Crucifer Host Plant Suitability for Bertha Armyworm (*Mamestra configurata*) and Diamondback Moth (*Plutella xylostella*)

A Thesis Submitted to the College of

Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy

in the Department of Biology

University of Saskatchewan

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Spring 2002

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Abstract

Crucifer host-plant suitability for bertha armyworm, *Mamestra configurata* Walker, and diamondback moth, *Plutella xylostella* L., was examined using cultivars and breeding lines of *Brassica napus* L., *B. juncea* L., *B. rapa* L., *B. carinata* L. and *Sinapis alba* L. Larval growth, development and survival, as well as feeding and oviposition preferences, were examined on physically and chemically distinct cruciferous plants using choice and no-choice experiments.

In choice and no-choice experiments with entire plants, single leaves and leaf discs, the *B. juncea* lines (AC Vulcan and H-Allyl) and the *S. alba* lines (AC Pennant and L-GS) were the poorest host plants in terms of bertha armyworm larval weight gain, development and feeding preference. The results indicate that specific foliar glucosinolates such as sinigrin, which is predominant in *B. juncea*, and sinalbin, which is abundant in *S. alba*, may provide crucifer crops with some protection from bertha armyworm feeding.

Bertha armyworm oviposition preferences were examined on representative cultivars from *B. juncea*, *B. carinata, B. napus* and *S. alba*. *S. alba* AC Pennant received the greatest number of eggs despite being relatively resistant to larval feeding. Oviposition was substantially greater on *B. napus* than on *B. juncea* in all choice and no-choice experiments. Full-flower *B. napus* plants were significantly preferred for oviposition over plants in pre-flower or pod stages. Bertha armyworm laid most eggs in the upper portion of the crop canopy on the underside of leaves.

The effect of conspecific eggs on bertha armyworm oviposition site selection was also examined. In choice experiments, females showed a strong preference for plant material with conspecific eggs over plants without eggs. Females showed a much stronger preference for plants with eggs of a different female than for plants with their own eggs. Gravid females also showed a preference for leaves which had been treated with a methanol egg wash over control leaves, indicating that the source of attraction may be chemical.

There were no consistent differences in diamondback moth larval growth, development, survival or fourth-instar feeding preference among the crucifer lines tested. However, there were differences in oviposition and first-instar feeding preferences on 'Glossy' and 'Waxy' lines of *B. rapa*. Although females laid more eggs on 'Glossy' plants, there was a strong preference among first-instar larvae for 'Waxy' plants in a choice situation. The results indicate that *B. rapa* expressing the glossy leaf wax characteristic showed some resistance to diamondback moth, similar to that observed previously with glossy *B. oleracea*. The resistance appears to have a behavioural basis and is expressed against early-instar larvae.

Acknowledgments

My time as a graduate student has been enjoyable and expansive. The success of my project and the great time I had while I was here is a product of the amazing people I have been surrounded by.

Foremost among these are my supervisors, Martin Erlandson and Cedric Gillott. The graduate experience is governed to a great extent by one's supervisors and mine has been a fantastic one. Thank you. Scientifically, you challenged and 'edited' my work, giving me direction but allowing me the freedom to explore. Personally, you conveyed the value of dedication, patience, and a sense of humor. You are terrific scientists and more importantly exceptional people, I feel very fortunate to have had the opportunity to work with and get to know you both.

My sincere appreciation is extended to Alison Paton and Keith Moore. Alison, without your efforts and the bertha armyworm colony much of my work would not have been possible. Keith, your insight and experience spared me a lot of time, energy and anguish. Assistance with insects, plants and everyday details could always be depended on, as could amusing conversation.

My thanks to the members of my advisory committee, Owen Olfert, Gerhard Rakow and Peta Bonham-Smith, for your time and constructive involvement in my project.

I thank the Department of Biology at the University of Saskatchewan and the Canada-Saskatchewan Agri-Food Innovation Fund for providing financial assistance.

I also extend my appreciation to countless people at AAFC and the Department of Biology who helped me every day. My project would not have been started or completed without your advice and assistance. More importantly your smiles, your witty banter and our friendships made my time here meaningful and pleasurable. It is said that something is only work if you don't enjoy it and thanks to all of you I didn't have to work a day while I was here.

Thank you Colleen, Ivor, Kato, Linus and all my friends who made sure I maintained a 'healthy' balance of work and play. Who knows where I might have ended up without you!

Finally I thank my family for the years of unconditional support and patience. To my parents, Jim and Marilyn, I thank you for instilling in me the confidence that anything can be accomplished. Through your example, you have evoked my curiosity and commitment. You have encouraged and inspired me from the beginning and any accomplishments in my life I owe to you. To my sisters, Marcie and Alison, thank you for believing in me and being there for me always. To my grandfather, Art, thank you for being the greatest teacher I ever had.

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1 Introduction

1.1 The Problem

Bertha armyworm (*Mamestra configurata*) and diamondback moth (*Plutella xylostella*) are the two most significant lepidopteran pests of oilseed production on the Canadian prairies. In outbreak years these insects cost producers tens of millions of dollars in crop losses and chemical controls (Mason et al., 1998; Anonymous, 1995a). At present, insecticides are the only reliable and effective means of controlling these insects in the field. However, the exclusive dependence on broad spectrum chemical insecticides is becoming less acceptable due to a range of environmental and human health concerns. Although pesticide resistance among bertha armyworm has not yet become a concern, there is ongoing development of resistance to many insecticides among diamondback moth populations around the world (Shelton et al., 1993). Thus, there are major incentives to develop pest management strategies that are more sustainable and environmentally benign.

1.2 Integrated Pest Management

The modern concept of integrated pest management (IPM) was formulated in the 1970's, mainly in response to concerns about the environmental impacts of pesticides (Altieri, 1995). IPM can be defined as a pest management strategy where, in the socioeconomic context of farming systems, the associated environmental and the population dynamics of the pest species utilize all suitable and compatible techniques and

methods to maintain a pest population below those causing economic injury (Dent, 1995). IPM was envisioned to provide an alternative to the strategy of unilateral intervention with chemicals. This new pest control strategy was to rely on a deeper understanding of insect and crop ecology and on the use of several complementary tactics to control crop pests. IPM stresses pest management rather than eradication. IPM is oriented towards preventing the outbreak of pests and improving the stability of crop systems as opposed to dealing with pest problems after they have occurred.

The various components of an IPM program can be placed in five major groups:

1. Scouting and Thresholds

Although not an actual means of pest control, the establishment of economic and action thresholds and methods of measuring them is extremely important to IPM. The economic threshold is defined as the point at which the economic loss due to pest damage is equal to the cost of controlling that pest (Dent, 1991). The action threshold is the specific amount of insects or damage that can be tolerated before the economic threshold is reached (Dent, 1991). IPM systems should be geared towards balancing beneficial and pest populations based on the economic threshold. Perhaps the simplest, most important and cheapest way for producers to reduce pesticide use is to avoid unnecessary applications. To do this, the levels of pest infestation that require chemical control must be established. Producers then must be able to assess their crops and determine when the thresholds are reached. Thus, producers must scout their fields consistently and use measurement techniques specific for each species.

2. Biological Control

Biological control is the control of agronomic pests with the use of natural enemies, notably parasitoids, predators and pathogens. This may be achieved through five strategies, introduction, augmentation, inoculation, inundation and natural enemy conservation depending on the characteristic of the biological control agent under consideration (Dent, 1995).

Biological control holds great promise in IPM programs, and in some agricultural systems such as orchards, vineyards and greenhouses, it has been a successful, environmentally and economically sound alternative to chemical pest control (Lenteren et al., 1997). However, in many large-scale agricultural operations biological control has yet to play a major role.

3. Cultural Control

Cultural control of pests is achieved by altering the environment in a way that leaves it less desirable to the pests or more favourable to their natural enemies (Dent, 1991). This can be accomplished in a multitude of ways including crop rotation, tillage, alteration of planting date, use of trap crops, weed-management strategies, interplanting crops, fertilization and irrigation, all of which influence insect diversity.

One of the most influential cultural practices is the cropping system. The cropping system in place for most major North American crops is monoculture in which large tracts of land are planted to one cultivar of one crop. This system is not conducive to IPM; monoculture provides concentrated resources and uniform physical conditions which often meet the entire life-cycle requirements of specialized insect pests allowing populations to explode to devastating levels (Altieri, 1995). Monoculture not only provides ideal

conditions for pests but the lack of diversity also inhibits the establishment of their natural enemies.

4. Pesticides

For the past few decades, pesticides have been and still are the single most important and effective method of pest control in North American agriculture. Pesticides have provided producers with convenient, simple, flexible, effective and relatively inexpensive control of agricultural pests (Dent, 1995). However, misuse as well as over-reliance and ecological concerns have led to the questioning of chemical control methods. Overuse of insecticides has been ecologically unsound, leading to insect pest resistance, secondary pest outbreaks, adverse effects on non-target organisms, direct hazards to the user and contaminating pesticide residues.

Although the goal of IPM systems is to minimize chemical pest control, it is very unlikely that the use of pesticides will be eliminated. Even in well developed IPM systems, pesticide use, although reduced, still plays a very important role.

5. Plant-based Resistance

Plant resistance has much potential in IPM systems and provides an advantage over some of the other IPM components. Plant resistance is generally very inexpensive for the producer; ideally, insect control is achieved for the cost of the seed alone. Plant resistance to insects is also compatible with insecticide use, while methods such as biological controls in many cases are not. Plant resistance to insects is not density-dependent, whereas biological controls often are. It has also been shown that plant-based insect resistance yields higher returns per dollar invested than those spent on insecticide development

(Smith, 1989).

Plant resistance can be defined as the collective heritable plant qualities which allow a plant species, race, or individual to reduce the possibility of successful utilization of that plant as a host by an insect (Dent, 1991). Plant resistance to insects is relative and in practice a resistant crop cultivar is one which yields more than a susceptible cultivar when exposed to similar insect populations.

The mechanisms of plant resistance can be divided into three categories, antixenosis (non-preference), antibiosis, and tolerance (Painter, 1951). In antixenosis, plant characters and insect responses deter the insect from using the plant for oviposition, food, and/or shelter. With this type of resistance, certain chemical or physical plant characteristics are detectable by and deterrent to the insect relative to a susceptible plant. Antibiosis mechanisms affect the physiology of the insect and result in adverse effects on the insect life history when a resistant plant is used as a host. These adverse effects could be in the form of increased mortality, reduced longevity, and/or reduced reproduction for the insect pest compared to those noted on a susceptible host. Tolerance involves the plant response to an insect pest. Tolerance is the ability of the plant to withstand and/or recover from damage caused by an insect population equal to that damaging a non-resistant plant. Plant resistance occurs in varying degrees and although resistance may not offer complete protection from an insect pest, in many instances any amount of resistance will raise the action threshold to some degree and benefit an IPM program.

Although there are examples of plant-based resistance to insects being utilized in crop production for over 200 years in North America (Wiseman, 1985), plant-based insect

resistance has not played a large role in crop selection and development. Many crop plants have been selected and bred based on characteristics such as yield, time to maturity and taste rather than insect resistance. In order to achieve these desired traits, intentional reduction of factors that were coincidently involved in pest resistance often occurred (Smith, 1989). For example, a selection process based on yield may favour plants which focus their resources in this area rather than those which emphasize metabolically expensive defences. Many plant defences which evolved to deter insects and other herbivores are also unfavourable to the humans and livestock for which the plants are designed and thus are selected against in breeding programs. The trend of selecting crops based on factors other than insect resistance was magnified by the cheap and effective use of insecticides.

Although it is unlikely that complete IPM systems will be adopted on a large scale for most major North American crops, IPM is a continuum rather than an all-or-none process and IPM programs of varying degrees will have an important role in North American agriculture. Insect-resistant crops are a component of IPM that could have a large impact on pesticide use, even in the absence of a fully developed IPM system.

1.3 Bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae)

1.3.1 Biology

Adult bertha armyworm emerge from early June to early August (Turnock, 1984a). The moth is predominantly gray with flecked patches of black, brown, olive and white and has a wing span of approximately 4 cm (Anonymous, 1995b) (Figure 1.1d). Adults are

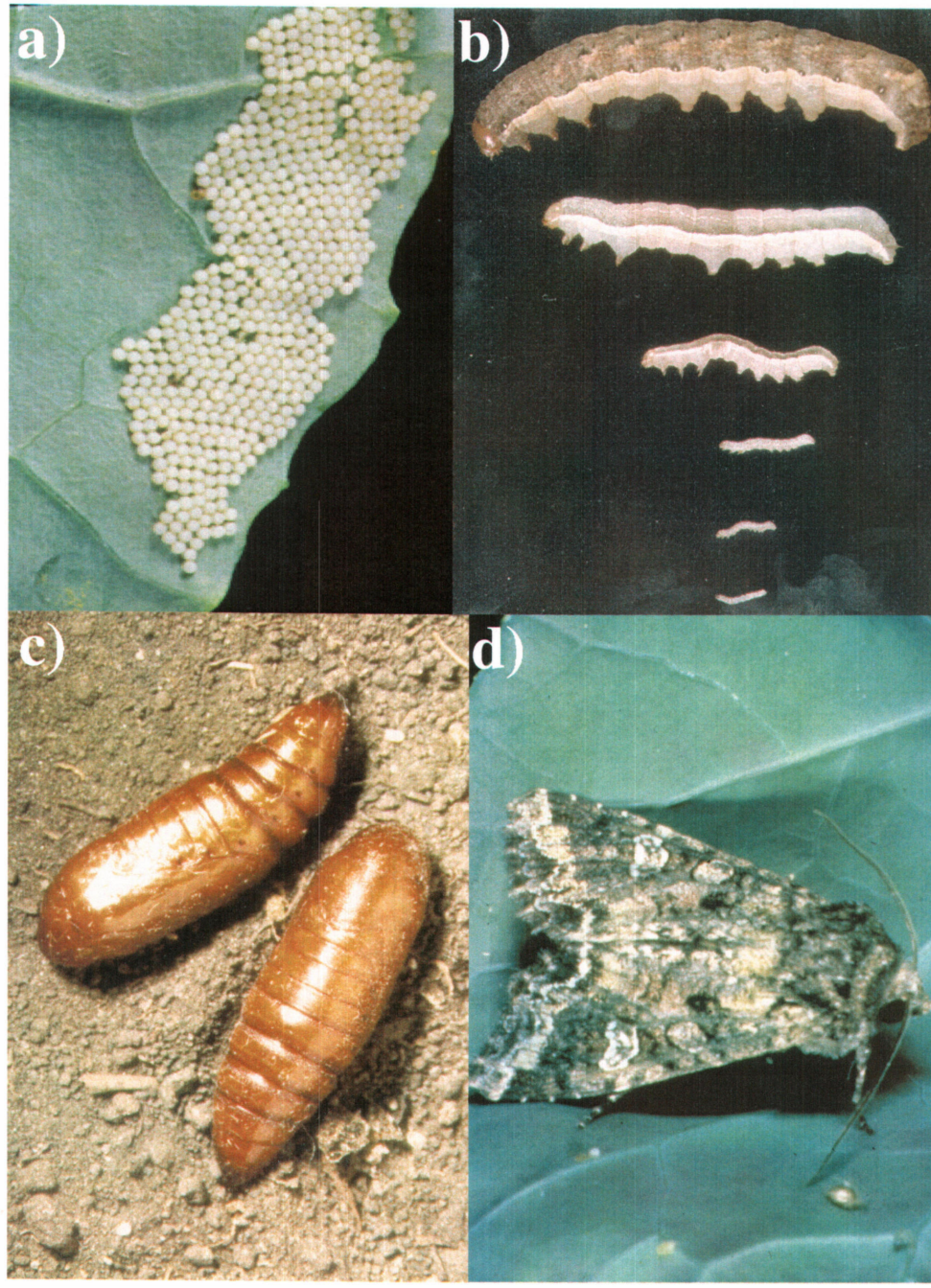

Figure 1.1. Stages in the life cycle of bertha armyworm: a) eggs, b) larval instars, c) pupae, d) adult.

active at night when egg laying, calling and mating take place. Females begin calling during the second or third scotophase and mate for the first time during the second to fifth scotophase, they lay their first eggs the same night and lay every night following until death (Howlader and Gerber, 1986). Each female moth lays approximately 2200 eggs over about 10 nights. Adults feed on nectar and may be attracted to flowering crops (Turnock, 1984b).

Eggs are laid in single-layer masses of approximately 125 eggs on the lower surface of host plant leaves. The eggs are less than 1 mm across and ridged. Eggs are white when laid and become darker as they develop (Figure 1.1a). Eggs hatch 4-10 days after they are laid, depending on the temperature.

Newly hatched larvae are pale in color with a black head capsule and are about 3 mm long (Figure 1.1b). Second- to fifth-instar larvae are green with a brown head capsule. Sixth-instar larvae are about 4 cm long and usually become brown or velvety black with a yellow stripe down each side. When disturbed, early-instar larvae may drop off the leaves by a silken thread. Larger larvae will drop off the plant and curl up, a defensive behavior typical of armyworms (Anonymous, 1995b). Early-instar larvae feed on the leaf surface, usually the underside, and produce 'shot holes' through the leaves. As the larvae grow, they become edge feeders and later instars are capable of feeding on all parts of the host plant. Larvae take about 6 weeks to complete development, depending on the temperature, after which they burrow 5 to 16 cm into the soil to pupate.

Pupation usually begins in mid August and all larvae will have pupated by mid-September. The reddish-brown pupae are 0.5 to 1.8 cm long and tapered with flexible, terminal abdominal segments (Figure 1.1c). They are identical to other cutworm pupae

(Anonymous, 1995b). Bertha armyworm survive the winter as pupae. However, if unusually warm conditions occur in the fall, some adults will emerge and be winterkilled.

1.3.2 Distribution, Host Plants and Damage

The bertha armyworm is native to North America and is found from British Columbia eastward across the Canadian prairies and as far south as Mexico along the Rocky Mountains. Although southern populations of the species are bivoltine, bertha armyworm populations on the Canadian prairies complete only one life cycle per year (Turnock, 1984a).

Bertha armyworm larvae are generalist feeders. Larvae will feed on most dicotyledonous plants growing in vegetable gardens and cultivated fields and have also been reported on some monocotyledons (King, 1928). Crucifers, which include crops such as canola (*Brassica napus, B. rapa*), rapeseed (*B. napus, B. rapa*), mustard (*B. juncea*) and cabbage (*B. oleracea*), are among the preferred hosts of bertha armyworm but larvae are also found on many other cultivated and weed species (Anonymous, 1995b).

Crop damage is a result of larval feeding. The level of damage depends on the host plant and its growth stage, as well as the number and growth stage of larvae present.

1.3.3 Outbreak History in Saskatchewan

Bertha armyworm has been recorded as a pest on the Canadian prairies since the early 1920's (King, 1928). The first outbreak of economic significance occurred in 1922 when flax (*Linum* spp.) crops in the Moose Jaw area were heavily damaged. Economically

significant outbreaks occurred again in 1925, 1927-28-29, and 1939-40 when crops of flax and garden vegetables were extensively damaged (King, 1928; Mason et al., 1998). Another outbreak in 1943-44 caused considerable damage but was also significant in that it was the first time bertha armyworm damage was recorded on rapeseed (Mason et al., 1998). Minor outbreaks occurred in the 1950's and 1960's mainly in Alberta. In 1971-72 a severe outbreak occurred across Alberta, Saskatchewan and Manitoba which affected over 75% of the rapeseed acreage, requiring insecticide control on up to 250 000 hectares (Mason et al., 1998). There were sporadic outbreaks in the late 1970's and the 1980's across the prairies. The most economically significant outbreak to date occurred in 1994-95-96 when canola and flax crops were devastated in Saskatchewan and Manitoba. During this outbreak, the cost of chemical control for prairie producers was as high as $52 million annually, and damage to crops resulting in yield losses cost producers up to $60 million annually (WCCP, 1995).

1.3.4 Management of Bertha Armyworm

Environmental factors are important in determining population densities of bertha armyworm. Neonate larvae are vulnerable to inclement weather, and cold winters with light snow cover can cause significant mortality of the overwintering pupae (Anonymous, 1995b). Other natural controls include predatory arthropods and vertebrates as well as parasitic insects which utilize bertha armyworm eggs, larvae or pupae as hosts (Mason et al., 1998). Diseases resulting from viral or fungal infection are also important factors in bertha armyworm mortality and may be largely responsible for the cyclical pattern of

outbreaks. Cultural controls can also influence population levels. Tillage, weed control, crop rotation, seeding and swathing date can all have a significant impact on bertha armyworm populations and damage (Anonymous, 1995b; Mason et al., 1998). However, most of the natural controls do not act early enough or quickly enough to control bertha armyworm in outbreak years and the results of many of the cultural controls are unpredictable. As a result, chemical control is often the only reliable solution to bertha armyworm damage in outbreak years. There are several insecticides registered for use in Canada, such as Lorsban, Decis, Lannate, Monitor and Pyrinex (Anonymous, 1995b).

