. Even outside of greenhouse refugia, mild winters, as those resulting from climate change, may provide conditions for DBM to successfully overwinter in Ontario, and lead to increases in fecundity and population growth (Marchioro and Foerster 2012). Although Dosedall (1994) reports survival of DBM in canola fields in Alberta in 1992, subsequent field studies for six consecutive years (1993-1998) have shown no survival, indicating overwintering is a rare event in that province (Dosedall et al. 2001).

DBM populations in temperate habitats may therefore, at least seasonally (and possibly longer in microclimates), become isolated from source populations, and can have up to six generations per year. This isolation and breeding can lead to further specialization on available host plants. The green mirid (*Creontiades dilutes*) displays a similar dispersal cycle to that of DBM from source populations, infesting agricultural areas in Australia at certain points of the year, but also utilizing wild host plants, leading to populations that are isolated geographically and by host-plant preference (Hereward et al. 2013). An analysis of mitochondrial DNA showed that temporal differences in genetic structure were related to host-associated differentiation, and could lead to genetic bottlenecks and drift associated with reduced gene flow (Hereward et al. 2013). All DBM in Canada are thought to originate in the southern United States and disperse northward each spring. In China, some return migration occurs in fall, but little data have been collected to support this phenomenon in North American DBM (Hopkinson and Soroka 2010;

Dosdall et al. 2001). Migration and dispersal affect gene flow between populations, which has evolutionary consequences, such as adaptation ability to various host plants (Wei et al. 2013a). Further genetic testing of DBM populations in North America will provide information on how population isolation due to seasonal northward dispersal, population isolation, and host-plant preference and specificity affect gene flow.

The number of eggs laid by adult female DBMs on the upper leaf surfaces was higher than lower surfaces of all plant species, except for ornamental kale. Zago et al. (2010) have reported more eggs laid on the upper surfaces for cabbage leaves. More eggs were reported to be oviposited on upper leaf surfaces than lower, but only on outer plant leaves of Chinese cabbage (Talekar et al. 1994). Other species of Lepidoptera, such as *Anticarsia gemmatalis*, *Rachiplusia nu*, *Spodoptera latifascia* and *Chrysodmeixis includens* were all reported to prefer upper over lower leaf surfaces of soybean plants for oviposition (Pansera-de-Araujo et al. 1999). DBM may prefer upper leaf surfaces due to higher concentrations of glucosinolates present in these surfaces, as illustrated by Traw and Feeney (2008) in *Brassica nigra* and *B. kabra*. Alternatively or simultaneously, they could be assessing qualities such as leaf waxiness or glossiness, or trichome abundance (Justus et al. 2000; Traw and Dawson 2002). It is important to note that the methodology of this experiment involved leaving plants for four consecutive nights and then counting the eggs. However, research indicates that oviposition causes chemical changes in host plants that could influence oviposition choice and behaviour of insects (Wielgoss et al. 2012). Therefore subsequent experiments should replace plants daily to reduce this confounding effect.

DBM is a very adaptable pest that is difficult to control due to its rapid life cycle and ability to develop insecticide resistance (Sarfranz and Keddie 2005). In addition, the use of nonhost interplanting for control purposes has been ineffective for this pest (Finch and Kienegger 1997). Trap cropping with other Brassicaceae species may become one of the most effective means of control of DBM populations (George et al. 2009; Satpathy et al. 2010). Results generated here provide a better understanding about DBM oviposition preference on potential host plants such as garden cress, aubretia, and ornamental kale, which had not been examined before.