1.4 Diamondback Moth, *Plutella xylostella* (Lepidoptera: Plutellidae)

1.4.1 Biology

The adult *P. xylostella* is a slender grayish-brown moth with a wing span of approximately 1.5 cm and a body length of 1 cm (Anonymous, 1996) (Figure 1.2d). Adults usually emerge during the first part of the day and first mating occurs at dusk of the same day with oviposition occurring immediately after mating (Pivnick et al., 1990a). The average life span of the adult is 16 days but most eggs are laid in the first 3 days after emergence (Harcourt, 1957). It is the small, weak flying adult stage which allows this insect to be carried from warmer climates to the Canadian prairies on southerly winds in the spring and summer.

Diamondback moth eggs are 0.44 x 0.26 mm, oval shaped and somewhat flattened (Harcourt, 1957) (Figure 1.2a). The pale green to yellow eggs are laid on both the upper and lower leaf surfaces, usually along veins or uneven parts of the leaf. Incubation time

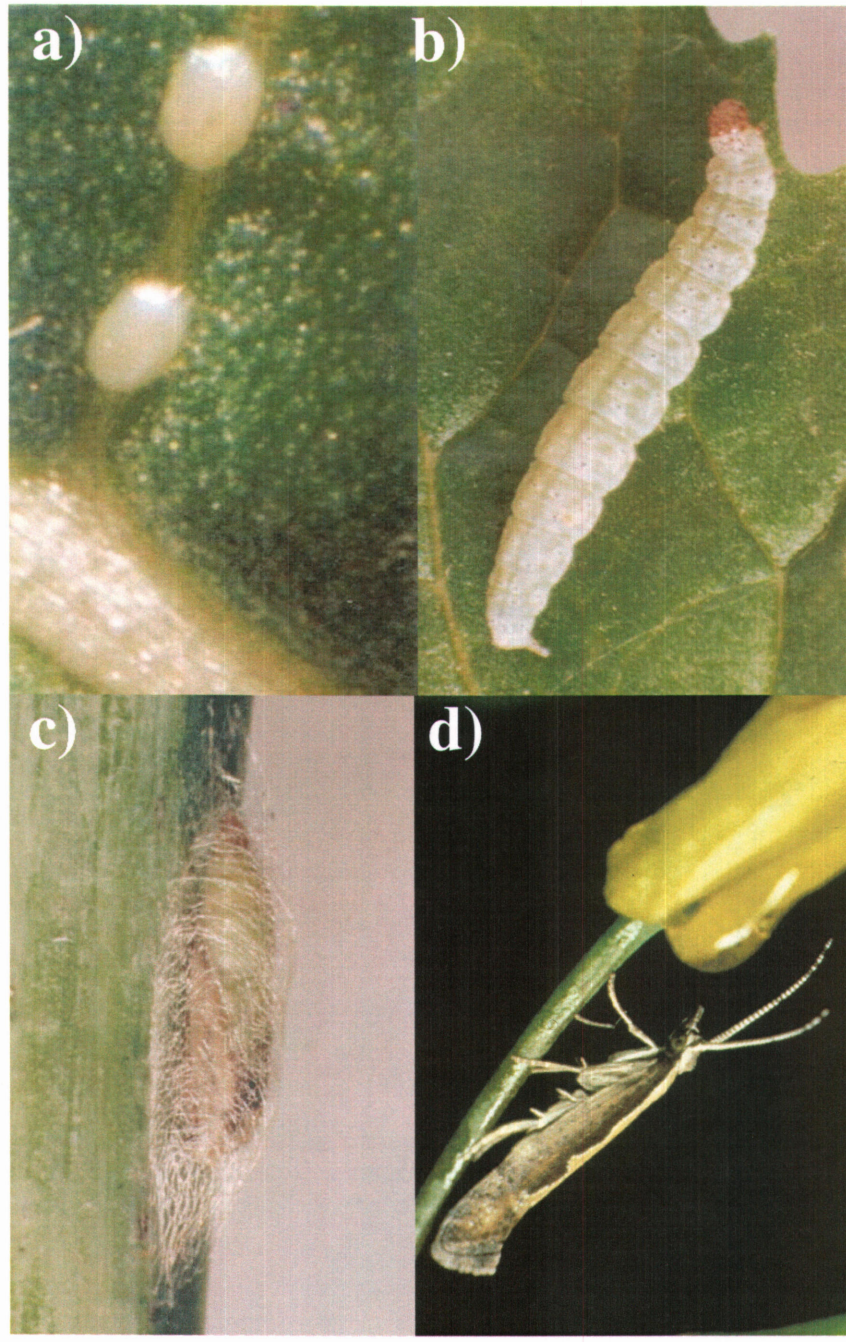

Figure 1.2. Stages in the life cycle of diamondback moth: a) eggs, b) larva, c) pupa, d) adult.

ranges from 4 to 6 days depending on the temperature.

Immediately after hatching larvae burrow into the leaf and feed on spongy mesophyll within the leaf until the end of the first stadium (Harcourt, 1957). The remaining larval instars feed on the surface of the leaves and other plant parts. Larvae are pale yellow-green to green caterpillars with a forked posterior end (Figure 1.2b). The larval stage lasts 10-21 days depending on temperature and nutrition (Anonymous, 1996). When disturbed, larvae will wriggle vigorously and/or drop from the plant on a silken thread.

When ready to pupate, diamondback moth spin a delicate, white, open-mesh cocoon which may be attached to various parts of the host plant (Figure 1.2c). Initially pupae are light green but become brown as they mature over the course of 5 to 15 days, depending on environmental conditions (Anonymous, 1996).

1.4.2 Distribution, Host Plants and Damage

Diamondback moth occur throughout the world. They specialize on cruciferous plants and in North America they occur wherever their host plants grow. Important host plants for diamondback moth in Canada include *Brassica* crops such as canola, mustard, cabbage, broccoli and several greenhouse plants. The widespread cultivation of cruciferous crops and the mobility of the adult stage have been major factors increasing the range and pest status of this insect (Talekar and Shelton, 1993). Although small numbers of diamondback moth may survive Canadian winters if conditions are ideal, populations on the Canadian prairies are usually extinguished over the winter months (Dosdall, 1994).

However, the short generation time and high fecundity of the diamondback moth have allowed it to become a significant pest of oilseed crops in this region.

Damage by first-instar larvae is characterized by the white markings their 'mines' create on the leaves. Later-instar larvae skeletonize the leaves and will also feed on buds, flowers, seed pods and the outer part of the stem of canola plants (Anonymous, 1996). The extent of crop damage depends on the plant growth stage and larval density. Because infestation on the Canadian prairies is due to immigrant moths, several or all life stages of diamondback moth may be present at the same time.

1.4.3 Management

Environmental factors play a significant role in determining populations of diamondback moth. Adults are less active in the cold and wind, and rainfall can also drown small larvae. Diamondback moth larvae are prey for many species including birds, spiders and predatory insects; there are also some species of parasitic wasps which use diamondback moth larvae as hosts, and fungal diseases can cause significant mortality, especially when populations are high (Anonymous, 1996). Despite these natural control agents, pesticides are the most reliable and effective way of controlling diamondback moth in outbreak years. There are several insecticides registered for use in Canada, such as Lorsban, Decis, Pyrinex, Sniper, Dylox, Malathion and Guthion (Anonymous, 1996). However, aside from the health and environmental concerns with insecticides, diamondback moth populations around the world are showing resistance to many of the insecticides registered for their control (Shelton et al., 1993).

1.5 Cruciferous Host Plants and Glucosinolates

Glucosinolates are secondary metabolites produced by cruciferous plants. It is glucosinolates and their products which give members of this group of plants their distinctive smell and taste. Glucosinolates and their products function as a component of plant defence against consumers, including mammals, birds, insects, bacteria and fungi (Louda and Mole, 1991). The universal products of glucosinolate hydrolysis are glucose, sulfate and a mustard oil. The biological activity of glucosinolates usually depends on their hydrolysis to mustard oils. Glucosinolates are stored separately from the degradative system and come into contact with the hydrolases when mechanical rupture of plant tissue occurs (Louda and Mole, 1991).

Insects which specialize on cruciferous plants, such as the diamondback moth and the cabbage butterflies (*Pieris* spp.), rely on glucosinolates as host finding and acceptance cues (Pivnick et al., 1990b; Traynier and Truscott, 1991; Du et al., 1995; Huang and Renwick, 1994). However, glucosinolates and their products are deleterious and deterrent for many vertebrate and invertebrate generalist feeders. Consequently, lowered levels of glucosinolates often result in greater susceptibility to herbivores among cruciferous plants (Giamoustaris et al., 1995), while high levels of glucosinolates in *Brassica* crops are known to influence the feeding activity and growth of many herbivores, including the bertha armyworm (Cheeke and Shull, 1985; Bodnaryk, 1991; McCloskey and Isman, 1993, 1995; Campbell and Schone, 1998). The most documented effects of glucosinolates in mammals are reduced animal performance and inhibition of thyroid function. High levels of glucosinolates in feed have also been associated with lower egg

production and liver haemorrhage mortality in laying hens (Campbell and Schone, 1998). Glucosinolates and their derivatives can be transferred through the placenta of mammals, as well as in the milk, and to the chicks of hens fed rapeseed meal (Cheeke and Shull, 1985). Due to the detrimental effects of glucosinolates in animal feed, ruminant livestock can only tolerate 10-20% dietary rapeseed meal with no ill effect and non-ruminant animals can tolerate only 5-10% (Cheeke and Shull, 1985).

Thus, reducing seed glucosinolate levels in oilseed rape was one of the aims of plant breeders in the development of canola or "double-zero" rapeseed. This breeding effort was so successful that canola meal can be used as a total replacement for soybean meal in some classes and types of livestock feed and is also a very important edible oil for human consumption (Cheeke and Shull, 1985). However, because there is a strong correlation between seed and foliar glucosinolate levels, the effort to lower glucosinolate levels in the seed of *Brassica* crop plants has also affected the foliar glucosinolate levels of these plants (McGregor and Love, 1987). While these changes have been positive for human and livestock consumption, their effects on insect pests are not fully understood.

1.6 Project Rationale

Currently, a number of crucifer species and breeding lines are being assessed for the development of elite lines of canola-type oilseed crops. In the present series of experiments the host plant suitability of existing and experimental plant lines from five cruciferous species was assessed as to suitability as host plants for the bertha armyworm, a generalist feeder, and for the diamondback moth, a crucifer specialist. The plant lines

were chosen based on seed glucosinolate level. Where available, a high and a low glucosinolate line of each species was selected, though it should be noted that the designations 'high' and 'low' are relative only within each species. Additional lines were also used in some experiments based on specific physical or chemical characteristics.

In recent years plant-based resistance in crucifers to both bertha armyworm and diamondback moth has been under investigation. To date, host-plant resistance studies involving the bertha armyworm have focussed on the larval stage. There is evidence that specific glucosinolates may offer plants some protection against larval feeding (Bodnaryk, 1991; McCloskey and Isman, 1993 & 1995; Shields and Mitchell, 1995a & b). Plant lines which contain sufficient levels of these chemicals show some resistance relative to those which do not and there appears to be potential for larval feeding resistance based on plant chemistry. The present study will expand on previous work by evaluating existing and experimental crucifer lines for differences in attractiveness, palatability and suitability for larval feeding. Although feeding by the larval stage causes the crop damage, ultimately the adult female determines which plant/crop will be infested. Thus, host plant suitability for oviposition will also be examined on different growth stages and lines of crucifer plants. The oviposition biology of the bertha armyworm will also be investigated. Oviposition site selection on individual host plants and within the crop canopy will be examined, as will the effect of conspecific eggs on oviposition.

Host plant resistance to diamondback moth has been studied much more extensively due to its worldwide pest status. However, this insect specializes on cruciferous plants and has proven to be very well adapted to a wide range of changes in

plant chemistry and morphology among this group of plants. The most promising evidence of success in this area to date involves glossy lines of *Brassica oleracea* which show considerable resistance to diamondback moth compared to the waxy wild-type lines (Lin et al., 1984; Eigenbrode and Shelton, 1990; Eigenbrode et al., 1991b; Eigenbrode and Pillai, 1998). As with the bertha armyworm, the present study will expand on previous work by evaluating existing and experimental crucifer lines, including glossy and waxy *B. rapa* lines, for differences in attractiveness, palatability and suitability for larval feeding. Host plant suitability for oviposition will also be examined on the experimental *B. rapa* lines.

2 Crucifer Host-Plant Suitability for Larval Bertha Armyworm

2.1 Introduction

Bertha armyworm, *Mamestra configurata* Walker (Lepidoptera: Noctuidae), is a major insect pest of oilseed crops, including canola, *Brassica napus* L. and *B. rapa* L., on the Canadian prairies. In outbreak years, bertha armyworm can cost tens of millions of dollars in crop losses and chemical control (Anonymous, 1995a). Economic damage results when larvae feed on foliage and developing seed pods (Bracken and Bucher, 1977).

It is known that glucosinolates influence the feeding activity and growth of bertha armyworm (McCloskey and Isman, 1993, 1995; Bodnaryk, 1991). Increased levels of certain glucosinolates in the foliage may provide plants with relative resistance to larval feeding. However, in the process of lowering seed glucosinolate levels for human and livestock consumption the foliar glucosinolate levels of these plants have been affected. While there is a correlation between seed and foliar glucosinolate levels, it is complicated by changes in plant phenology and leaf expansion which influence foliar glucosinolate levels (McCloskey and Isman, 1995; McGregor and Love, 1987).

Currently, a number of additional crucifer species and breeding lines are being assessed for the development of elite lines of canola-type oilseed crops. In the present study, dual-choice experiments were undertaken to determine the relative feeding preference of *M. configurata* for crucifer lines, with a range of glucosinolate profiles in similar breeding lines, from five crucifer species. In addition, no-choice experiments were

carried out on individual plants (or plant parts) to determine the suitability of these plant lines for larval growth, development, survival and nutritional value. The relationship between foliar glucosinolate levels and larval feeding preference, growth and development was also examined.

2.2 Materials and Methods

Insects. A laboratory colony of bertha armyworm was reared on a semi-synthetic diet (Bucher and Bracken, 1976) at 21°C, 60% RH and under 20L:4D photoperiod. The genetic diversity of the colony was maintained by annually mating colony insects with moths derived from field-collected pupae. The colony cycles through five to six generations per year. Adults mated and laid eggs on potted canola plants (AC Excel) in cages. Egg masses were transferred to diet cups and maintained at room temperature until hatching. Large egg masses were selected so that enough individuals for an entire experimental replicate could be obtained from a single cohort of eggs.

Host Plants. Fourteen lines from five species of Brassicaceae were selected for testing based primarily on their relative seed glucosinolate contents. Where available, a high and a low glucosinolate line of each species was selected, though it should be noted that the designations 'high' and 'low' are relative only within each species. The plant lines selected were: *Sinapis alba* L. (AC Pennant, Low-glucosinolate or L-GS [Line#93-0860]), *Brassica carinata* L. (Dodolla, S-67), *B. rapa* L. (AC Boreal, Echo, Glossy [Line#CB9625], Waxy [Line#CB9626]), *B. napus* L. (Midas, AC Excel), *B. juncea* L. (AC

Vulcan, H-Allyl [Line#89-102], H-butenyl [Line#60143], Low-glucosinolate or L-GS [Line#T097-3413-1]).

Seeds were sown directly in a soil-less mix and grown at 22°C, 16L:8D photoperiod in a greenhouse. Plants were grown in 12.7 cm pots, two plants per pot, and watered daily.

Leaf glucosinolate levels. The designations 'high' and 'low' glucosinolate were based on seed content, but in these experiments *M. configurata* were offered foliage. Thus, the foliar glucosinolate profiles of the 14 plant lines were determined by the staff of the Oilseeds Chemistry Laboratory at the Agriculture and Agri-Food Canada, Saskatoon Research Centre, by gas chromatography of the trimethylsilyl derivatives of desulfated glucosinolates (Hewlett Packard 5890A Gas Chromatograph) (Daun and McGregor, 1981). Benzyl glucosinolate and allyl glucosinolate were used as the internal standards for the *Brassica* and *S. alba* lines, respectively. These profiles were developed for fully expanded leaves at growth stage 3.2 (Harper and Berkenkamp, 1975), the same plant stage used for the feeding preference, growth and development studies. Each test involved two samples (approximately 0.2 g leaf material per sample) from each plant line and the test was repeated three times for each of the plant lines examined.

Dual-choice leaf disc experiment. Leaf discs (15 mm diameter) were cut from true leaves (third or fourth from bottom) of each of the 14 plant lines. One experimental disc and one control (AC Excel) disc were placed in a 4.5 cm diameter plastic petri dish, lined with moistened filter paper and covered with a tightly fitting lid (Figure 2.1). As an internal

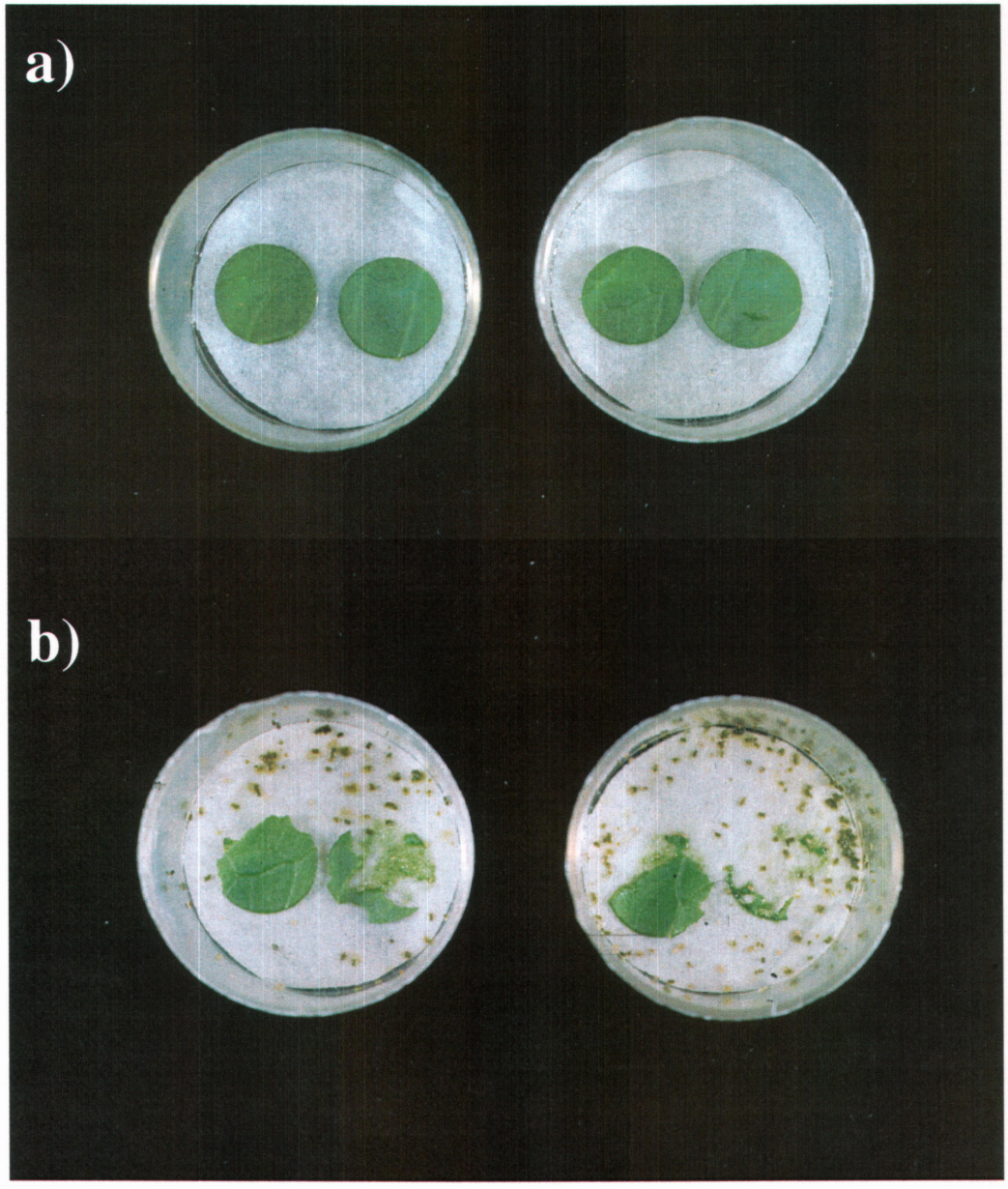

Figure 2.1. Dual-choice leaf-disc feeding experiment with an AC Vulcan leaf disc on the left paired with an AC Excel leaf disc on the right of each dish. a) Paired leaf discs before feeding. b) Paired leaf discs after feeding

check, AC Excel was also treated as an experimental line and paired with itself. Three early third-instar larvae, with no feeding experience on plant material, were randomly placed in each petri dish with unrestricted access to both discs. Larvae were allowed to feed for 18 h at 21°C. The area of each disc was determined at the start and end of the feeding period using image analysis software (AgImage Plus Version 1.08. ©Decagon Devices, Inc. 1989-1991). Food consumption was measured from the absolute area of the leaf discs consumed (original area less the area after 18 h of feeding). Biomass per unit area was not significantly different among the *Brassica* lines; however, the *S. alba* leaf discs had significantly less biomass per unit area than the *Brassica* lines. A conversion factor of 0.85 was used to adjust the feeding index values for the *S. alba* lines. Feeding preference was measured as the amount of the control disc consumed minus the amount of the experimental disc consumed; thus, positive values indicate a preference for the control disc. Each experiment consisted of five pairs of discs for each plant line and the experiment was replicated four times with different cohorts of insects and plants.

No-choice leaf-disc experiment. A newly molted fourth-instar larva, with no feeding experience on plant material, was offered a 22-mm diameter leaf disc in a 4.5-cm petri dish as described above. Both the larva and the leaf disc were weighed at the start of the experiment. After 24 h, the disc was removed, weighed and replaced by another weighed leaf disc from the same plant line. The experiment was carried out over 4 consecutive days at which time larval weight was again determined. Larvae which molted to the fifth-instar or died were not included in the analysis.

Larval food consumption was measured as the total initial leaf disc dry weight less the total final leaf disc dry weight over the 4-day period. The wet/dry ratio for each plant line was obtained by weighing, drying (60°C for 48 h) and re-weighing 10 leaf discs of each line for each replicate. The wet/dry weight ratio for each line was used to determine the initial dry weight for the discs that were actually fed to the larvae. After the feeding period, the remaining portions of the leaf discs were dried under the same conditions to determine final leaf disc weight. The experiment was conducted at 21°C and 16L:8D photoperiod. Each experiment consisted of five larvae for each plant line and was replicated four times with different cohorts of insects and plants.

Nutritional indices were also calculated for the plant lines tested including:

a) efficiency of conversion of ingested material (ECI) = $\triangle B/I$, where $\triangle B$ = change in body weight and I = weight of food ingested.

b) relative consumption rate (RCRi) = I/(Bi)(T), where I = weight of food ingested, Bi = initial weight of the larva and T = feeding period in days.

c) relative growth rate (RGRi) = $\triangle B/(Bi)(T)$, where $\triangle B$ = change in body weight, Bi = initial weight of the larva and T = feeding period in days.

These indices, which are based on initial larval weights, were deemed most appropriate for this short-term experiment ($\leq$ one instar). They allow for comparison of consumption of different host plants without the potentially confounding influence of postingestive effects (Farrar et al., 1989).

Individual leaf experiment. Plants from each line were selected at growth stage 3.2.

Transparent perforated plastic bags, approximately 11 x16 cm, were used to restrict larvae to an individual leaf. Five early second-instar larvae, with no feeding experience on plant material, were placed in a bag fitted over a single leaf (third or fourth from bottom) and secured with a twist tie. Two plants from each of the plant lines were included in each replicate, which was conducted at 21°C and 16L:8D photoperiod. The bagged leaves were checked daily and feeding damage was rated visually on a scale from 0-10 each day (10 being complete defoliation). After 7 d, the bags were removed and larval survival, growth (final weight) and development (progression to specific instar) were noted, with survivors being weighed individually and their instar determined by head capsule measurement (Arthur and Moore, unpublished data). Two sets of experiments were carried out using the same protocol, Experiment 2 being conducted later when additional *B. juncea* lines (H-Allyl, H-Butenyl and L-GS) with varied concentrations of foliar sinigrin became available for testing. Both Experiment 1 and 2 were replicated five times with different cohorts of insects and plants.

Whole plant experiment. Plants from each line were selected at growth stage 3.2. Twenty neonate larvae, with no feeding experience on plant material, were placed on a single plant and transparent perforated plastic bags, 30 x 75 cm, were used to restrict larvae to an individual plant. The bags were held fully expanded and upright by a modified wire tomato hoop set in the soil and were secured tightly around the pots with rubber bands. The bags remained sealed for the duration of the experiment, the plants being watered from the bottom. The experiment was conducted at 21°C with a 16L:8D photoperiod. After 14

days, the bags were removed, larval survival and development were noted, and each larva was weighed. Each experiment consisted of one bagged plant from each line and the experiment was replicated four times with different cohorts of insects and plants.

Data analysis. An ANOVA was performed to determine treatment (plant) and replicate effects, as well as interactions for each experiment (Statistix 4.0, Analytical Software, Tallahassee, Florida.). No significant replicate effects were detected and data were pooled in each experiment. The least significant difference (LSD) method was used for comparisons of means.

2.3 Results

Foliar glucosinolate profiles of the plant lines examined. The 14 lines had distinct foliar glucosinolate composition (Table 2.1). The most notable variants among the plant lines were the concentrations of sinigrin (allyl- or 2-propenyl glucosinolate) and sinalbin (*p*-hydroxybenzyl glucosinolate). Two *B. juncea* lines, AC Vulcan and H-Allyl, contained far greater amounts of sinigrin in their foliage than any other line tested. The two *B. carinata* lines, Dodolla and S-67, and the *B. juncea* line H-Butenyl were the only other lines which contained appreciable amounts of sinigrin. The *S. alba* line AC Pennant was the only line which contained high levels of sinalbin in its foliage; sinalbin was not detectable in the other lines. The relative differences in various glucosinolates in foliage of different lines (Table 2.1) reflect data from seed glucosinolate profiles. While cultivars and breeding lines within each species were originally selected on the basis of the high and low (seed)

Table 2.1. Leaf tissue glucosinolate concentrations for *Brassica* spp. and *S. alba* (μmole/g of leaf material)

Species / Line	Glucosinolate: Allyl (Sinigrin)	3-Butenyl	Benzyl	HOBenzyl (Sinalbin)	Other	Total
Sinapis alba						
AC Pennant		0.05 [0.02]	3.47 [0.75]	7.56 [0.80]	0.05 [0.03]	11.13 [1.50]
Low-GS		nd	1.67 [0.72]	nd	nd	1.67 [0.72]
Brassica carinata						
Dodolla	1.09 [0.32]	0.01 [0.01]		nd	0.02 [0.02]	1.12 [0.34]
S-67	0.57 [0.19]	nd		nd	0.02 [0.01]	0.59 [0.19]
B. juncea						
AC Vulcan	2.50 [0.43]	0.03 [0.02]		nd	0.05 [0.02]	2.58 [0.47]
H-Allyl	1.80 [0.19]	0.03 [0.00]		nd	0.01 [0.01]	1.84 [0.19]
H-Butenyl	0.15 [0.06]	1.57 [0.41]		nd	0.08 [0.08]	1.80 [0.55]
L-GS	nd	0.01 [0.01]		nd	0.02 [0.02]	0.03 [0.02]
B. rapa						
AC Boreal	nd	nd		nd	0.04 [0.02]	0.04 [0.02]
Echo	nd	nd		nd	0.02 [0.01]	0.02 [0.01]
Glossy	nd	nd		nd	0.01 [0.01]	0.01 [0.01]
Waxy	nd	nd		nd	0.05 [0.02]	0.05 [0.02]
B. napus						
Midas	0.02 [0.02]	0.01 [0.01]		nd	0.16 [0.14]	0.19 [0.03]
AC Excel	nd	nd		nd	0.03 [0.02]	0.03 [0.02]

Note: Values are mean ± SE. The limit of detection was 0.01μmol/g; nd, nondetectable level.
No value indicates the substance was used as an internal standard.

glucosinolate range for that species, it must be noted that there were large variations in total foliar glucosinolates for lines designated as 'high' or 'low' (seed) glucosinolate across species.

Dual-choice leaf-disc feeding experiment. In all experiments there was evidence of larval feeding on both control, AC Excel, and test plant line discs. However, third-instar larval feeding preferences were detected among the plant lines tested ($p<0.0001$, $F_{15,354}=10.88$) (Figure 2.2). The *B. juncea* lines AC Vulcan and H-Allyl were significantly less preferred than all other lines tested. On average, larvae consumed less than one-fifth the leaf area of these experimental lines relative to AC Excel. Other lines were less preferred, but not significantly so, than AC Excel including AC Pennant (*S. alba*), the two *B. carinata* lines and the *B. juncea* H-Butenyl line. Generally, lines within a species were similar in terms of bertha armyworm feeding preference. For example, within *B. rapa*, *B. napus* and *B. carinata*, all lines showed similar values. However, in *S. alba* and *B. juncea,* which had the largest range of foliar glucosinolate levels, there were significant differences in feeding preference among lines.

No-choice leaf-disc feeding experiment. The no-choice leaf-disc experiment showed significant differences among lines for food consumption ($p=0.017$, $F_{8,32}=2.83$) and weight gain ($p=0.013$, $F_{8,32}=2.97$) (Table 2.2). Food consumption values were linked closely to the weight gain of larvae and the differences among lines were similar for these two parameters. Weight gain and consumption were lowest on the *B. juncea* line AC Vulcan.

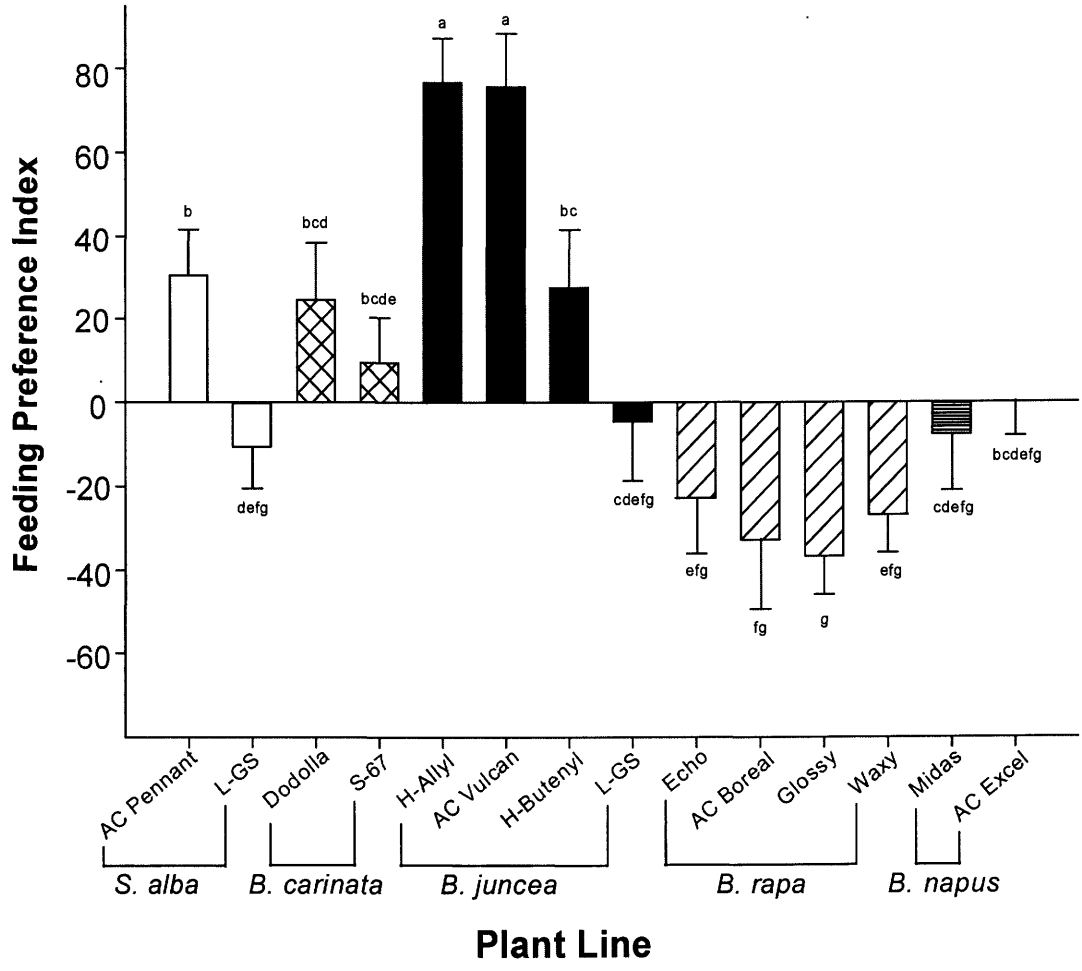

Figure 2.2. Consumption of leaf discs by third-instar bertha armyworm larvae in a dual choice disc assay. Feeding preference index is measured as the difference in absolute area (mm^2) between the AC Excel disc consumed and the experimental disc consumed over 18 h by three third-instar larvae. Positive values indicate that larvae ate less of the experimental disc than the control AC Excel disc. Negative values show that more of the experimental disc than the AC Excel disc was consumed. Means with the same letter are not significantly different (LSD p=0.05), lines above or below means indicate SE.

Table 2.2. Weight gain (wet weight) and plant material consumed (dry weight) by bertha armyworm in a 4 d no-choice leaf disc feeding experiment

Species	line	larval wt gain wet wt (mg)	plant material consumed dry wt (mg)
S. alba			
	AC Pennant	48.4 [5.6] ^{bc}	17.9 [1.7]^{c}
	L-GS	48.3 [5.0] ^{bc}	19.3 [1.7] ^{bc}
B. carinata			
	Dodolla	57.6 [4.7] ^{abc}	21.9 [1.7] ^{abc}
	S-67	65.6 [5.9] ^{a}	26.6 [3.1] ^{a}
B. juncea			
	AC Vulcan	46.7 [5.3] ^{c}	18.3 [1.5] ^{c}
B. rapa			
	Echo	59.6 [6.8] ^{ab}	22.6 [1.6] ^{abc}
	AC Boreal	62.8 [5.8] ^{a}	22.7 [1.8] ^{abc}
B. napus			
	Midas	64.5 [8.7] ^{a}	24.9 [2.2] ^{ab}
	AC Excel	62.8 [7.4] ^{a}	23.9 [2.5] ^{ab}

Note: Values are mean ± SE. Means for each line are based on five larvae per replicate in four replicates (n =20). Means within columns followed by the same letter are not significantly different (LSD, $P > 0.05$)

The two *S. alba* lines (AC Pennant and L-GS) also had low values for both food consumption and weight gain.

There were significant differences in relative consumption rate (RCR*i*) ($p=0.004$, $F_{8,155}=2.97$) and relative growth rate (RGR*i*) ($p=0.022$, $F_{8,155}=2.33$) among fourth-instar larvae reared on the different plant lines (Table 2.3). Larvae fed AC Pennant had the lowest RCR*i* followed by those on AC Vulcan. Larvae on these two lines also had the lowest values for RGR*i*. Larvae on AC Excel had the highest value for efficiency of conversion of ingested food (ECI) and larvae on S-67, which had the highest values for RCR*i* and RGR*i*, had the lowest value for ECI (Table 2.3). However, the differences in ECI were not significant ($p=0.74$, $F_{8,155}=0.65$).

Individual leaf experiment. The 7 d individual leaf experiment also revealed that there were significant differences among the plant lines tested (Table 2.4). Second-instar larvae restricted to the *B. juncea* lines AC Vulcan and H-Allyl showed the lowest final weight ($p=0.001$, $F_{8,32}=4.27$) and slower development ($p<0.0001$, $F_{8,32}= 10.99$) after 7 d. These lines also suffered the least amount of feeding damage with 30-50% less defoliation than the most susceptible lines (AC Excel, AC Boreal and the L-GS *B. juncea*). As in the previous experiment, larvae on the *S. alba* lines (AC Pennant and L-GS) had the next lowest weights, although leaf damage on these two lines was high. There were no significant differences in larval survival among the plant lines in either Experiment 1 or Experiment 2. The results again appeared to be grouped by plant species except for the *B. juncea* lines in Experiment 2 where the L-GS line, which lacks foliar sinigrin, produced

Table 2.3. Efficiency of conversion of ingested food (ECI), relative consumption rate (RCR*i*) and relative growth rate (RGR*i*) of fourth instar bertha armyworm larvae on each plant line

Species	line	ECI	RCR*i*	RGR*i*
S. alba				
	AC Pennant	0.393 [0.028]^{a}	27.3 [2.75]^{b}	10.5 [1.33]^{c}
	L-GS	0.378 [0.029]^{a}	36.3 [3.47]^{ab}	12.8 [1.05]^{bc}
B. carinata				
	Dodolla	0.377 [0.033]^{a}	39.1 [2.83]^{ab}	13.9 [0.99]^{abc}
	S-67	0.341 [0.029]^{a}	49.7 [5.09]^{a}	16.9 [1.82]^{a}
B. juncea				
	AC Vulcan	0.358 [0.025]^{a}	31.9 [2.34]^{b}	10.6 [0.53]^{c}
B. rapa				
	Echo	0.357 [0.028]^{a}	39.7 [3.03]^{ab}	14.7 [1.90]^{ab}
	AC Boreal	0.390 [0.034]^{a}	35.4 [2.66]^{ab}	13.3 [1.14]^{abc}
B. napus				
	Midas	0.394 [0.031]^{a}	39.5 [5.63]^{ab}	14.8 [1.92]^{ab}
	AC Excel	0.422 [0.029]^{a}	39.9 [5.59]^{ab}	15.4 [1.76]^{ab}

Note: Values are mean ± SE. Means for each line are based on five larvae per replicate in four replicates (n=20). Means within columns followed by the same letter are not significantly different (LSD, $P > 0.05$)

ECI = larval dry weight gain/dry weight of food consumed

RCR*i* = dry weight of food consumed/(initial larval dry weight)(number of days)

RGR*i* = larval dry weight gain/(initial larval dry weight)(number of days)

Table 2.4. Bertha armyworm final weight, percent larvae reaching 4th instar, percent survival and leaf damage rating after 7 d feeding on an individual leaf on each plant line

Species	Line	mean larval wt (mg) [SE]	% larvae reaching 4th instar [SE]	% survival [SE]	damage rating
Experiment 1					
S. alba					
	AC Pennant	28.9 [4.2] ^{bcd}	48 [8.5]^{c}	90 [5.4]	9.4
	L-GS	23.6 [1.2] ^{cd}	48 [6.7]^{c}	94 [6.0]	9.1
B. carinata					
	Dodolla	37.4 [3.1] ^{abc}	54 [8.4]^{bc}	86 [8.0]	6.9
	S-67	35.8 [3.9] ^{abc}	62 [9.0]^{abc}	90 [6.1]	7.3
B. juncea					
	AC Vulcan	18.8 [1.6] ^{d}	2 [2.0]^{d}	96 [4.0]	6.4
B. rapa					
	Echo	44.2 [5.8] ^{a}	56 [11.0]^{abc}	80 [10.0]	7.5
	AC Boreal	43.7 [3.0] ^{a}	78 [5.4]^{a}	98 [2.0]	9.1
B. napus					
	AC Excel	49.2 [7.7] ^{a}	68 [10.8]^{abc}	86 [6.1]	7.8
	Midas	39.4 [5.1] ^{ab}	74 [9.8]^{ab}	98 [2.1]	7.6
Experiment 2					
B.juncea					
	AC Vulcan	24.6 [5.8] ^{c}	3 [2.5]^{c}	83 [4.4]	4.1
	H-Allyl	26.5 [6.5] ^{c}	15 [5.0]^{c}	83 [4.5]	4.6
	H-Butenyl	36.0 [6.0] ^{bc}	18 [5.8]^{bc}	70 [9.2]	4.9
	L-GS	52.0 [8.5] ^{ab}	38 [9.4]^{ab}	83 [7.8]	7.6
B.napus					
	AC Excel	56.3 [7.2] ^{a}	50 [9.2]^{a}	95 [3.2]	8.3

Note: Values are mean (damage rating) of mean ± SE.

For each experiment, means within columns followed by the same letter are not significantly different (LSD $P>0.05$)

Mean damage rating based on two leaves in five replicate assays ($n=10$). Damage rating: 0=no feeding, 10=complete defoliation

Each mean value for larval weight is based on the number of survivors of 10 larvae in 5 replicate assays ($n=50$)

higher larval weights and development rates than the others.