Lastly, the exploration of wild and ornamental hosts as alternate oviposition choices for DBM suggests that they are potential hosts for low to high levels of oviposition and may serve to support DBM when more preferred plants are not available. DBM may choose from a variety of crop, weed, and ornamental plants within a relatively small environment (Sarfranz et al. 2010a;

Zhang et al. 2012). Muhamed et al. (1994) discuss in detail the potential of wild Brassicaceae species to act as refugia for DBM in the spring, before crop hosts are planted. Their experimentation with wild and cultivated Brassicaceae species showed that, in general, DBM fitness was higher on cultivated Brassicaceae, however, they note that this does not diminish the refuge potential of wild host plants when crops are not available (Muhamed et al. 1994). Kahuthia-Gathu et al. (2008) also emphasize the role of wild Brassicaceae as DBM refugia, but also as refugia for several of their parasitoids, thus promoting biological control of DBMs. Other reports have also established the refugia status of wild host plants for insect species in the interim between access to more preferred plant hosts (Kalaitzaki et al. 2013; Villegas et al. 2013). Dispersing DBM are able to colonize seasonal habitats through the use of a variety of host plants. Further investigation of DBM preferences and fitness levels on additional Brassicaceae will provide a thorough understanding of how this agricultural pest is able to thrive in many places, and lead to control opportunities through the use of trap crops.

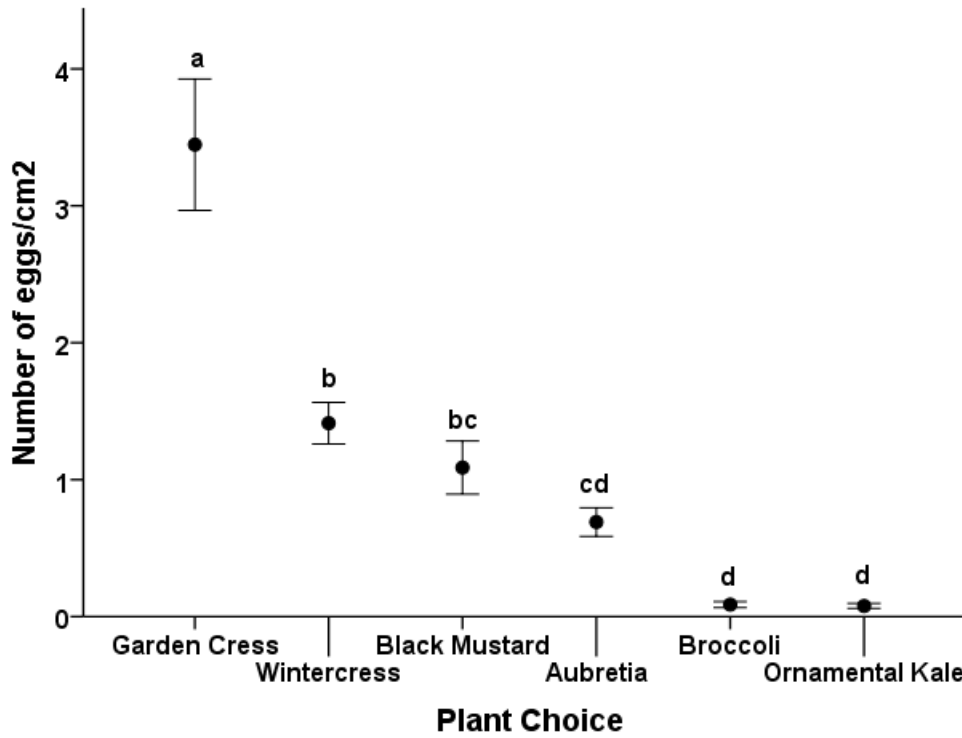


Figure 3.1 Mean number of eggs oviposited per cm² on various plant species by DBM females from five populations (Alberta, Saskatchewan, Ontario Crop, Ontario Wild and Ontario Ornamental) over four nights (n=240). Letters denote significant differences between plant species. Error bars represent one standard error above and below the mean.