Whole plant experiment.

Data from this experiment (Table 2.5) were similar to that from the single leaf experiment. Two *B. juncea* lines, AC Vulcan and H-Allyl, and both *S. alba* lines were the least suitable for bertha armyworm growth and development. In this longer-duration feeding trial, starting with neonates, there was a greater difference between the larval final weights on the two *S. alba* lines; larvae on AC Pennant showed lower final weights compared to those on the L-GS line. Although after 14 days there were no significant differences in survival of larvae on the 14 lines, both development ($p=0.023$, $F_{13,31}=2.40$) and final weights ($p<0.0001$, $F_{15,1053}=24.0$) were significantly reduced on AC Vulcan and H-Allyl.

2.4 Discussion

The present study was designed to evaluate feeding preferences and development of bertha armyworm larvae on five crucifer species using a series of dual-choice and no-choice feeding experiments. Two to four plant lines, with glucosinolate profiles differing in both quantity and makeup, were chosen for each species to evaluate the impact of foliar glucosinolates.

Our results showed that plant lines with substantial levels of sinigrin were less preferred than *B. napus* AC Excel by larvae. This was particularly evident in the *B. juncea* lines where the feeding preference index increased (i.e., plants were less preferred) with

Table 2.5. Bertha armyworm final weight, percent larvae reaching sixth-instar and percent survival after 14 d of feeding on intact plants of each plant line

Species	line	mean larval wt (mg) [SE]	% larvae reaching 6th instar [SE]	% survival [SE]
S. alba				
	AC Pennant	190 [13]^{d}	49 [7.2]^{abc}	84 [4.5]
	L-GS	280 [15]^{c}	47 [15.1]^{abc}	90 [6.1]
B. carinata				
	Dodolla	383 [24]^{b}	54 [10.1]^{ab}	89 [5.9]
	S-67	370 [22]^{b}	59 [9.2]^{ab}	95 [9.4]
B. juncea				
	AC Vulcan	183 [7]^{d}	22 [9.3]^{c}	83 [15.1]
	H-Allyl	200 [9]^{d}	27 [7.1]^{c}	80 [3.5]
	H-Butenyl	258 [12]^{c}	44 [4.2]^{bc}	93 [4.8]
	L-GS	365 [19]^{b}	55 [4.9]^{ab}	90 [5.9]
B. rapa				
	Echo	386 [22]^{b}	59 [20.0]^{ab}	65 [17.1]
	AC Boreal	363 [16]^{b}	72 [6.0]^{a}	92 [1.4]
	Glossy	350 [18]^{b}	74 [8.3]^{a}	89 [4.8]
	Waxy	440 [21]^{a}	57 [6.0]^{ab}	82 [4.3]
B. napus				
	Midas	373 [19]^{b}	50 [10.0]^{abc}	89 [7.5]
	AC Excel	373 [23]^{b}	67 [8.6]^{ab}	85 [8.3]

Note: Values are mean ± SE. Each mean is based on the number of survivors of 20 larvae per plant in four replicates (n=80). Means within columns followed by the same letter are not significantly different (LSD, $P > 0.05$).

increasing sinigrin levels. For example, AC Vulcan and H-Allyl, which had the highest levels of foliar sinigrin, had significantly higher feeding indices than all other lines tested, while the *B. juncea* H-Butenyl and L-GS lines, with moderate and no detectable levels of sinigrin, respectively, had feeding indices that were not significantly higher than that of AC Excel. Consistent with previous results showing sinigrin and its metabolite, allyl isothiocyanate, decreased bertha armyworm feeding when incorporated in artificial diet (Shields and Mitchell, 1995a & b; McCloskey and Isman, 1993), our results indicated that in the same plant germplasm increasing sinigrin concentrations increasingly inhibited bertha armyworm feeding. The two *B. carinata* lines, Dodolla and S-67, also contained detectable amounts of sinigrin in leaf tissue and also were less preferred than the AC Excel control. However, sinigrin concentration alone cannot fully explain feeding preferences as the *B. carinata* lines had substantially higher levels of sinigrin than the *B. juncea* H-butenyl line, yet gave very similar feeding preference values. Nevertheless, sinigrin generally appears to provide those plants which produce it with some resistance to bertha armyworm.

Sinapis alba also showed a large difference in bertha armyworm feeding preference between the selected lines and this was related to the level of sinalbin in the foliage. High levels of sinalbin appeared to give AC Pennant a greater level of protection from bertha armyworm compared to the L-GS line which lacked it. This is consistent with previous work which indicated that high concentrations of sinalbin in the cotyledons of *S. alba* deterred neonate bertha armyworm feeding and offered some resistance to *S. alba* over *B. napus* species (Bodnaryk, 1991). Our study extends these observations in that we tested

differing sinalbin levels within the same plant species and used a plant stage which bertha armyworm normally attacks rather than the cotyledon stage which occurs much earlier in the season than the larval stage of the insect. Thus, high levels of sinalbin in foliage may be a source of relative resistance against bertha armyworm. Interestingly, although the *S. alba* L-GS line contains high levels of benzyl-glucosinolate it was no less preferred than all the lines with low total glucosinolate. This indicates that the quantities of specific foliar glucosinolates, notably sinigrin and sinalbin, may be more important to larval deterrence than is the overall glucosinolate level.

There were significant differences among the plant lines tested in terms of bertha armyworm weight gain and development in all the no-choice experiments. Bertha armyworm final weights in the bag enclosure experiments as well as weight gain in the no-choice leaf-disc experiment were lowest on the *B. juncea* lines AC Vulcan and H-Allyl. The growth-inhibiting effects of the high foliar sinigrin content of these lines were seen in the 7-d individual leaf experiment and were magnified in the 14-d whole plant experiment. It is likely that few larvae on AC Vulcan or H-Allyl would survive through pupation. Similar antibiotic resistance to bertha armyworm larvae was observed by McCloskey and Isman (1993) in high sinigrin *B. juncea* lines. It is also noteworthy that long-term larval weight gain, but not development and survival, was significantly inhibited by the H-Butenyl line of *B. juncea*.

The *S. alba* lines AC Pennant and L-GS consistently ranked immediately behind the high sinigrin *B. juncea* lines AC Vulcan and H-Allyl as the poorest hosts for *M. configurata* in terms of larval weight gain. Larvae on AC Pennant and AC Vulcan also

showed the lowest relative growth rate and relative consumption rate among the lines tested in the no-choice leaf disc experiment. This is consistent with previous studies showing lower survival and weight gain of larvae on *S. alba* than on *B. napus* (Bodnaryk, 1991; McCloskey and Isman, 1993). Bodnaryk (1991) suggests that sinalbin is an antibiotic factor reducing the fitness of *M. configurata* larvae based on lower survival and weight gain on *S. alba* cotyledons compared to older *S. alba* plants in which sinalbin concentrations are lower. Our data from the 14-d feeding experiment supports this suggestion, in that larvae on AC Pennant were significantly lighter than those on L-GS which has no detectable sinalbin. However, in all cases the larval weights on *S. alba* L-GS were significantly lower than on the *B. rapa* and *B. napus* lines, indicating that other features of *S. alba* aside from sinalbin make it a less suitable host for *M. configurata* development. L-GS does produce a large amount of foliar benzyl glucosinolate. Though apparently not a feeding deterrent, the benzyl derivative may prevent normal weight gain when ingested by larvae.

The present investigation employed both commercial cultivars and experimental crucifer lines with a range of glucosinolate profiles. This study has shown that bertha armyworm larvae have significantly different feeding preferences for these lines which appear to be due to specific foliar glucosinolates, such as sinigrin and sinalbin, rather than simply total glucosinolate content. Sinigrin and sinalbin, as well as possibly 3-butenyl glucosinolate and benzyl glucosinolate, also influence the ability of larvae to utilise the food they ingest, suppressing weight gain and development. Maintaining adequate levels of certain glucosinolates in the foliage of cruciferous crops may offer some resistance to

generalist insect feeders such as *M. configurata*. Foliar levels of these glucosinolates may also be useful in predicting the attractiveness and suitability of plants to bertha armyworm.

3 Crucifer Host-Plant Suitability for Bertha Armyworm Oviposition

3.1 Introduction

Economic damage is the result of larval bertha armyworm feeding on the foliage and developing seed pods of canola plants (Bracken and Bucher, 1977). Consequently, this stage has been the focus of bertha armyworm host-plant studies to date. However, it is the adult female which selects the host plant on which the larvae will feed. Early-instar larvae are unable to search for a suitable host plant and must feed on, or very near, the plant on which the eggs were laid. Thus, host-plant resistance as a defence strategy against bertha armyworm must include development of plant lines less attractive for oviposition.

The present study was undertaken to determine if *B. juncea* and *Sinapis alba*, which have been shown to be relatively resistant to bertha armyworm larvae (Bodnaryk, 1991; McCloskey and Isman, 1993), also deter ovipositing females and to establish which canola growth stages are most attractive to ovipositing females. A series of choice experiments were carried out in field cages to evaluate host-plant attractiveness among different crucifer hosts using cultivars of *B. napus*, *B. juncea, B. carinata* and *S. alba* in full-flower. The *B. napus* and *B. juncea* cultivars were also tested in dual-choice (field-cage) and no-choice (greenhouse) experiments using full-flower plants. Choice experiments were also undertaken to determine the most attractive plant growth stage for bertha armyworm oviposition, comparing oviposition on plants at the pre-flower, full-flower and pod stage in field cages with the *B. napus* cultivar AC Excel. Bertha armyworm oviposition

preferences for different plant parts and regions of the crop canopy were also evaluated.

3.2 Materials and Methods

Insects. A laboratory colony of bertha armyworm was reared on a semi-synthetic diet (Bucher and Bracken, 1976) at 21°C, 60% RH and under a 20L:4D photoperiod. The genetic diversity of the colony was maintained by annually mating colony insects with moths derived from field-collected pupae. The colony cycles through five to six generations per year. Upon pupation, individuals were sexed and transferred to 500 mL plastic tubs with screened lids at 21°C, 60% RH and a 16L:8D photoperiod until adults emerged. The male and female moths were mated in mesh cages (38 x 26 x 26 cm) within 2 days of eclosion at 21°C, 60% RH and a 16L:8D photoperiod. After a 48 h mating period, adults were released into cages containing plants. Thus, females were exposed to plant material during the third, fourth or fifth scotophase after eclosion when bertha armyworm lay the most eggs (Howlader and Gerber, 1986).

Host Plants. Seeds of *B. napus* AC Excel, *B. juncea* AC Vulcan, *B. carinata* Dodolla and *S. alba* AC Pennant were sown, two per 12.7-cm pot, directly in a soil-less mix and grown at 22°C and a 16L:8D photoperiod in a greenhouse and watered daily. For the cultivar choice and no-choice experiments, plants of each cultivar were grown to full-flower (stage 4.2 [Harper and Berkenkamp, 1975]). For the phenology experiment, AC Excel plants were grown until they reached the appropriate growth stage, pre-flower, full-flower and pod (stages 3.1, 4.2 and 5.2, respectively) (Figure 3.1). For the field-cage experiments,

Figure 3.1. Growth stages of *B. napus* AC Excel used in the plant phenology oviposition preference experiment: pre-flower, full-flower, pod.

greenhouse-grown potted plants were transferred to outdoor cages. The pots were placed in large galvanized trays and bottom-watered for the 48 h period of the experiment.

Leaf Area Available for Oviposition. Because 97% of bertha armyworm oviposition occurred on the leaves (see Results), only leaf material was used to determine the amount of substrate available for oviposition on the different crucifer cultivars and the different growth stages of AC Excel. Leaf material was collected from 10 plants of each cultivar. Leaves were separated from the plant at the base of the leaf blade. Dry weight was used as a measure of the approximate leaf area available for oviposition. The plant material was dried at 60°C for 72 h in paper bags, after which dry weight was determined. Dry weight per unit area of leaf material was not significantly different among the *Brassica* lines; however, *S. alba* AC Pennant had significantly less (0.85 times) dry weight per unit leaf area than the *Brassica* lines. For the plant phenology experiment, 10 AC Excel plants from each growth stage were used. The dry weight of leaf material for each vertical third of the plants was measured.

Cultivar Preference Experiments. To examine oviposition preferences between the *B. napus* cultivar AC Excel and the *B. juncea* cultivar AC Vulcan, experiments were conducted in field cages (1.65 m high x 1.60 m wide x 5.95 m long) at the AAFC Research Farm near Saskatoon (N 52° 09' lat., W 106° 34' long.) (Figure 3.2). For each cultivar, a group of 16 plants was placed randomly at either end of the cage. Moths (10 of each sex) were mated for 48 h with no exposure to plant material before being released into the

Figure 3.2. Field cages used for bertha armyworm oviposition experiments

middle of the cage and allowed access to the plants for 48 h. Plants were then divided into vertical thirds and destructively sampled to collect egg masses. The position (plant structure, abaxial or adaxial surface) of each egg mass on the plant was also noted. Eggs were counted from an enlarged digital image of each egg mass. The experiment was replicated three times (using four cages in each replicate), two times in the last 10 days of June and once in the first week of August 2000.

A similar experiment was carried out with four crucifer cultivars. A group of 16 full-flower plants of each of *B. napus* AC Excel, *B. juncea* AC Vulcan, *B. carinata* Dodolla and *Sinapis alba* AC Pennant was placed in each cage. The protocol was similar to that in the above experiment except: (a) the four groups (cultivars) of plants were arranged randomly at equal distance along the length of the cage, (b) 12 mated pairs of moths were released into each cage, and (c) the plants were not divided into vertical thirds when sampled. The experiment was replicated three times (using four cages in each replicate) from June 23 to July 4, 2001.

No-choice Experiment. The *B. napus* cultivar AC Excel and the *B. juncea* cultivar AC Vulcan were tested for attractiveness for bertha armyworm oviposition in a greenhouse no-choice experiment. Two full-flower plants, in one pot, were placed in a 40x42x75 cm mesh cage. Moths (three of each sex) were mated for 48 h with no exposure to plant material and then released into the cage. On each of five consecutive days, plants were inspected for eggs, and egg masses were cut from the leaves with scissors removing as little leaf material as possible. Eggs within each egg mass were counted using a dissecting

microscope. During mating and throughout the experiment, a 10% sucrose-honey solution was offered to the moths as food by means of a wick in a covered plastic bottle. The experiment was conducted with a 16L:8D photoperiod and at temperatures ranging from 15°C at night to 20°C during the day. The experiment was repeated six times with AC Vulcan and seven times with AC Excel.

Plant Phenology Preference Experiment. To determine the effect of plant phenology on oviposition, experiments were conducted in field cages (1.65 m high x 1.60 m wide x 5.95 m long) at the AAFC Research Farm near Saskatoon. Sixteen plants from each of three growth stages (pre-flower, full-flower, pod) were placed in three distinct groups arranged randomly at equal distance along the length of the cage, each group consisting of one growth stage. Moths (10 of each sex) were mated for 48 h with no exposure to plant material before being released into the middle of the cage and allowed access to the plants for 48 h. Plants were then divided into vertical thirds and destructively sampled to collect egg masses. The position of each egg mass on the plant was noted and eggs were counted from an enlarged digital image of each egg mass. The experiment was replicated three times (using four cages in each replicate) from July 6 to July 20, 2000.

Temperature. Air temperature was recorded during the field-cage experiments using a Campbell Scientific CR10 datalogger with a HMP45C Temperature sensor (shielded) placed at 1.4 m above the ground surface. Recordings were made every 20 minutes (based on the mean of readings every 10 seconds).

Data Analysis. An ANOVA was performed to determine treatment and replicate effects, as well as interactions for the plant phenology experiment and the four-cultivar experiment. A one-way ANOVA was performed for the leaf dry weights, the number of eggs per egg mass on different cultivars and the egg count data for each vertical third of the phenology experiment. The least significant difference (LSD) method was used for comparison of means. A paired t-test was used for the dual-choice oviposition preference experiment and a two-sample t-test was used for the no-choice experiment involving AC Excel and AC Vulcan cultivars (Statistix 4.0, Analytical Software, Tallahassee Florida).

3.3 Results

Cultivar Preference Experiments. In the dual-choice field-cage experiment female moths laid significantly more eggs per cage on AC Excel (2111±346) than on AC Vulcan (1340±207) (T_{23}=-8.09, P<0.0001) (Figure 3.3). Females laid a total of 190 egg masses on AC Excel with 133±7.5 eggs per mass and 143 egg masses on AC Vulcan with 112±7.9 eggs per mass. The number of eggs per egg mass was not significantly different between the two cultivars ($F_{1,331}$=3.6, P=0.058). Most eggs were found in the middle vertical third of the plants followed by the top and bottom thirds on both AC Excel and AC Vulcan (P=0.0007and P=0.010, respectively) (Figure 3.3).

There were significant differences in the number of eggs laid on the four crucifer cultivars tested in the field-cage choice test ($F_{2,6}$=5.52, P=0.036) (Table 3.1). AC Pennant received the most eggs per cage followed by AC Excel, AC Vulcan and Dodolla, respectively. Females laid a total of 164 egg masses on AC Pennant with 129±9.1 eggs per

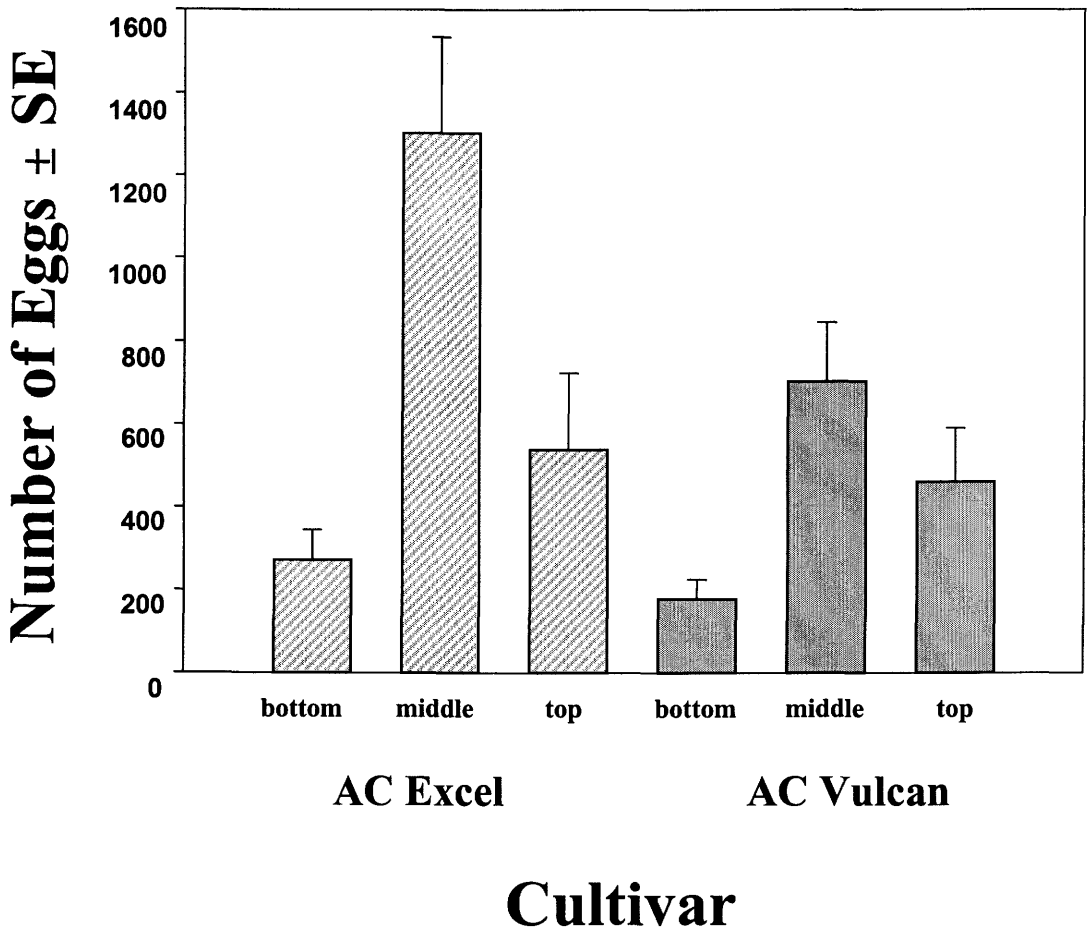

Figure 3.3. Mean number of bertha armyworm eggs laid on each vertical third of *B. napus* AC Excel and *B. juncea* AC Vulcan in a dual-choice field-cage experiment

Table 3.1. Available leaf material and bertha armyworm oviposition on four crucifer cultivars

Species	Cultivar	Number of Eggs per cage ±SE	Leaf Material per plant (g dry wt) ±SE
Brassica carinata	Dodolla	523±158a	2.91±0.44a
Brassica juncea	AC Vulcan	730±184ab	2.52±0.22a
Brassica napus	AC Excel	1208±193bc	2.74±0.44a
Sinapis alba	AC Pennant	1767±464c	*1.48±0.23b

Means followed by the same letter are not significantly different (LSD, p>0.05).
A cage contained 16 plants of each cultivar.
*AC Pennant had more leaf surface area per unit dry weight than the Brassica cultivars, thus to convert to relative leaf area divide 1.48 by 0.85.

mass, 147 egg masses on AC Excel with 99±6.9 eggs per mass, 93 egg masses on AC Vulcan with 94±8.8 eggs per mass and 60 egg masses on Dodolla with 105±10.2 eggs per mass. The number of eggs per egg mass was not significantly different among the four cultivars ($F_{3,415}$=2.2, P=0.086).