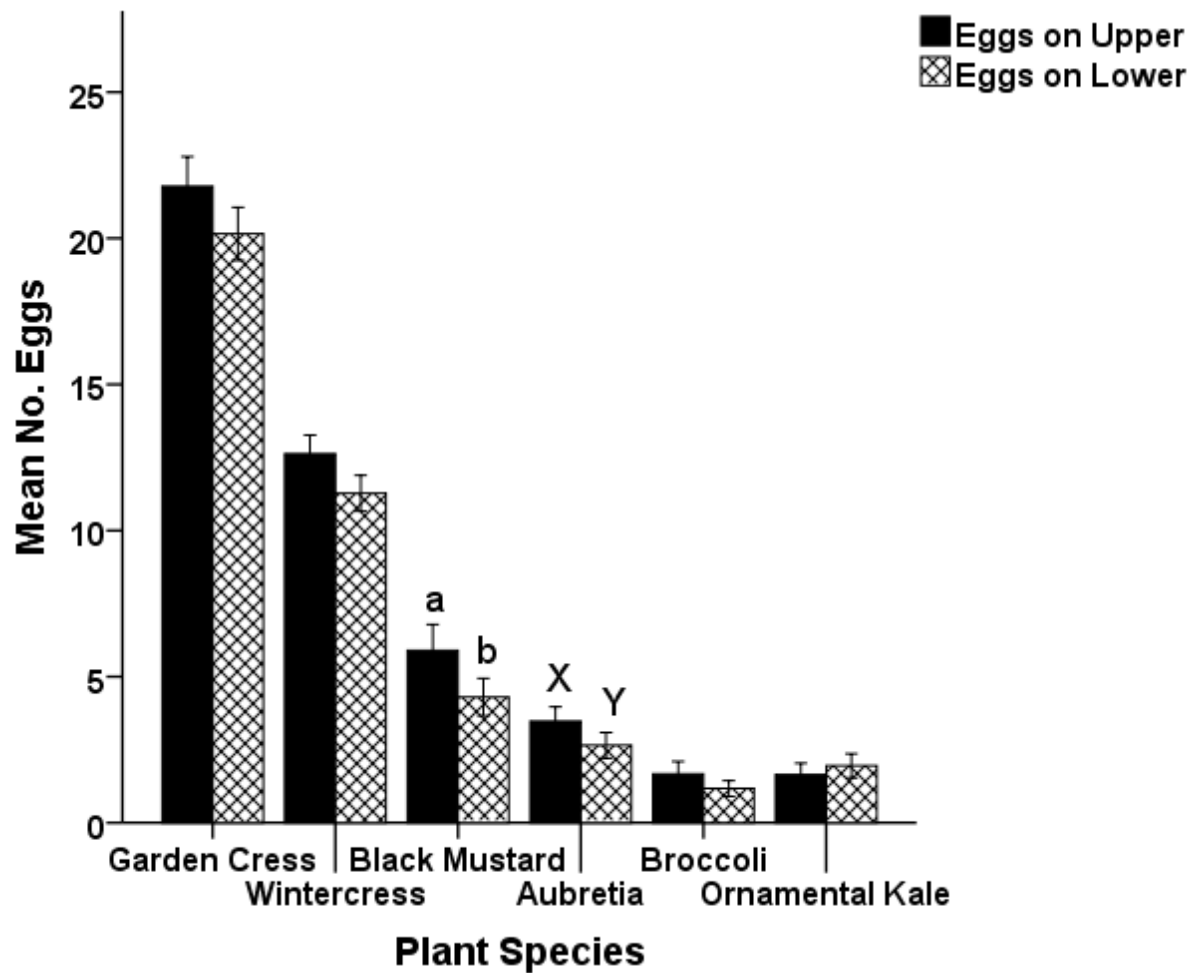


Figure 3.2 The mean numbers of DBM eggs oviposited on the upper and lower surfaces of each plant species (n=40) from all populations (Alberta, Saskatchewan, Ontario Crop, Ontario Wild and Ontario Ornamental) combined. Letters denote significant differences. Error bars represent one standard error above and below the mean.

Table 3.1 Generalized linear model (GLZM) summary statistics for the number of eggs laid on each of six different plant species (garden cress, wintercress, black mustard, aubretia, broccoli and ornamental kale) by DBM females from five populations (Alberta, Saskatchewan, Ontario Crop, Ontario Ornamental, Ontario Wild) combined. Significance levels were set to $\alpha < 0.05^*$, $< 0.01^{**}$, and $< 0.001^{***}$.