There were also significant differences in the amount of leaf material on the four cultivars ($F_{3,16}$=3.36, P=0.045) (Table 3.1). AC Pennant had the least leaf material followed by AC Vulcan, AC Excel and Dodolla, respectively.

In the no-choice greenhouse experiment significantly more eggs were laid on AC Excel than on AC Vulcan after five nights of oviposition ($F_{6,5}$=4.82, P=0.05) (Figure 3.4).

Plant Phenology Preference Experiment. In the field-cage choice experiment female moths laid significantly more eggs on plants in full-flower (2663±388) than on pre-flower (1045±152) or pod plants (549±129) ($F_{2,33}$=19.24, P<0.0001) (Figure 3.5a).

On full-flower plants, significantly more eggs were found in the middle third of the crop canopy than on the top or bottom ($F_{2,33}$=7.56, P=0.002) (Figure 3.5a). Significantly more eggs were found on the top third of pre-flower plants than in the middle or bottom thirds ($F_{2,33}$=3.63, P=0.037) (Figure 3a). There were no significant differences in the number of eggs on each vertical third of pod plants ($F_{2,33}$=1.93, P=0.16) (Figure 3.5a).

Full-flower plants had significantly more leaf material in the middle vertical third than in the bottom or top third ($F_{2,27}$=19.26, P<0.0001) (Figure 3.5b). However, pre-flower plants had significantly more leaf material in the top third of the plants than in the middle or bottom third ($F_{2,27}$=15.26, P=0.0001) (Figure 3.5b). Pod plants had more leaf material

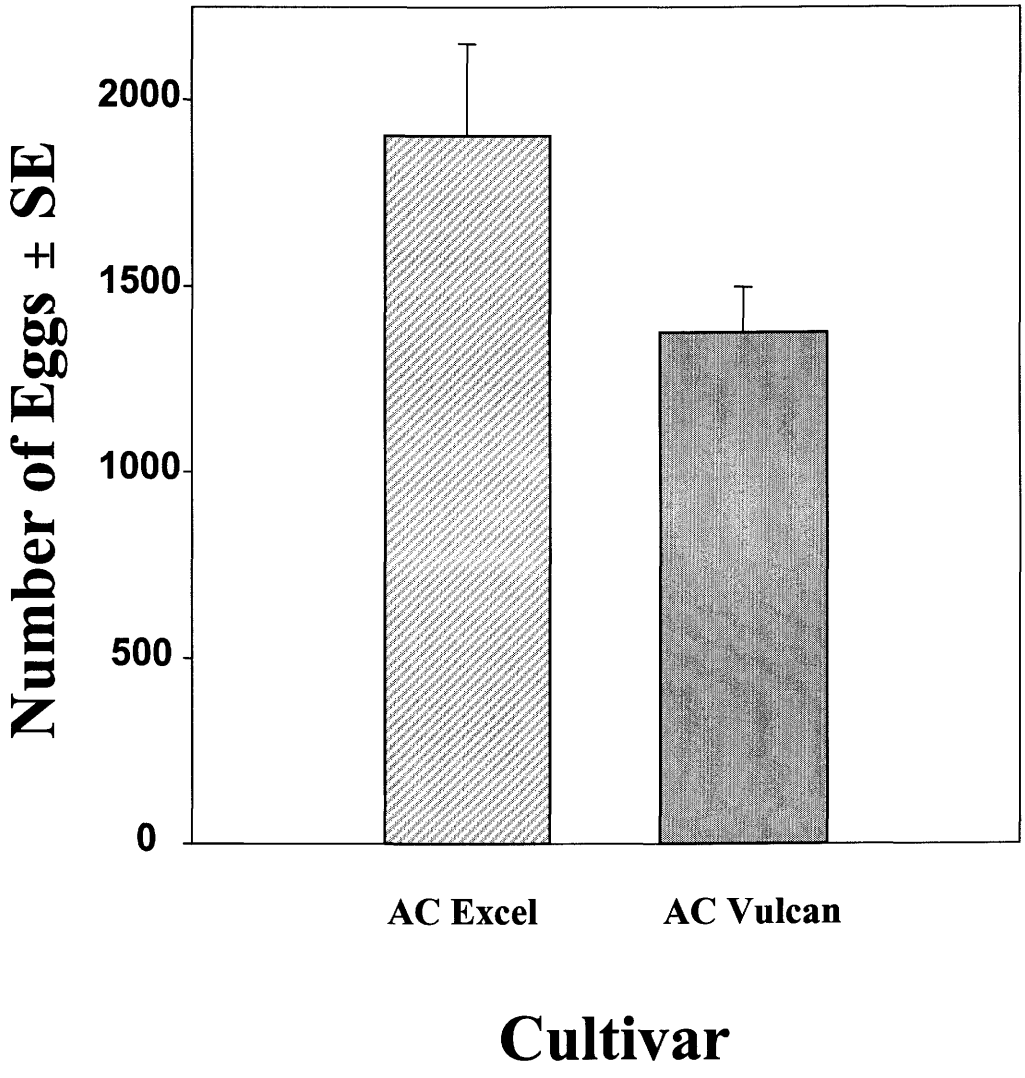

Figure 3.4. Mean number of bertha armyworm eggs laid on *B. napus* AC Excel and *B. juncea* AC Vulcan in a 5-day no-choice experiment.

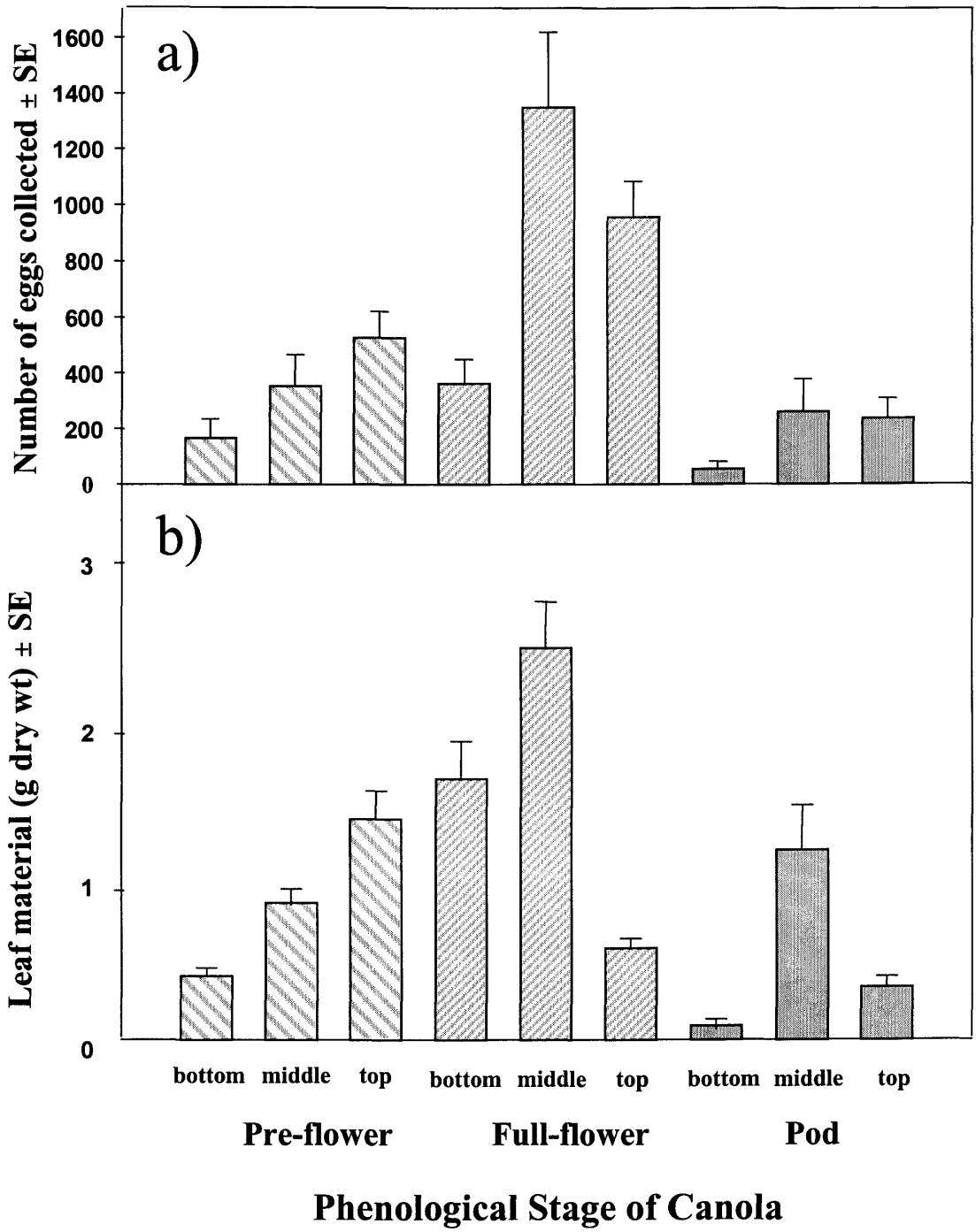

Figure 3.5. (a) number of bertha armyworm eggs collected and (b) amount of leaf material available for oviposition on each vertical third of pre-flower, full-flower and pod *B. napus* AC Excel plants in a field-cage experiment

in the middle vertical third than in bottom or top thirds ($F_{2,27}$=12.21, P=0.0002) (Figure 3.5b).

Egg Mass Position. In the dual-choice and plant phenology field cage experiments 751 egg masses containing 92,506 eggs were collected; thus, a bertha armyworm egg mass averaged 123±3.9 eggs. Egg mass size ranged from 1 to 778 eggs with a median value of 103 eggs per mass. Significantly more egg masses were laid on the lower surface of leaves (92%) than on any other plant part ($F_{3,15}$=13.65, P<0.0001) (Table 3.2). Small numbers of eggs were laid on the upper surface of leaves (5%) as well as on stems, petioles, flowers and pods (Table 3.2).

Temperature Data. There was no apparent relationship between temperature and bertha armyworm oviposition in the present study. The mean 48 h temperature for the outdoor experiments ranged from 14.1 to 22.0 °C. The maximum temperature recorded during the field experiments was 29.1 °C and the minimum temperature was 7.8 °C. These temperatures are well within the range at which bertha armyworm calling, mating and oviposition occur (Howlader and Gerber 1987).

3.4 Discussion

Ovipositing bertha armyworm laid more eggs on the *B. napus* cultivar AC Excel than on the *B. juncea* cultivar AC Vulcan in the no-choice experiment with full-flower plants, in the dual-choice field experiment and in the field experiment with four crucifer

Table 3.2. Oviposition site selection on the plant types tested in the 2000 field season, measured as the number of egg masses collected from each location

	bottom of leaf	top of leaf	stem/petiole	flower/pod
Phenology study				
pre-flower	103	5	3	n/a
flower	245	4	8	1
pod	36	7	1	5
Dual-choice cultivar study (full-flower plants)				
B. juncea AC Vulcan	136	6	1	0
B. napus AC Excel	173	15	2	0
Totals	693[a]	37[b]	15[b]	6[b]

Means followed by the same letter are not significantly different (LSD, $p>0.05$)

cultivars. The lower oviposition preference of bertha armyworm for AC Vulcan compared to AC Excel parallels results of previous larval feeding experiments in which *B. juncea* lines were less preferred by feeding larvae relative to several other crucifer plant lines, including AC Excel, and slowed larval growth and development (McCloskey and Isman, 1993; Section 2.3). The resistance to larval feeding has been attributed to high levels of sinigrin, the predominant foliar glucosinolate among the *B. juncea* lines tested (McCloskey and Isman, 1993; Shields and Mitchell, 1995a; Section 3.2). Sinigrin is found in a wide range of plant species which produce glucosinolates (Daxenbichler et al., 1991), and it is likely that bertha armyworm has evolved with plants containing this compound. Larval bertha armyworm have been shown to have sinigrin-sensitive sensilla, the stimulation of which deters feeding (Shields and Mitchell, 1995b). It would be valuable to examine whether adult females are also able to detect sinigrin and are deterred from ovipositing on those plants that contain high levels of this glucosinolate.

Sinapis alba cultivars, including AC Pennant (Section 2.3), have also been shown to be relatively resistant to larval feeding (Bodnaryk, 1991). Though *S. alba* cultivars lack sinigrin, the glucosinolate sinalbin, which is present in the foliage of *S. alba* cultivars, has been linked to this resistance. Larvae which feed on these plants are destined to slower development and lower survival than those on a more suitable host (Bodnaryk, 1991). However, in the present study AC Pennant was the most attractive host plant for oviposition. Although sinalbin is detrimental to larval development and survival, adult females may not be equipped with sensilla that detect sinalbin. This apparent contradiction may be a result of the relatively short, if any, evolutionary history that the bertha

armyworm has with *Sinapis* spp. and their unique glucosinolate profiles. *Sinapis arvensis* is thought to have been present in the northeastern United States as early as 8000 years b.p. (Warwick, 1993), though it is unclear if or when *Sinapis* spp. occurred in the range of the bertha armyworm in the past. Host-plant acceptance is a trade-off between attractants and deterrents; thus, even if adult females could detect sinalbin, perhaps there are other host-plant characteristics which are so strongly attractive that any inhibitory effect of sinalbin is overridden. For example, compared to the other cultivars tested in the present study, AC Pennant plants were distinct in terms of leaf architecture and had a more than 10 times greater density of foliar trichomes relative to the *Brassica* cultivars tested (Ulmer, unpublished data). Increased trichome density has been shown to be attractive to several other noctuids during oviposition site selection (Hillhouse and Pitre, 1976; Navasero and Ramaswamy, 1991; Mascarenhas and Pitre, 1997). Female bertha armyworm may show a similar preference for the increased trichomes on *S. alba* plants.

Brassica napus AC Excel plants in full-flower are the most attractive growth stage for ovipositing bertha armyworm. Significantly more egg masses and eggs were laid on full-flower plants than on pre-flower or pod plants. Although full-flower plants have more available leaf material for oviposition than the pre-flower or pod plants, this difference is relatively small when compared to the number of additional eggs found on the full-flower plants. The oviposition preference for flowering plants in the present study is consistent with previous pheromone trap-count data which indicated that flowering plants may be more attractive to adult bertha armyworm than other growth stages (Turnock, 1984b). A similar oviposition preference for flowering plants has also been observed for other

polyphagous noctuids. Soybean looper, *Pseudoplusia includens,* shows a strong preference for flowering soybean, its preferred host, relative to pre-flower plants (Felland et al., 1992) and corn earworm, *Heliothis zea*, lays significantly more eggs on flowering snap beans than on pre-flower or post-bloom plants (McLeod, 1988). Corn earworm has also shown a similar preference for the flowering stage of several other host plants (Johnson et al., 1975).

In the present study bertha armyworm moths were observed feeding on the nectar of flowering plants and this source of food may be in part responsible for the attraction to this growth stage. The potential importance of nectar to ovipositing *H. zea* was suggested by McLeod (1988) in a no-choice study in which females on flowering snap bean plants maintained egg production throughout the 6-day study while those on pre-flower and post-bloom plants showed dramatically reduced egg production after the third day. Crop canopy development has also been associated with soybean looper oviposition preferences, females preferring plants at the flower or early pod stage which provide the most dense crop canopy (Felland et al., 1992; Mascarenhas and Pitre, 1997). Similarly, flowering canola plants provide a more dense crop canopy than either the pre-flower or pod plants which may be attractive to ovipositing bertha armyworm. The increased foliage on flowering plants relative to other growth stages may also protect eggs and neonate larvae and provide them with the micro-environment they require for optimal development, as well as providing the largest potential food source.

In this study the preferred site for bertha armyworm oviposition within the crop canopy was related to the amount of leaf material in each vertical third of the crop. Most

oviposition among full-flower and pod plants occurred in the middle vertical section of the crop canopy where leaf material was greatest. Likewise, oviposition on pre-flower plants was greatest on the top third where most leaf material occurred. However, the available leaf material did not fully explain the observed oviposition behaviour. Among full-flower plants, the top third of the canopy received more than twice as many eggs as the bottom third despite having less than half as much leaf material. A similar preference for the upper part of the host plant has also been observed among other noctuids (Hillhouse and Pitre, 1976; Mascarenhas and Pitre, 1997; Sappington et al., 2001). Ovipositing bertha armyworm may be attracted to canola flowers and as a result lay most eggs on the upper part of the plant. Leaf tissue in the upper part of the canopy is also younger and as a result the leaves are more densely covered with trichomes which may be an oviposition stimulant. This behavior would also benefit larvae which are negatively geotropic and may find the younger tissues near the top of the plant easier to ingest.

Although eggs were laid on all parts of the crucifer plants tested, bertha armyworm laid 92% of their eggs on the underside of leaves. A preference for laying on the underside of leaves also occurs among other noctuids (Pansera-de-Araujo et al., 1999; Mascarenhas and Pitre, 1997; Sappington et al,. 2001). The lower surface of the leaf may protect eggs from adverse weather conditions and may also reduce the chances of detection by natural enemies. Increased oviposition on the underside of the leaf by other noctuids has also been associated with an increased trichome density on the lower leaf surface (Hillhouse and Pitre, 1976; Navasero and Ramaswamy, 1991; Mascarenhas and Pitre, 1997). Trichome density may be responsible for increased oviposition on the lower surface of hairy cultivars

such as AC Pennant, which has a greater density of trichomes on the lower leaf surface (Ulmer, unpublished data). However, females also preferred the lower leaf surface of cultivars which had relatively few trichomes ($<2/cm^2$) on either leaf surface such as AC Excel.

This study has shown that bertha armyworm moths prefer flowering crucifer plants for oviposition, most of which occurs on the underside of leaves in the upper part of the crop canopy. Females also show oviposition preferences among different cultivars of cruciferous plants, though further study is required to determine which characteristics are responsible for attracting and deterring oviposition on these cultivars.

Providing information on the most susceptible *Brassica* growth stages and cultivars, coupled with pheromone trap-count data, will assist in alerting canola producers to economically important bertha armyworm infestations earlier in the insect's development. Knowing the preferred location for oviposition will also facilitate development of more efficient scouting protocols.

4 The Effect of Conspecific Eggs on Bertha Armyworm Oviposition Site Selection

4.1 Introduction

The bertha armyworm, *Mamestra configurata*, is a noctuid moth native to Canada, found from British Columbia eastward across the Canadian prairies. Bertha armyworm oviposit at night and lay their eggs in masses or clusters on the undersides of host-plant leaves. Larvae are generalist feeders, although crucifers are among their preferred hosts and in outbreak years bertha armyworm cause significant damage to *Brassica* crops on the Canadian prairies.

The majority of Lepidoptera lay their eggs singly and their larvae develop solitarily (Stamp, 1980; Herbert, 1983). However, approximately 5% of the North American butterflies lay their eggs in clusters (Stamp, 1980) and a similar frequency of egg-clustering species (8%) has been shown among forest-inhabiting moths in Canada (Herbert, 1983). Laying eggs singly is considered the ancestral state and the proportion of Lepidoptera which adopt this strategy would indicate that laying eggs singly is generally advantageous; however, egg clustering has evolved independently several times in Lepidoptera (Sillen-Tullberg, 1988), suggesting that under certain circumstances, this may be more beneficial.

The strategy of egg-clustering may hold advantages for the eggs, adults or larvae. Egg clustering may protect eggs from desiccation as well as from environmental factors including egg parasites. Energy conservation may be a driving force for adult females

which cluster eggs. Species which do not feed as adults, species which oviposit or feed on patchily distributed host plants, or species which are at high risk of predation while in flight may benefit from laying many eggs at one time (Stamp, 1980; Herbert, 1983).