	Wald Chi-Square	df	p
Plant Choice	166.444	5	<0.001***
Population	1.302	4	0.861

Table 3.2 The average number of eggs (on upper and lower leaf surfaces together) oviposited by DBM adult females (n=40) on each plant species (garden cress, wintercress, black mustard, aubretia, broccoli and ornamental kale) for all populations combined (Alberta, Saskatchewan, Ontario Crop, Ontario Wild and Ontario Ornamental). Mean eggs per plant and mean eggs per cm² are shown.

Plant Species	Mean Eggs/Plant ± SE	Mean Eggs/cm² ± SE
Garden Cress	41.70 ± 1.51	3.45 ± 0.48
Wintercress	23.90 ± 1.02	1.41 ± 0.15
Black Mustard	10.20 ± 1.36	1.089 ± 0.19
Aubretia	6.12 ± 0.86	0.69 ± 0.10
Ornamental Kale	3.62 ± 0.77	0.08 ± 0.02
Broccoli	2.85 ± 0.61	0.09 ± 0.02

Table 3.3 Chi-square test summary statistics for DBM eggs laid on upper and lower surfaces of all plant species together (garden cress, wintercress, black mustard, aubretia, broccoli and ornamental kale) for all populations combined (Alberta, Saskatchewan, Ontario Crop, Ontario Wild and Ontario Ornamental). Significance levels were set to $\alpha < 0.05^*$, $< 0.01^{**}$, and $< 0.001^{***}$.

	Chi-Square	df	p
Eggs on Upper Leaf Surface	1342.557	32	$< 0.001^{***}$
Eggs on Lower Leaf Surface	1185.029	28	$< 0.001^{***}$

Chapter 4. Discussion and Conclusion

Diamondback moths display differential preferences for various Brassicaceae host plant species (De Bortoli et al. 2013; Sarfraz et al. 2010a). Understanding these preferences provides information on where DBM may be found at various time points. It also indicates how their abundance is controlled naturally due to nutritional and chemical components of their host plants. In agricultural settings, as well as along dispersal routes, DBM are exposed to a wide variety of wild, cultivated and ornamental Brassicaceae species (Muhammed et al. 1994; Zhang et al. 2012; Cloyd 2013). DBM fitness varies on these species, from lower developmental time leading to increases in population abundance on certain species to lower feeding rates causing the limitation or, in some cases, elimination, of insect populations (Saeed et al. 2010; Cunningham 2011; Sarfraz et al. 2011). The use of alternate host plants, even those that merely sustain low levels of DBM, is important as it shows that there are refugia for DBM in between crop availability and, perhaps, during mild Ontario winters and in greenhouses.

Phytophagous insects, particularly specialists, are limited by their host plants (Futuyma and Agrawal 2009; Leimu et al. 2012). This limitation is often a combined effect of plant nutritional composition, enemy avoidance potential, shelter from climatic elements, and the ability of the insect to bypass plant defense mechanisms (Thompson and Pellmyr 1991; Sarfraz et al. 2009; Steinbauer and Carroll 2011). Overcoming these barriers can give access to plant surfaces for feeding and oviposition. Several hypotheses have been suggested to explain how these factors can define a host plant's suitability for an insect. The plant stress hypothesis states that when under stress, plants produce fewer defense chemicals, leading to increased herbivory (White 1974). The plant vigour hypothesis postulates that stressed plants produce more vegetation, leading to increased herbivory (Price 1991; Sarfraz et al. 2009). The nutrient stress hypothesis states that herbivorous insects are constantly under pressure to acquire sufficient nutrients from their host plants and that, given choices, will prefer plants with the best nutrients for their current developmental needs (Joern and Behmer 1998; Frago and Bauce 2014). The mother-knows-best hypothesis states that adults oviposit on host plants that increase offspring fitness (Valladares and Lawton 1991; Vencel et al. 2013). Insect behaviour supporting this latter hypothesis would include adult oviposition on plant species that are good food sources for their offspring. If this is not the case, other factors such as enemy avoidance, competition, or access to adult resources may play important roles in influencing oviposition location (Scheirs et al. 2000; Lee et al. 2011; Buser et al. 2014). It has also been suggested that insects are constrained by their adaptive specialization on certain host plant species and that this drives them to choose

plants that may not be the best developmental choices (Cunningham 2011; Badenes-Perez et al. 2014).

DBM is seasonal in Canada, dispersing from southern USA and Mexico populations, but relatively little data have been collected as to source populations for various regions in Canada or the potential for isolation within microclimates (De Bortoli et al. 2013; Sarfraz et al. 2010a). If source origins and dispersal routes of Canadian DBM differ by province, or if isolation within provinces is maintained, then localized environmental factors could create natural selection pressures resulting in different ecotypes (Ruiz-Montoya and Núñez-Farfán 2013). Reduced gene flow among populations can also result in the eventual evolution of specific traits and gradually speciation (Mullen and Shaw 2014). This has been seen in Australia where a new species of DBM was recently discovered (Landry and Hebert 2013). Therefore, an investigation of host plant preference differences in populations, along with life history trait differences, such as larval body weight, length of instar periods and pupal weight, could reveal underlying phenotypic differentiation.

4.1 Host Plant Preference

Interestingly, garden cress and wintercress were shown to be preferred by most DBM in this study. These results are of particular interest because at least one genotype of wintercress has been documented to be unsuitable for DBM larval growth due to the chemical concentrations of saponin (Shelton and Nault 2003; Badenes-Perez et al. 2014). This study found that wintercress could support both 3rd and 4th instar larvae to the adult stage. Results showed that 1st instar larvae were seen to drop onto leaves from eggs, however, no 1st and 2nd instar larvae were observed to survive over time. This indicates that larvae may not be able to digest wintercress or garden cress in earlier life stages. The increased ability of larvae to detoxify chemicals as they age is supported by Vallau et al. (2013) in their study of the common heath moth (*Ematurga atomaria*) wherein it was discovered that younger larvae preferred and had higher fitness outcomes on host plants with fewer chemicals (*Calluna vulgaris*) compared to one with more chemical defenses (*Vaccinium myrtillus*), while at more advanced larval stages their preferences and fitness levels were reversed on these two host plants.

This information is important, as wintercress has been used as a dead-end trap crop alongside cash crops (Lu et al. 2004; Badenes-Perez et al. 2014). If it is true that later life stages can survive on wintercress, then there is the possibility that mobile larvae could move from cash

crops to wintercress and survive to adulthood, lay eggs on cash crops and continue to show population growth instead of control. The possibility of this larval movement was illustrated by placing DBM eggs on non-host tomato plants (*Lycopersicon esculentum* (Mill.)) in juxtaposition to cauliflower (*Brassica oleracea* var. *Botrytis*). Eight days later, 15% of neonatal larvae were observed on cauliflower, indicating the ability of neonatal DBM to move up to one meter to locate appropriate host plants (George et al. 2011). Thus, DBM larvae can move between plants to some extent. It is therefore important for field managers to understand the potential for some wintercress plants, even genotypes with saponin, to support DBM population growth. Garden cress is also reported to contain saponins (Hussein et al. 2011), which also appeared to act as feeding deterrents to DBM larvae. However, similar to wintercress, our experiments also showed growth and development of 3rd and 4th instar larvae on garden cress, but no viable younger larvae were observed from the eggs on garden cress. If these plants are located close to cash crops they may result in population control when eggs are laid on leaves, but also as reservoirs if mobile 3rd and 4th instars move to them from cash crops to feed and return to crops to lay eggs in adulthood.

Black mustard is also preferred by DBM more than broccoli or ornamental kale, a fact of importance as this weed is abundant in Ontario, and often found near agricultural crops. Furthermore, the feeding ability of larvae over one hour on black mustard was low. Previous literature reports that black mustard is high in glucosinolates responsible for attracting DBM, but that the mechanical defenses of black mustard, specifically trichomes in younger leaves, may reduce survival and growth of herbivores (Traw and Dawson 2002). Thus, black mustard may be an effective trap crop that attracts DBM adults, but reduces growth and survival of larval offspring. The prevalence of black mustard in wild settings around crops may therefore be a beneficial occurrence during growing seasons when pests may move from crops to more desirable black mustard where their numbers could decline. Many wild Brassicaceae have the potential, however, to serve as intermediary hosts in times when Brassicaceae crops are not present (Muhamad et al. 1994). Sarfraz et al. (2011) called these wild Brassicaceae species “bridge hosts” as they maintain populations of DBM in between crop availability.