Egg clustering usually leads to larval aggregations (Clark and Faeth, 1997) which often positively affect larval development and survival. Larval feeding aggregations have been associated with advantages such as increased growth and survival, as well as reduced predation and parasitism (Lawrence, 1990; Clark and Faeth, 1997; Denno and Benrey, 1997). Larval advantages that accrue from egg clustering could also be achieved or enhanced by species which oviposit near previously laid conspecific eggs.

Bertha armyworm oviposition habits and larval development appear to be similar to those observed in other egg-clustering Lepidoptera whose larvae gain advantages from feeding in aggregations. Casual observations during the studies of bertha armyworm oviposition (Chapter 3) suggested that gravid females may preferentially lay eggs near conspecific eggs (Figure 4.1). In this study we examined whether gravid bertha armyworm are attracted to conspecific eggs, both their own and those of other females. Bertha armyworm oviposit at night on the undersides of leaves; thus, it is likely that if conspecific eggs are attractive to gravid females, the attraction is a chemical cue. We investigated this phenomenon using egg washes and choice tests similar to those used to examine oviposition-deterring pheromones in other Lepidoptera (Klijnstra, 1986; Schoonhoven, 1990).

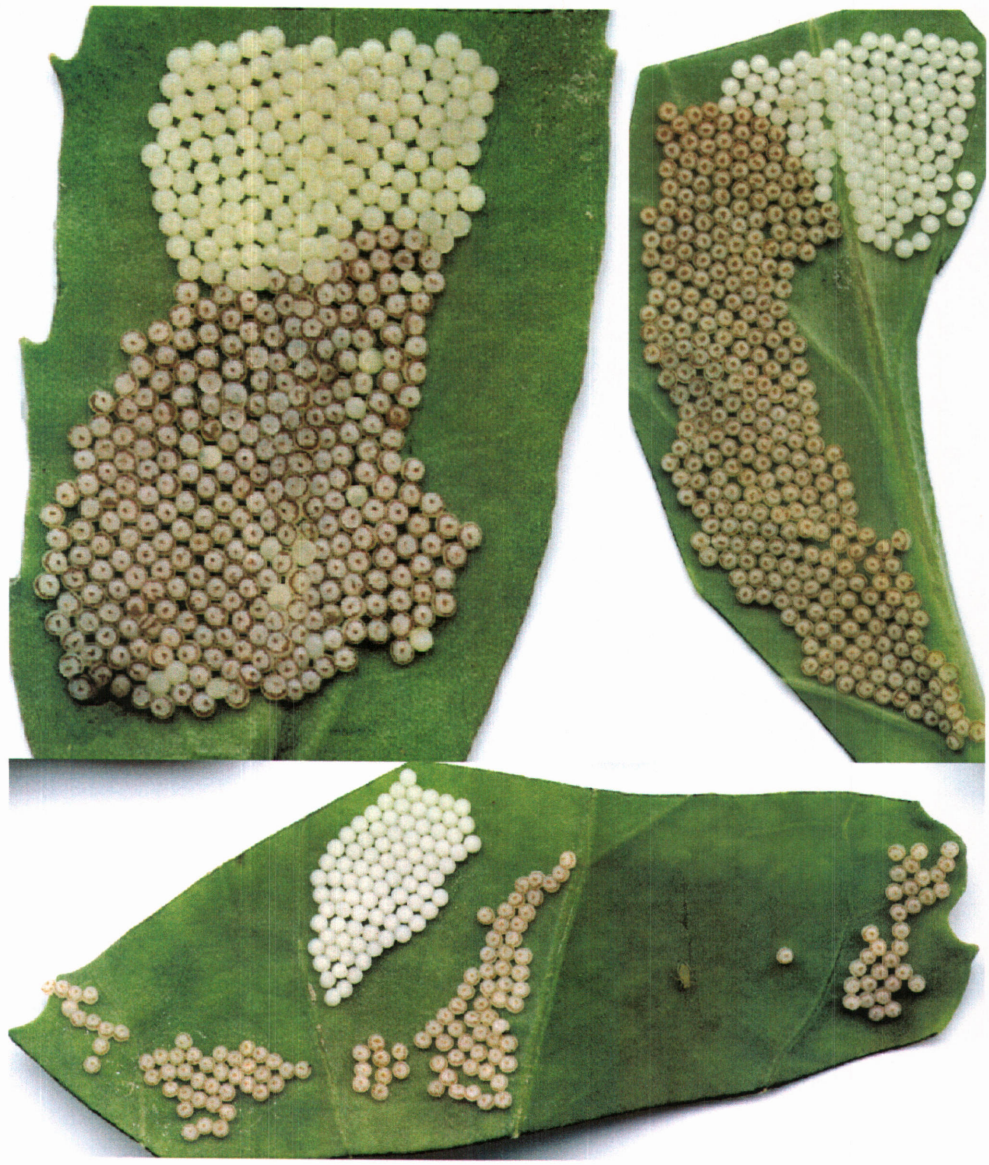

Figure 4.1. Bertha armyworm egg masses (light) laid beside conspecific egg masses which were laid 24 h earlier (dark)

4.2 Materials and Methods

Insects. A laboratory colony of bertha armyworm was reared on a semi-synthetic diet (Bucher and Bracken, 1976) at 21°C, 60% RH and under a 20L:4D photoperiod. The genetic diversity of the colony was maintained by annually mating colony insects with moths derived from field-collected pupae. The colony cycles through five to six generations per year. Upon pupation, individuals were sexed, transferred to 500 mL plastic tubs with screened lids at 21°C, 60% RH and a 16L:8D photoperiod, and held until adults emerged. The male and female moths used in the present experiments were introduced into a screened cage (38 x 26 x 26 cm) for mating within 2 days of eclosion. After a 48-h mating period, adults were exposed to plant material. Thus, females were exposed to plant material during the third, fourth or fifth scotophase which is when bertha armyworm lay the most eggs (Howlader and Gerber, 1986).

Field-cage experiment. The oviposition experiment reported here was carried out during the course of a more extensive study of bertha armyworm site selection on crucifer host plants at the AAFC Research Farm near Saskatoon (see Section 3.2, plant phenology preference experiment).

Effect of conspecific eggs on oviposition using excised leaves. Individual leaves of *B. napus* AC Excel were used to test the effect of previously laid eggs on bertha armyworm oviposition. Seeds of *B. napus* AC Excel were sown directly in a soil-less mix and grown in 10-cm pots, two plants per pot, at 22°C and a 16L:8D photoperiod in a greenhouse. A

single virgin female moth was placed in a cage (38 cm high x 26 cm wide x 26 cm long) with 2 males for a 48-h mating period with no exposure to plant material. A single plant (growth stage 3.2; Harper and Berkenkamp, 1975) was then introduced into the cage and inspected for eggs after 24 h. If a leaf with an egg mass of about average size (100-150 eggs) was found, it was cut off at the base of the petiole. An egg-free leaf of approximately the same size and age was then cut from the same plant. The petiole of each leaf was secured with a foam-rubber plug and immediately inserted into a 50-mL flask of water. The two leaves from the same plant were then offered to the same female in the same cage for 24 h, then inspected for new eggs. If new egg clusters were present, the leaves were removed and the eggs were counted on each leaf. If no new egg clusters were present, the leaves were left in the cage for a further 24 h, after which eggs were counted. If no new egg clusters were found after the second day, the experiment was terminated with that female. A 10% sucrose-honey solution was offered to the moths as food by means of a wick in a covered plastic bottle. The experiment was conducted in a growth chamber with a 16L:8D photoperiod and at temperatures ranging from 17°C (night) to 25°C (day) and was repeated 16 times.

A similar protocol was used to test the effect of previously laid eggs on a different female. A leaf with eggs laid by one female during the previous scotophase was paired with an egg-free leaf from the same plant and offered to a different female which had also laid eggs for the first time during the previous night. The experiment was repeated 22 times.

Methanol egg wash experiment. Eggs laid by colony-derived females on AC Excel host-plants were collected and removed with a small metal spatula, causing as little damage to the leaf surface as possible. To prepare the egg wash, 0.2 g of freshly collected eggs (approximately 2000 eggs) were washed in 1.5 mL of methanol (Klijnstra, 1986). The eggs were gently agitated in the methanol for 5 minutes in a 1.5 mL microfuge tube, after which the mixture was centrifuged at low speed to pellet the eggs.

Individual leaves of *B. napus* AC Excel, grown under the same conditions as those in the above excised leaf experiment, were used to test the effect of methanol egg wash on bertha armyworm oviposition. A single virgin female moth was placed in a cage (38 cm high x 26 cm wide x 26 cm long) with two males for a 48-h mating period with no exposure to plant material. Two leaves of approximately the same size and age were cut from an AC Excel plant. The petiole of each leaf was secured with a foam rubber plug and immediately inserted into a 50-mL flask of water. A 200μL aliquot of the methanol egg-wash was pipetted evenly to the underside of one leaf: A 200μL aliquot of methanol was applied to the underside of the control leaf. Fifteen minutes after the methanol was applied, the pair of leaves was placed in a cage with a mated female for 24 h, after which the leaves were inspected for eggs. If eggs were present on either leaf, the leaves were removed and eggs were counted. If no eggs were present after 24 h, the female was excluded from the experiment. During the mating period and throughout the experiment, a 10% sucrose-honey solution was offered to the moths as food by means of a wick in a covered plastic bottle. The experiment was conducted in a growth chamber with a 16L:8D photoperiod at 21°C and repeated 21 times.

Data Analysis. A paired t-test was used for the dual-choice oviposition preference experiments testing the effect of conspecific eggs on single leaves, and the Sign Test was used for the methanol egg wash experiment. A one-way ANOVA was performed on the field experiment data (Statistix 4.0, Analytical Software, Tallahassee Florida).

4.3 Results

Field-cage experiment

Flowering plants are the most preferred growth stage for bertha armyworm oviposition and in the present study received significantly more eggs than the other growth stages (see Section 3.3). As a result, only the data from flowering plants are presented, though the trends were similar for the pre-flower and pod plants. Significantly more egg masses were deposited on plants which previously contained at least one conspecific egg mass, a total of 159 egg masses, than on plants that contained no previously laid eggs, a total of 79 egg masses ($P=0.001$, $F_{1,22}=7.98$) (Figure 4.2). Significantly more (3.5 times) plants received two or more egg masses than plants which received only one egg mass ($P<0.0001$, $F_{2,33}=31.65$) (Figure 4.3). These results are not due to saturation of the system as 20% of flowering plants received no egg masses and many of the plants at the other growth stages received no eggs.

Effect of conspecific eggs on oviposition using excised leaves

Bertha armyworm laid more eggs on leaves which already had their own eggs than they did on leaves from the same plant which had no eggs; however, the difference was not

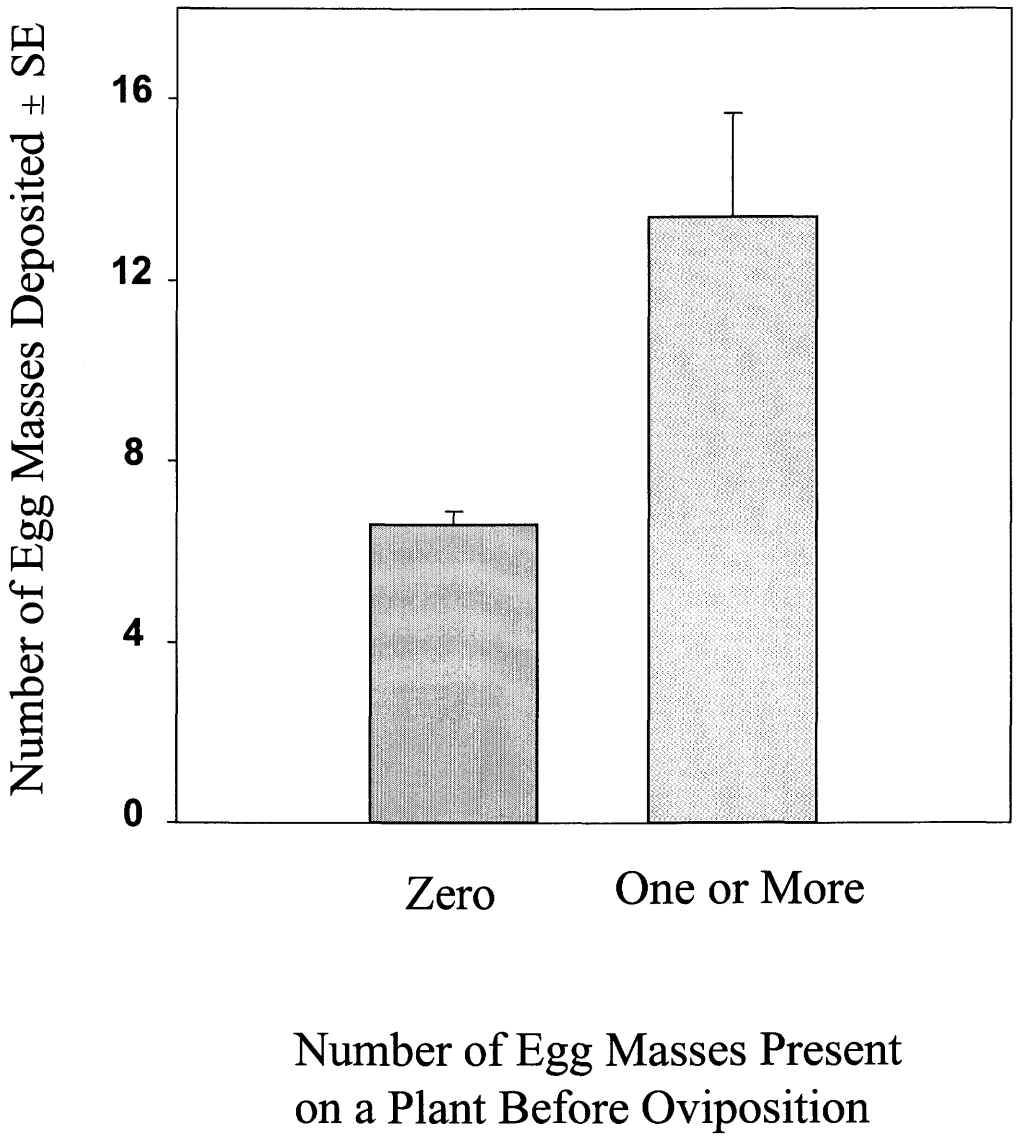

Figure 4.2 Bertha armyworm oviposition in the field-cage experiment. The mean number of egg masses deposited per cage on flowering canola plants which had at least one conspecific egg mass is compared to the number of egg masses deposited on flowering canola plants which previously had no eggs.

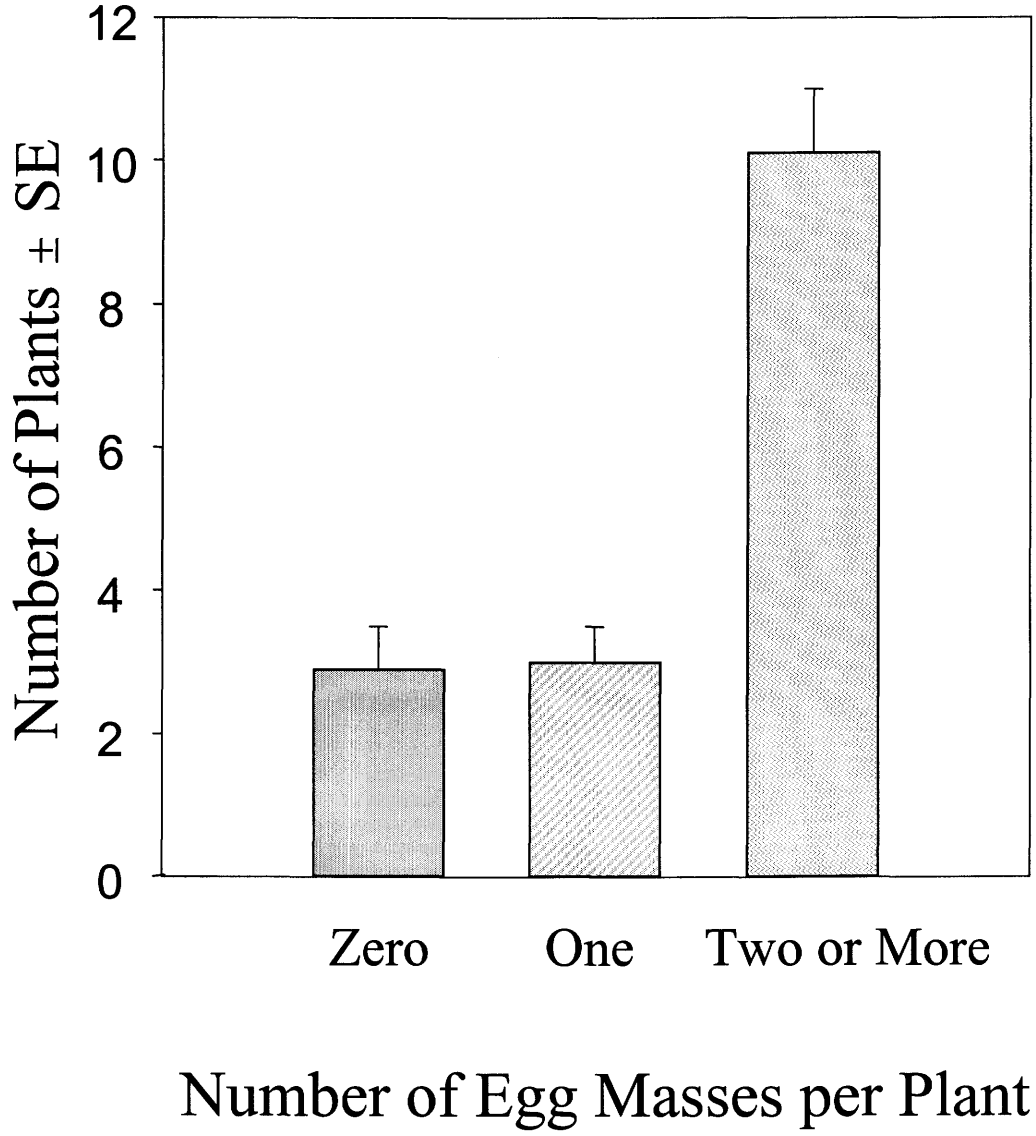

Figure 4.3. Bertha armyworm oviposition in the field-cage experiment. The mean number of flowering canola plants per cage with zero egg masses, one egg mass or more than one egg mass are compared.

significant (t=0.2, df=15, P=0.84) (Figure 4.4). Sixteen females laid 22 egg masses containing 2078 eggs on leaves which had their own previously laid eggs and laid 16 egg masses containing 1648 eggs on the control leaves which had no previously laid eggs.

Females laid significantly more eggs on leaves which had another female's eggs than on leaves from the same plant which had no previously laid eggs (t=2.46, df=21, P=0.02) (Figure 4.4). Twenty-two females laid 28 egg masses containing 4520 eggs on leaves which had eggs laid previously by another female but laid only 12 egg masses containing 1618 eggs on the control leaves which had no previously laid eggs.

Methanol egg wash experiment

Bertha armyworm laid significantly more eggs on leaves treated with methanol egg wash (27 egg masses containing 4709 eggs) than on control leaves treated with methanol (14 egg masses containing 2760 eggs) (Sign Test, P=0.04, 15 positive: 6 negative) (Figure 4.5).

4.4 Discussion

In both field and laboratory experiments female bertha armyworm showed an oviposition preference for host-plants on which conspecific eggs had been laid over host-plants which had no eggs on them. Gravid females also showed a preference for host-plants which had been treated with methanol egg wash over a control host-plant, indicating that the basis for the attraction is at least to some degree based on chemical cues.