Another study on DBM choice between wild mustard (*Sinapsis alba*) and cauliflower resulted in more eggs being oviposited on the wild mustard than cauliflower (George et al. 2009). In a comparison of two cultivated (*Brassica oleracea* var. *Capitata* and *B. oleracea* var. *Acephala*) and four wild (*Erucastrum arabicum*, *Raphanus raphanistrum*, *Rorippa nudiuscula* and *Rorippa micrantha*) Brassicaceae in Kenya, Africa, results show that *R. micrantha* is the most preferred and *B. oleracea* var. *Capitata* and var. *Acephala* were the least preferred. Highest

fecundity was seen on wild *R. nudiuscula* (Kahuthia-Gathu et al. 2008). Niu et al. (2014) confirmed that eight wild Brassicaceae species were able to support DBM populations and could contribute to maintaining DBM numbers when cultivated crops were not in season.

For DBM, ornamental Brassicaceae plants may provide additional host opportunities near agricultural fields and in greenhouses (Sarfraz et al. 2011; Zhang et al. 2012). Other studies have indicated that ornamentals are a major source of exotic introductions, both in and of themselves, and when they inadvertently transfer insects with them during delivery to other nurseries or planting sites (Smith et al. 2007). In this context, landscape ornamental plants such as aubretia and ornamental kale, may also serve as bridge hosts when grown near Brassicaceae crops. Aubretia has not, to our knowledge, been tested before in relation to its suitability as a host to DBM. As a landscape ornamental, aubretia is planted regularly in Ontario and elsewhere in the world (Pilon 2009). Although ornamental kale was only preferred by DBM as much as broccoli and less than the other plants simultaneously offered, it is important to note that high larval infestation of ornamental cabbage and kale were seen in site surveys of nurseries in Niagara (Newman, unpublished). Cloyd (2013) also described extensive damage within plant nurseries due to DBM. It is therefore important for nurseries to check ornamental and vegetable Brassicaceae plants often and to utilize trap crop and parasitoid options if possible to control DBM populations (Cloyd 2013).

The mother-knows-best theory of host plant preference was not illustrated here, as adult female DBM chose to oviposit more eggs on garden cress, wintercress, and black mustard instead of species conducive to larval survival and higher fitness, such as broccoli, ornamental kale and aubretia. Similar observations have been made in other insect species, such as the Mediterranean pine engraver (*Orthotomicus erosus*), that finds some host conifers acceptable for oviposition, but are unsuitable for larval growth and development (Walter et al. 2010). *Helicoverpa armigera* females also prefer to oviposit on a host plant species (*Solanum viarum*) that are unsuitable for offspring development (Srinivasan et al. 2013). It is possible that olfactory constraints are driving females to choose plant species that are high in glucosinolates, despite the fact that those same plants also contain saponins. With regards to larvae, the majority also chose garden cress. In this case, too, it seems that adaptation to glucosinolate signaling and identification cues from host plants drive preference in DBM, despite the resulting fitness consequences (Cunningham 2011).

4.2 Population Differences

We hypothesized that preference differences among populations from different provinces would show geographic differentiation. If so, these different preferences could be linked to population isolation, producing ecotypes, a term first coined by Turesson in 1922, to describe phenotype differentiation (Turrill 2006). Alternatively, but not mutually exclusive to geographic differentiation, we hypothesized that preference and life history differences among populations from different host plant origins (crop, ornamental, wild) could demonstrate host adaptation differentiation (HAD), likely caused by sympatric (occurring in the same geographic region) adaptation events. In the case that no differences could be found among the populations, our hypothesis was that high gene flow with southern USA and Mexico source populations was occurring. Our results indicate no differences, either geographically or related to host plant type among the populations tested. Ruiz-Montoya and Núñez-Farfán (2013) suggest that no difference among populations regarding host plant preference may indicate high gene flow among populations or high phenotypic plasticity.

As a dispersing specialist herbivore, the DBM is subjected to various adaptive forces, such as isolation of populations, geographical and climatic characteristics, and host plant defense chemical variability. Although our tests were inconclusive, further testing will help to identify populations of DBM that are becoming isolated from source populations. Increasing isolation of DBM populations in Canada from their source populations further south, in particular due to overwintering in greenhouses or microclimates or climate change, and in refugia created by ornamental and wild Brassicaceae, may create selection pressures which cause further specialization on specific host plants.

4.3 Instar Differences

Larval food preference trials showed no significant differences in host plant preference depending on larval instar. Both 3rd and 4th instars preferred garden cress followed by wintercress, followed by black mustard, with broccoli, ornamental kale and aubretia less preferred. This indicates that the attraction to plants is similar regardless of latter larval life stage. Furthermore, adult trials with the same larval experiment plant species, showed the same trend in species choices, indicating that larval and adult DBM have similar host plant preferences.

There were significant differences between 3rd and 4th instar larvae in weight gain over one hour on various plants. Third instars had higher weight gain on more plants compared to 4th instars. This result indicates that 3rd instars are more voracious eaters while fourth instars may

be less inclined to eat, perhaps because they are preparing to pupate (Mason et al. 2003; Shintani et al. 2003). This result is different than those found for other Lepidopteran species, where the 4th instar is often the most voracious (Scriber and Slansky 1981). Results may have been biased if the choice of individuals tended to be in latter 4th instar, close to pupation, as opposed to earlier in the stage when they were more voracious. Further testing should strive to include individuals from both 3rd and 4th instars. Although the trend was for lower growth rates on black mustard in 3rd instars, the relative growth rate difference between 3rd and 4th instars was not significant on black mustard. In addition, growth rates were lowest on black mustard than any other plant. This indicates that black mustard is deterring feeding by both instars and especially 3rd instars, perhaps due to the surface trichomes found on this species (Traw and Dawson 2002; Mathur et al. 2011).

4.4 Conclusion and Future Research Directions

In this study, food and oviposition preferences of five populations of DBM were analyzed. When given six plant choices simultaneously, third and fourth instar larvae and adult moths preferred relatively the same species (i.e. garden cress and wintercress). When considering the ovipositioning theories, DBM preference illustrated here supports the concept that preference is driven by olfactory constraint due to the adaptation of DBM for attraction to glucosinolates. While DBM have the ability to digest glucosinolates, high concentrations of these chemicals in host plants also reduce larval and pupal weights and increase developmental times (Li et al. 2000). It is likely that DBM preference is linked to an adaptive response to glucosinolate cues, regardless of the underlying nutritional value of their food source or of the suitability for larval offspring. This effect has been noted in other cases as an explanation of preference to an unsuitable host plant (Cunningham 2011, Badenes-Perez et al. 2014). The high level of preference of DBM for garden cress, however, may provide an opportunity to use this plant species as a dead-end trap crop to control this insect pest species.

Ultimately this study identifies a newly examined host plant, garden cress, which is highly attractive to DBM. It also identifies aubretia as an acceptable host plant and confirms the potential of ornamental kale and black mustard to act as bridge hosts when crops, such as broccoli, are not available. Finally, this study suggests that garden cress, as well as wintercress, may be lethal to early DBM life stages, but 3rd and 4th instars are capable of eating and developing on these plant species. Further research on the genotypes and saponin content of

these species as they relate to all life stages of DBM would clarify the ability of these plants to act as dead-end trap crops.

Additional research should continue to examine the prevalence of DBM populations in Canada during all months of the year, and in various refugia, such as greenhouses and microclimates. Further establishment of food and oviposition preferences of DBM in various life stages and resulting life history traits will provide a thorough understanding of the population dynamics of DBM in Canada. This research will contribute to life history data on host plant preferences and performance of DBM on various crop, ornamental and wild Brassicaceae. It may also contribute to management practices to control DBM in agricultural environments.

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