Egg clustering and the attraction of laying females to conspecific eggs observed in

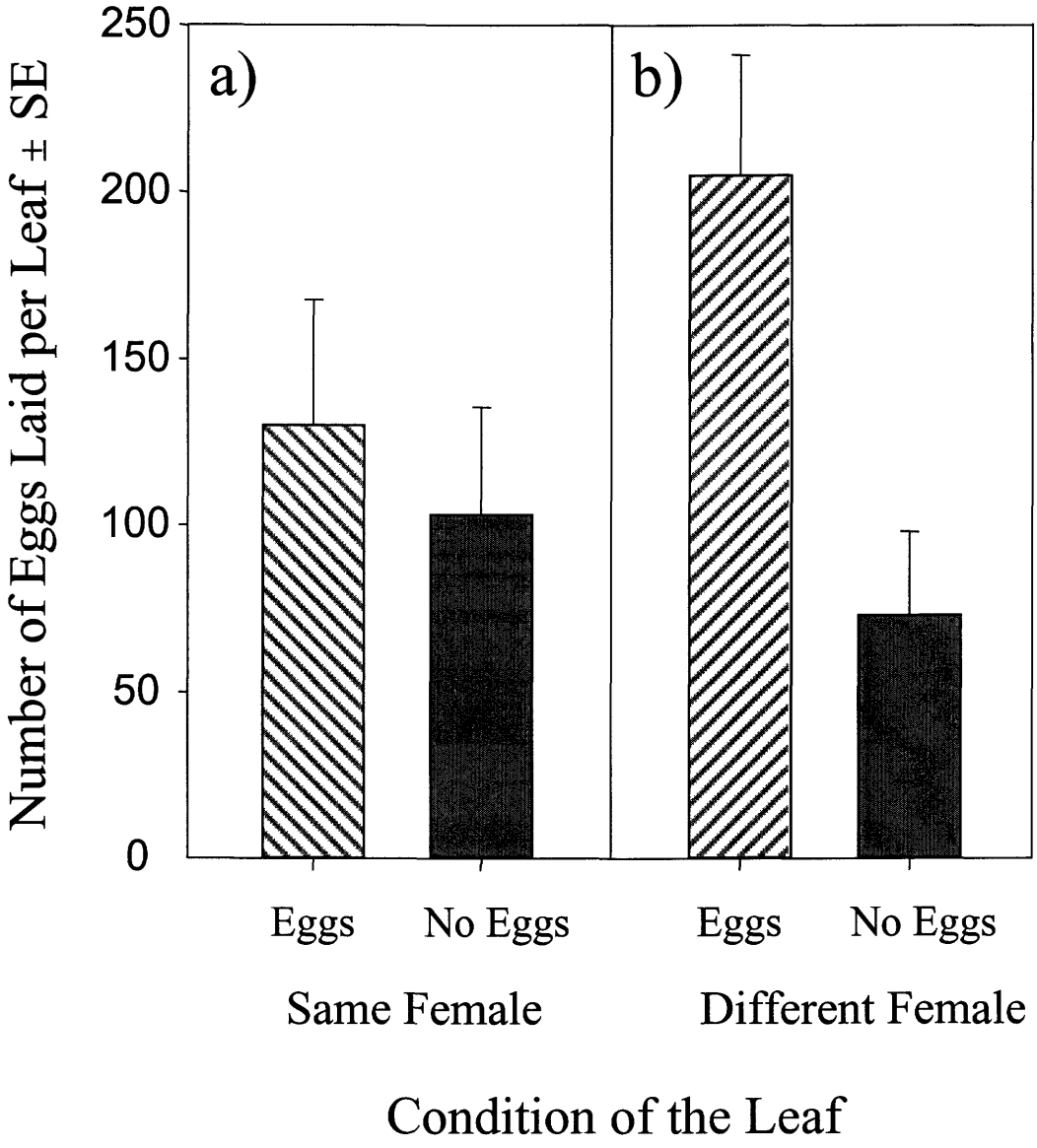

Figure 4.4. Bertha armyworm oviposition in the dual-choice single-leaf experiment. Females were offered a leaf with conspecific eggs and a leaf with no eggs. (a) Mean number of eggs laid by females offered a leaf with their own eggs and a leaf with no eggs. (b) Mean number of eggs laid by females offered a leaf with another females eggs or a leaf with no eggs.

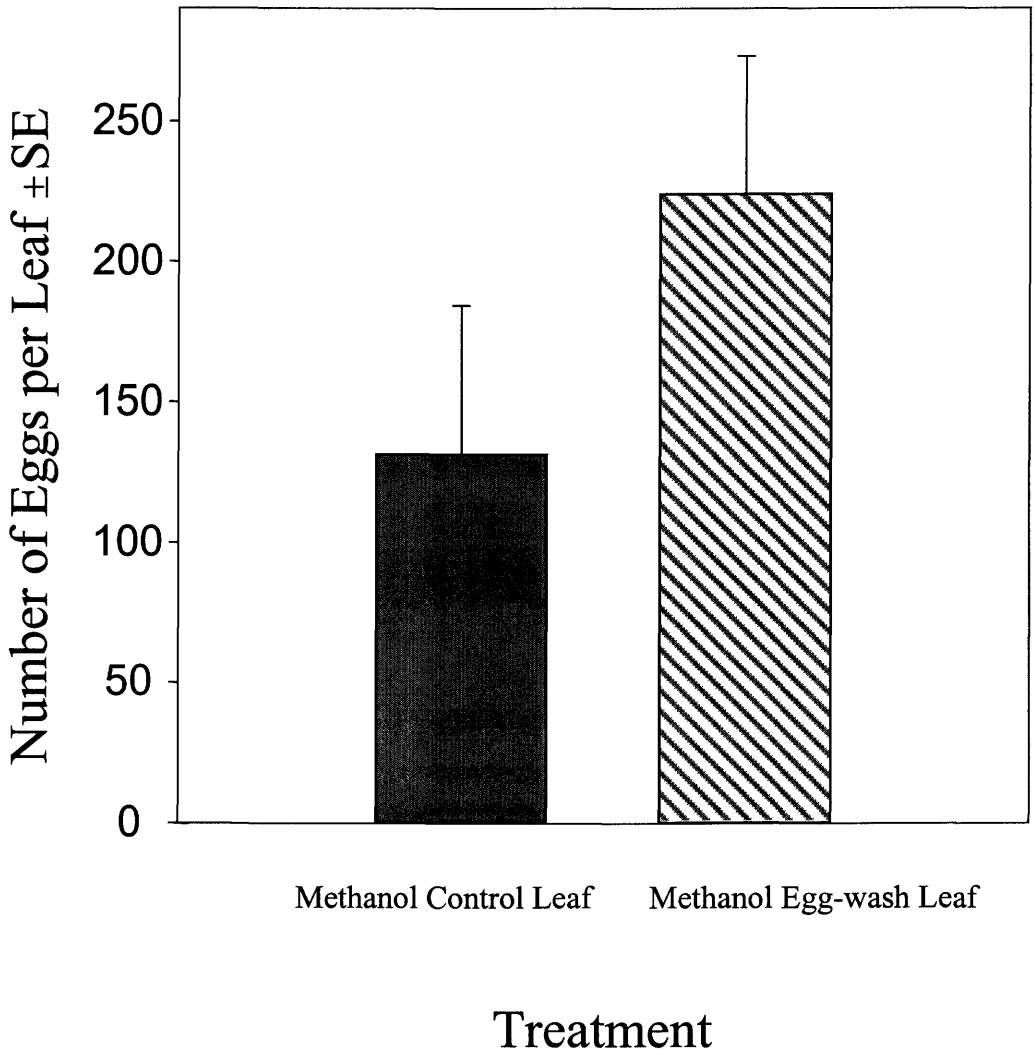

Figure 4.5. Mean number of eggs laid by bertha armyworm in the dual-choice methanol egg-wash experiment. Females were offered a leaf treated with methanol egg-wash and a control leaf.

the bertha armyworm may benefit the adult, the eggs or the larvae. Although the ancestral host plants of the bertha armyworm are unclear, the host plants today tend to be annual or biennial plants, including many crucifers, which are often colonizers of disturbed areas (Harris and Rogers, 1988). Before modern agriculture, such plants probably would have been patchily distributed. Thus, a chemical cue from conspecific eggs may have aided females in locating suitable host plants which were isolated in distribution. Egg clustering might also reduce flight time spent searching for host plants, lowering energy consumption as well as risks associated with flight, notably predation (Stamp, 1980). However, bertha armyworm adults are strong fliers and are able to feed which would reduce the drawbacks of increased flight. Egg clustering could also help prevent egg desiccation (Clark and Faeth, 1998) and reduce egg parasitism (Stamp, 1980), though bertha armyworm lay eggs in a single layer which would reduce these benefits. Thus, egg clustering may offer greater benefits to the larval stage and these benefits may be further exploited by female attraction to conspecific eggs for oviposition.

Although the attraction of gravid females to conspecific eggs is not common among Lepidoptera studied to date, there are some neotropical butterflies which are attracted to conspecific eggs or lay multiple egg clusters on the same host plant (Mallet, 1980; Clark and Faeth, 1997). The larvae of these butterflies, and others which lay their eggs in clusters, have been shown to gain several advantages from the resulting larval feeding aggregations (Lawrence, 1990; Denno and Benrey, 1997; Clark and Faeth, 1997). These larval aggregations have been associated with feeding facilitation, increased growth rates and decreased development time (Lawrence, 1990; Clark and Faeth, 1997; Denno and

Benrey, 1997). Larval aggregations have also been shown to reduce predation and parasitism, which may be a result of predator/parasitoid dilution, increased effectiveness of larval defense postures in large groups, or predator avoidance, especially among those species which are distasteful and conspicuously colored (Clark and Faeth, 1997). Larvae of these species generally spend the first few instars cryptically colored then become darkly colored and conspicuous in later instars (Lawrence, 1990; Clark and Faeth, 1997). The larvae are often distasteful and it is thought that the dark conspicuous coloration warns predators and also helps the larvae thermoregulate. The larval stage of bertha armyworm is similar in many ways to these egg-clustering neotropical butterflies. Although bertha armyworm are not truly gregarious, larvae do exhibit a highly clustered distribution, particularly in the early developmental stages. Bertha armyworm larvae also spend the first few instars cryptically colored and hidden and become darkly colored and conspicuous in later instars. It would be of interest to determine if the size of larval feeding aggregations affects bertha armyworm growth, development and survival in a manner similar to that in larvae of the neotropical butterflies which have similar oviposition and larval growth habits.

Larval rearing density has been investigated in a related species, the cabbage moth, *Mamestra brassicae,* with similar oviposition and larval feeding habits (Goulson and Cory, 1995). Larval density had a profound effect on several aspects of larval *M. brassicae* physiology including rate of development, susceptibility to disease, weight and colour. Cabbage moth larvae which were reared on artificial diet showed the highest weight, highest disease resistance and fastest development time at the second highest of five

rearing densities, though at the highest rearing density these advantages were no longer observed (Goulson and Cory, 1995). The advantages of larval feeding aggregations have also been shown to cease or be reversed at extremely high densities among other species which feed in aggregations (Denno and Benrey, 1997; Clark and Faeth, 1997). Cabbage moth larvae also became darker as density increased (Goulson and Cory, 1995).

Gravid bertha armyworm females in the present study were more attracted to the eggs of another female than they were to their own eggs. Another female's eggs may indicate a plant has been evaluated and accepted as a suitable host-plant by a conspecific female, and thus may serve as an acceptance cue for other gravid females. Further, if large egg aggregations are beneficial to larvae, it might be advantageous for a gravid female to be more attracted by a different female's eggs than her own. If females are more strongly attracted to the eggs of another female, the number of eggs in an area and the subsequent larval aggregations have the potential to be much larger than that which a single female could produce.

The results of the methanol egg wash experiment indicate that the basis for the attraction of bertha armyworm females to conspecific eggs is at least in part chemically based. Females laid significantly more eggs on a host-plant leaf treated with methanol egg wash than on a host treated with methanol alone. Bertha armyworm oviposit at night and eggs are usually laid on the undersides of host plant leaves; thus, a chemical attractant may be an effective means of indicating the presence of eggs. This phenomenon may be a result of pheromones comparable to the oviposition-deterring pheromones found in *Pieris* spp. In choice tests similar to those in the present study (Klijnstra, 1986; Klijnstra and

Schoonhoven, 1987; Schoonhoven, 1990), *Pieris* spp. eggs were found to contain a non-volatile oviposition-deterring pheromone. The oviposition-deterring pheromone in *Pieris* spp. is thought to be produced in the accessory glands, which also produce the egg adhesive, and is released during egg deposition (Schoonhoven, 1990). Further study is required to determine the nature and source of the apparent oviposition-attracting pheromone acting in bertha armyworm.

The attraction of gravid bertha armyworm females to conspecific eggs is unique among noctuids studied to date. Although the attractant appears to be chemical, further investigation is required to determine the nature of the attraction and to isolate the compounds responsible. This would be of interest biologically but could also provide a means of attracting gravid females of this pest insect. Further study is also required to determine if bertha armyworm larvae gain the same advantages from larval feeding aggregations as the larvae of neotropical butterflies which share similar oviposition and larval growth habits.

5 Crucifer Host Plant Suitability for Diamondback Moth

5.1 Introduction

The diamondback moth, *Plutella xylostella* (L.), is a serious pest of crucifer crops in Canada and throughout the world. Diamondback moth populations on the Canadian prairies usually do not survive the winter months (Dosdall, 1994). However, they are a significant pest of *Brassica* oilseed crops in this region due to their short generation time and high fecundity. Adults are carried on winds from the south-western United States in the spring and where they arrive early and in sufficient numbers, their offspring cause significant damage to canola (*Brassica napus* L. and *B. rapa* L.). In years of population outbreaks these insects can cost oilseed producers tens of millions of dollars in crop losses and chemical control (Anonymous, 1995a). Though insecticides remain the primary defence against the diamondback moth, insecticide resistance has become a problem in many diamondback moth populations (Shelton et al., 1993). As a result, alternative methods of insect control, including plant-based resistance, are being investigated. One of the most promising examples of plant-based resistance is the reduced survival of larvae on glossy lines of *Brassica oleracea* (Lin et al., 1983; Eigenbrode and Shelton, 1990; Eigenbrode et al., 1991b; Eigenbrode and Pillai, 1998). The leaf-wax characteristics of the glossy lines affect the ability of first-instar larvae to mine the leaf tissue, leaving larvae exposed for extended periods and increasing larval mortality due to abiotic factors and natural enemies (Eigenbrode et al., 1991a; Eigenbrode et al., 1995).

In the present study 11 lines from five cruciferous species were tested for palatibility and suitability for larval feeding in choice and no-choice experiments with second- to fourth-instar larvae of diamondback moth. Additional experiments were carried out with the *B. rapa* lines to determine if glossy *B. rapa* expresses similar resistance to diamondback moth as that observed with glossy lines of *B. oleracea*. The *B. rapa* lines, including the near isogenic 'Glossy' and 'Waxy' lines, were tested for diamondback moth oviposition and first-instar feeding preference in choice experiments and in a no-choice situation to determine survival through the first instar on each plant line.

5.2 Materials and Methods

Insects. Cultures of diamondback moth were reared on whole plants (*B. napus* cv. Excel) at 21°C, 60% RH, and 16:8 L:D photoperiod. Plants grown in 10-cm pots, at the pre-bolt stage, were placed in mesh cages (38 cm high x 26 cm wide x26 cm long) into which approximately 50 adult moths were released to mate and oviposit. A 10% sucrose-honey solution was offered to the adults as food by means of a wick in a covered plastic bottle. As the larvae developed and defoliated a plant, new plants were placed in the cages.

Plant material. Eleven lines from five species of Brassicaceae were selected for testing based primarily on their relative seed glucosinolate content. Where available, a high and a low glucosinolate line of each species was selected, though it should be noted that the designations 'high' and 'low' are relative only within each species. The plant lines selected were: *Sinapis alba* L. [AC Pennant and Low-glucosinolate or L-GS (line 93-

0860)], *Brassica carinata* L. [Dodolla and S-67], *B. rapa* L. [AC Boreal, Echo, Glossy (line CB9625) and Waxy (line CB9626)], *B. napus* L. [Midas and AC Excel], and *B. juncea* L. [AC Vulcan]. *Brassica rapa* 'Glossy' and 'Waxy' near isogenic lines were developed by crossing CrGC line 1-13 (obtained from the Crucifer Genetics Cooperative, Department of Plant Pathology, University of Wisconsin) to AC Sunshine followed by two backcrosses to a white rust (*Albugo candida* (Pers. ex Lev.) Ktze.) race 7v resistant line derived from AC Sunshine. As the glossy character is recessive, at each generation the cross was grown to F_2 and the successive cross made to the glossy segregants. BC_2F_2 plants were grown in the greenhouse, separated based on foliage waxiness, and allowed to interpollinate. From the harvested material, two bulks of seed were created, designated CB9625 ('Glossy') and CB9626 ('Waxy'). These two bulks were increased in isolation in the field in 1997, with roguing based on leaf waxiness, to provide adequate seed for further testing.

All plant lines were sown directly into a soil-less mix and grown at 22°C, 16:8 L:D photoperiod in a greenhouse. Plants were grown in 10-cm pots, two plants per pot, and watered daily.

Oviposition and first-instar choice test. The 'Glossy' and 'Waxy' lines of *B. rapa* were offered to female diamondback moth in a choice experiment to measure oviposition. A 'Glossy' and a 'Waxy' plant were grown in the same 10-cm pot such that the plants were in physical contact with one another in several places. The plants were placed in a mesh cage (38 cm x 26 cm x 26 cm) at the bud stage. Thirty moths (1:1 sex ratio) were

introduced into the cage and allowed access to both plants for 4 days. The plants were then destructively sampled and eggs were counted on both *B. rapa* lines. The experiment was conducted at 23±2 °C and 16:8 L:D and was replicated 10 times.

The same protocol was used for the first-instar choice experiments. The 'Waxy' and 'Glossy' plants were destructively sampled. The first-instar larvae, which hatched from eggs laid in the first 48 h, were counted on each plant line 6 days after the adult moths were introduced. The experiment was conducted at 23±2 °C and 16:8 L:D and was replicated 10 times.

Survival on four *B. rapa* lines. Larval survival from hatch to second instar was determined for four *B. rapa* lines (AC Boreal, Echo, 'Waxy', 'Glossy') in a no-choice situation. A single potted plant of each line grown to the bud stage was placed in a mesh cage (38 cm high x 26 cm wide x 26 cm long). Diamondback moth eggs were collected on cabbage extract-treated aluminum foil exposed to females for 48 h and cut into strips of 150 eggs. Eggs were placed in the foliage of each plant line. Seven days after the eggs were introduced, the plants were destructively sampled to collect surviving larvae. The experiment was conducted at 23±2 °C and 16:8 L:D. The experiment was replicated four times with different cohorts of insects and plants.

7-Day no-choice leaf disc experiment. No-choice leaf disc feeding experiments with second-instar larvae were conducted in Nutrend 24-well trays with 1.25 mL of solidified bacto-agar per well. A 15-mm diameter leaf disc was placed on the agar and a single

second-instar larva was placed on the leaf disc. The entire plate was heat-sealed with thin transparent plastic wrap and small holes were made above each well to prevent condensation. Larvae were transferred twice to new leaf discs in new 24-well trays over the course of the experiment. After 7 days, surviving insects were counted and weighed. The experiment was conducted at 23±2 °C and 16:8 L:D. The experiment was replicated four times with different cohorts of insects and plants, each replicate included eight larvae fed on each of the 11 plant lines.

Dual-choice leaf disc experiment. Leaf discs (15-mm diameter) were cut from the third or fourth true leaves of each of the 11 plant lines. One experimental disc and one control (*B. napus* cv. AC Excel) disc were placed in a 4.5-cm diameter plastic petri dish. As an internal check, AC Excel was also treated as an experimental line and paired with itself. The dish was lined with moistened filter paper and had a tightly fitting lid. Three early fourth-instar larvae were placed in each petri dish with unrestricted access to both discs. The larvae were allowed to feed for 20 h at 23±2 °C. The surface area of each disc was determined at the start and end of the feeding period using image analysis software (AgImage Plus Version 1.08. ©Decagon Devices, Inc. 1989-1991). Food consumption was measured from the absolute area of the leaf discs consumed (original area less the area after 20 h of feeding). Biomass per unit area was not significantly different among the *Brassica* lines tested. Feeding preference was calculated as previously described for bertha armyworm (see Section 2.2) as the amount of the control disc consumed minus the amount of the experimental disc consumed (thus, positive values indicate a preference for the

control disc). Each experiment consisted of five pairs of discs for each plant line and the experiment was replicated four times with different cohorts of insects and plants.

Data Analysis. An ANOVA was performed to determine treatment (plant line) and replicate effects, as well as interactions for the dual-choice leaf disc feeding preference experiment, the 7-day no-choice leaf disc feeding experiment and the experiment to study first-instar larval survival on the four *B. rapa* lines. The least significant difference (LSD) method was used for comparison of means. A paired T-test was used for the first-instar feeding and oviposition preference experiments with the glossy and waxy lines. All analyses were carried out with Statistix 4.0, Analytical Software, Tallahassee, Florida.

5.3 Results

More eggs were laid on the 'Glossy' plants than on the 'Waxy' plants in the oviposition choice experiment (Figure 5.1), though the difference was not significant ($p=0.42$, $df=9$). Although ovipositing females did not discriminate between the 'Glossy' and the 'Waxy' *B. rapa* lines, there was a strong feeding preference for 'Waxy' plants over 'Glossy' plants among first-instar larvae ($p=0.0005$, $df=9$). Twice as many larvae were found on 'Waxy' *B. rapa* plants than on 'Glossy' plants (Figure 5.1).

In the no-choice feeding experiment on entire plants there were no significant differences in the survival of first-instar larvae among the four *B. rapa* lines tested ($p=0.98$, $F_{3,12}=0.04$). From 600 eggs, 45-49% of larvae reached the second-instar on the four *B. rapa* lines. The lowest survival was on Echo (45%) followed by AC Boreal (47%), 'Waxy'

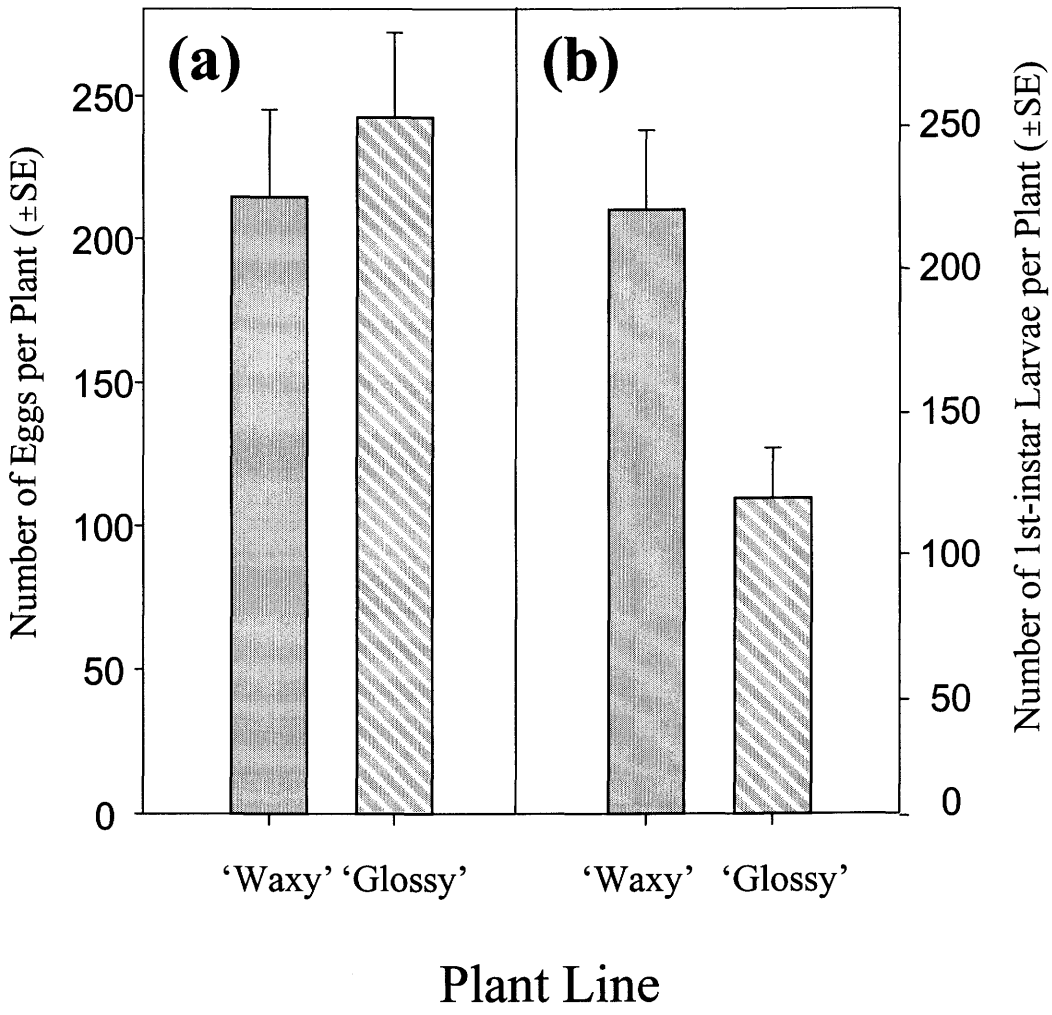

Figure 5.1. Diamondback moth oviposition and first-instar distribution on plants in the dual-choice experiment with entire plants of 'Waxy' and 'Glossy' *B. rapa*. (a) Mean number of eggs laid on 'Waxy' and 'Glossy' plants. (b) Mean number of first-instar larvae on 'Waxy' and 'Glossy' plants.

(48%) and 'Glossy' (49%).

In the 7-day no-choice leaf disc experiment all surviving larvae reached the fourth-instar or pupation on all 11 plant lines. Larval survival was lowest on AC Excel and the 'Glossy' *B. rapa* line, though not significant ($p=0.31$, $F_{10,33}=1.23$) (Table 5.1). Fourth-instar larval weight was significantly different among the plant lines. Larval weight was highest on AC Excel and lowest on the 'Glossy' *B. rapa* line ($p=0.01$, $F_{10,106}=2.48$) (Table 5.1). Pupae reared on the 'Glossy' line also had lower weights than those on the other lines (Table 5.1); however, the differences were not significant ($p=0.22$, $F_{10,127}=1.33$).

Fourth-instar larvae showed only minor differences in feeding preferences among the plant lines tested in the dual-choice leaf-disc experiment (Table 5.2). Larvae fed on leaf discs of all the lines tested and the area consumed for each of the lines was very close to that of the control, *B. napus* cv. AC Excel ($p=0.07$, $F_{10,165}=1.76$). Feeding preference indices ranging from 34.94 to -18.13 mm^2.

5.4 Discussion

Though more eggs were laid on the 'Glossy' *B. rapa* line than on 'Waxy' plants in the present study, the difference was not significant. However, an oviposition preference for glossy plants has been shown in studies of *B. oleracea* (Lin et al., 1984) and *B. napus* (Justus et al., 2000). Although larval survival is often lower on glossy plants, females do not discriminate against them as host plants for oviposition (Lin et al., 1984).

Despite females laying similar numbers of eggs on the 'Waxy' and 'Glossy' *B. rapa* lines, twice as many first-instar larvae were found on 'Waxy' plants. The difference in

Table 5.1. Diamondback moth mean (±SE) larval weight, pupal weight and percent survival after 7 days in a no-choice leaf disc experiment testing lines of *Brassica spp.* and *Sinapis alba*

Species	Line	% Survival	Fourth-instar weight (mg)	Pupal Weight (mg)
Sinapis alba	AC Pennant	71.9±3.1ab	5.54±0.3ab	6.25±0.3a
S. alba	L-GS	84.4±6.0a	6.24±0.7a	6.16±0.2a
Brassica carinata	Dodolla	71.9±10.7ab	6.01±0.5ab	6.23±0.3a
B. carinata	S-67	78.1±9.4ab	5.67±0.6ab	6.45±0.3a
B. juncea	AC Vulcan	78.1±6.0ab	5.53±0.3ab	6.39±0.4a
B. rapa	Echo	84.4±5.9a	5.36±0.2ab	5.96±0.3ab
B. rapa	AC Boreal	78.1±7.8ab	4.88±0.3b	5.89±0.4b
B. rapa	'Waxy'	71.9±11.8ab	5.32±0.4ab	5.68±0.4ab
B. rapa	'Glossy'	59.4±6.0b	4.19±0.2b	5.32±0.2ab
B. napus	AC Excel	56.3±8.0b	6.47±0.7a	5.75±0.2ab
B. napus	Midas	68.8±10.8ab	5.53±0.3ab	5.66±0.2ab

Means in each column followed by the same letter are not significantly different (LSD, $p>0.05$)

Table 5.2. Comparison of feeding preference (mean±SE) for fourth-instar larvae in a 20-hour dual-choice leaf disc assay using *Brassica napus* AC Excel as the control

Species	Line	[1]Feeding Preference
Sinapis alba	AC Pennant	18.31±10.7ab
S. alba	L-GS	21.13±11.0a
Brassica carinata	Dodolla	6.06±5.8ab
B. carinata	S-67	1.50±8.5ab
B. juncea	AC Vulcan	34.94±13.0a
B. rapa	Echo	-18.13±10.6b
B. rapa	AC Boreal	17.25±10.4ab
B. rapa	'Waxy'	-1.75±15.2ab
B. rapa	'Glossy'	16.63±8.9ab
B. napus	AC Excel	2.19±10.3ab
B. napus	Midas	26.69±15.6a

[1]Feeding preference is the amount of the control disc consumed minus the amount of the experimental disc consumed.
Means followed by the same letter are not significantly different (LSD, $p>0.05$).

larval numbers on the two lines is not due to mortality, as the survival through the first instar was equal on the two plant lines in the no-choice experiment. It is probable that the feeding preference is a reflection of increased larval dispersal and movement rates on the 'Glossy' line, similar to that observed with glossy lines of cabbage (Eigenbrode and Shelton, 1990; Eigenbrode et al., 1991a). The increased movement on the less acceptable 'Glossy' line resulted in larvae encountering the more acceptable 'Waxy' host plant where they then began to mine and feed. This conclusion is based on the fact that the proportion of first-instar larvae on the two lines does not reflect the relative number of eggs laid on each line.

There were no significant differences in survival from hatch to second-instar among the *B. rapa* lines tested in a no-choice situation. However, the no-choice experiment was carried out in a growth cabinet where the impact of environmental factors on mortality is greatly reduced. Under field conditions, resistance to diamondback moth in glossy *B. oleracea* depends largely on natural enemies and environmental factors such as desiccation and drowning (Eigenbrode et al., 1991a; Eigenbrode et al., 1995). The absence of these external factors in the present study with *B. rapa* lines may have had an effect similar to that observed in greenhouse experiments with *B. oleracea* (Eigenbrode et al., 1995).

Larval survival, pupal weight and fourth-instar weight were lower on 'Glossy' plants than on the other lines tested in the 7-day no-choice leaf-disc experiment. This may indicate that the second-instar larvae, which began the experiment, were negatively affected by the glossy characteristic. It is possible that the glossy characteristic delayed the initiation of feeding which slowed growth relative to the other lines.

In the dual-choice experiment fourth-instar larvae fed equally on all the plant lines tested and did not show the same preference for 'Waxy' relative to the 'Glossy' *B. rapa* as first-instar larvae. However, the relative resistance of glossy *Brassica* lines to diamondback moth is due to mortality and migration in the early instars (Eigenbrode et al., 1990). The results of the dual-choice test with *B. rapa* are consistent with previous work with *B. oleracea* in which fourth-instar diamondback moth larvae showed no preference between glossy and waxy cabbage lines (Eigenbrode et al., 1990). Thus, fourth-instar feeding preferences may not reflect the resistance of glossy plants to diamondback moth.

Diamondback moth thrived on all the plant lines tested and appears to be well adapted to a spectrum of cruciferous plants. None of the plant lines tested deterred larval feeding or reduced survival in laboratory tests. However, the present study indicates that glossy lines of *B. rapa* may show resistance to diamondback moth similar to that seen in glossy lines of other *Brassica* species. As in *B. oleracea*, this resistance appears to have a behavioural basis and is expressed against first-instar larvae (Eigenbrode et al., 1990). Evaluation of diamondback moth survival on glossy and waxy lines of *B. rapa* in the field in the presence of natural enemies and other environmental factors is necessary to confirm these findings.

6. General Discussion

Integrated pest management strategies in varying degrees of completeness are being examined for many North American cropping systems. Host-plant resistance is an important component of integrated pest management and insect resistant crops could have a large impact on pesticide use, even in the absence of fully developed integrated pest management systems.

In the present study several crucifer cultivars and breeding lines were examined for host-plant suitability for two economically important pests of oilseed production on the Canadian prairies, bertha armyworm and diamondback moth. Crop damage by these two insects is a result of larval feeding. Thus, the initial focus of the study was to determine the host-plant suitability of various physically and chemically distinct crucifers for larval feeding and development. Recognizing that it is the egg-laying female and not the larvae which determines the plants to be utilized as hosts, the study moved on to examine crucifer host-plant suitability for bertha armyworm oviposition. Both the larval feeding and the oviposition studies included choice and no-choice experiments with various crucifer host plants.

The results of the bertha armyworm larval feeding experiments showed that foliar levels of specific glucosinolates are important to larval host-plant suitability. All plant lines which contained detectable levels of sinigrin were less preferred for feeding than the *B. napus* standard, AC Excel. Those lines with the highest levels of foliar sinigrin, such

as *B. juncea* AC Vulcan and H-allyl, were least preferred. These results are consistent with previous results showing sinigrin and its metabolite, allyl isothiocyanate, decreased bertha armyworm feeding when incorporated in artificial diet (Shields and Mitchell, 1995a & b; McCloskey and Isman, 1993). Our results indicated that within similar crucifer breeding lines, increasing sinigrin concentrations increasingly inhibited bertha armyworm feeding.

High levels of the glucosinolate sinalbin appeared to give *S. alba* AC Pennant a greater level of protection from larval feeding compared to the *S. alba* L-GS line which lacked it. This is consistent with previous work which indicated that high concentrations of sinalbin deterred bertha armyworm feeding and offered some resistance to *S. alba* over *B. napus* species (Bodnaryk, 1991). In the present study these findings were extended to include the plant growth stages that larvae would naturally feed on. Interestingly, although the *S. alba* L-GS line contained high levels of benzyl-glucosinolate, it was no less preferred than all the lines with low total glucosinolate content. Thus, the quantities of specific foliar glucosinolates, notably sinigrin and sinalbin, may be more important to larval deterrence than is the overall glucosinolate level.

The no-choice larval feeding experiments also showed significant differences in host-plant suitability among the crucifer lines tested. Bertha armyworm weights were lowest on the high sinigrin *B. juncea* lines AC Vulcan and H-Allyl. Similar antibiotic resistance to bertha armyworm larvae was observed by McCloskey and Isman (1993) in high sinigrin *B. juncea* lines. The *S. alba* lines AC Pennant and L-GS consistently ranked

behind only the high sinigrin *B. juncea* lines as the poorest hosts for *M. configurata* in terms of larval weight gain. Larvae on AC Pennant and AC Vulcan also showed the lowest relative growth rate and relative consumption rate among the lines tested in the no-choice leaf-disc experiment. This is consistent with previous studies showing lower survival and weight gain of larvae on *S. alba* than on *B. napus* (Bodnaryk, 1991; McCloskey and Isman, 1993). The relative resistance of *S. alba* has been attributed to high levels of sinalbin (Bodnaryk, 1991), and larvae reared on the high sinalbin line AC Pennant weighed significantly less than those on L-GS which has no detectable sinalbin in the present study. However, in all cases the larval weights on *S. alba* L-GS were significantly lower than on the *B. rapa* and *B. napus* lines indicating that there are other features of *S. alba,* aside from sinalbin, which make it a less suitable host for *M. configurata* development.

The larval feeding study showed that bertha armyworm larvae have significantly different feeding preferences for different crucifer lines and these differences appear to be due to specific foliar glucosinolates, such as sinigrin and sinalbin. Sinigrin and sinalbin also influence the ability of larvae to utilize the food they ingest, suppressing weight gain and development. Maintaining certain levels of glucosinolates in the foliage of crucifer crops may offer some resistance to generalist insect feeders such as the bertha armyworm. Foliar levels of these glucosinolates may also be useful in predicting the attractiveness and suitability of plants to bertha armyworm. It would be of interest to study longer-term effects of high glucosinolate host plants on bertha armyworm to determine the effects these plants have on development through the pupal and adult stages as well as their effects on fecundity. It would also be interesting to determine if larval host plants affect choices

made by adult females during host-plant selection for oviposition.

In the experiments examining host-plant attractiveness for bertha armyworm oviposition, females laid more eggs on *B. napus* AC Excel than on *B. juncea* AC Vulcan in all situations. The relative resistance to bertha armyworm oviposition of AC Vulcan over AC Excel parallels results of the larval feeding experiments. The resistance to larval feeding among *B. juncea* lines has been attributed to high levels of sinigrin which can be detected by larvae by a specialized sinigrin-sensitive sensillum (McCloskey and Isman, 1993; Shields and Mitchell, 1995a & b). Sinigrin is found in a wide range of genera which produce glucosinolates (Daxenbichler et al., 1991) and it is likely that bertha armyworm evolved with plants containing this compound. Bertha armyworm adults may be equipped with sensilla that are able to detect sinigrin and are deterred by it; however, further investigation of the role of this glucosinolate in host-plant selection is needed. A study examining the effect of sinigrin on adult host-plant selection could be accomplished in the laboratory with commercially available sinigrin or in the field with crucifer lines that differ mainly in their glucosinolate profiles, such as the *B. juncea* lines in the present study.

Although the *S. alba* line AC Pennant was among the poorest hosts for bertha armyworm larval feeding, AC Pennant was the most attractive host-plant for oviposition. This apparent contradiction may be a result of the relatively short, if any, evolutionary history that the bertha armyworm has with *Sinapis* spp. and their unique glucosinolate profiles. AC Pennant plants were also very different in terms of leaf architecture and had a much greater density of foliar trichomes relative to the *Brassica* cultivars tested. Increased trichome density has been shown to be attractive to several other noctuids during

oviposition site selection (Hillhouse and Pitre, 1976; Navasero and Ramaswamy, 1991; Mascarenhas and Pitre, 1997) and female bertha armyworm may show a similar preference for the increased trichomes on *S. alba* plants.

Flowering AC Excel plants were the most attractive growth stage for ovipositing bertha armyworm. The oviposition preference for flowering plants in the present study is consistent with previous pheromone-trap counts which indicated that flowering plants may be more attractive to adult bertha armyworm than other growth stages (Turnock, 1984). A similar oviposition preference for flowering plants has also been observed for other polyphagous noctuids (Felland et al., 1992; McLeod, 1988; Johnson et al., 1975). The nectar food source may be in part responsible for the attraction to flowering plants. Flowering canola plants also provide the most dense crop canopy, which has been associated with oviposition preferences among other noctuids (Felland et al., 1992; Mascarenhas and Pitre, 1997), and may attract adult bertha armyworm in a similar manner.

Most bertha armyworm eggs were laid in the vertical third of the crop canopy which contained the most foliage for each growth stage. However, the available leaf material did not fully explain the observed oviposition behaviour. Among flowering plants, the top third of the canopy received more than twice as many eggs as the bottom third despite having less than half as much leaf material. A similar preference for the upper part of the host plant has also been observed among other noctuids (Hillhouse and Pitre, 1976; Mascarenhas and Pitre, 1997; Sappington et al., 2001). Ovipositing females may be attracted to the flowers or the younger leaf tissue, which has a higher trichome density, on the upper part of the plant and as a result lay most eggs in this region. This behaviour

would also benefit neonate larvae which may find the younger tissues near the top of the plant easier to ingest. It would be of interest to investigate the role of the flowers themselves in the attraction of gravid females to flowering plants.

Bertha armyworm lay most of their eggs on the underside of leaves, which is consistent with the oviposition habits of many other noctuids (Pansera-de-Araujo et al., 1999; Mascarenhas and Pitre, 1997; Sappington et al., 2001). The lower surface of the leaf protects eggs from adverse weather conditions and may also reduce the chances of detection by natural enemies. Among other noctuids, a preference for the underside of the leaf as an oviposition site has also been associated with increased trichome density on the lower leaf surface (Hillhouse and Pitre, 1976; Navasero and Ramaswamy, 1991; Mascarenhas and Pitre, 1997) and trichomes may have a similar affect on bertha armyworm oviposition. The role of trichomes in bertha armyworm host-plant suitability for oviposition is unclear, but, considering the apparent impact of trichome density on other noctuids, further investigation seems warranted.

Bertha armyworm females showed an oviposition preference for host plants on which conspecific eggs had been laid over host plants on which no eggs had been laid. The oviposition habit of egg clustering and the attraction of gravid females to conspecific eggs may benefit the eggs, the adult, or the larvae. The ancestral host plants of bertha armyworm were likely colonizers of disturbed areas and patchily distributed (Harris and Rogers, 1988). A chemical cue from conspecific eggs may have aided females in locating suitable host plants. Egg clustering would also limit flight time spent searching for host plants, reducing energy consumption as well as risks associated with flight (Stamp, 1980).

However, bertha armyworm adults can feed and are strong fliers which would reduce the drawbacks of increased flight. Egg clustering could also help reduce egg desiccation and parasitism (Stamp, 1980; Clark and Faeth, 1998), though bertha armyworm lay eggs in a single layer which may reduce these benefits. Thus, egg clustering may offer the greatest benefit to bertha armyworm larvae and these benefits may be further exploited by female attraction to conspecific eggs for oviposition.

The attraction of gravid females to conspecific eggs is not common among Lepidoptera, though, it is known to occur among some neotropical butterflies (Mallet, 1980; Clark and Faeth, 1997). The larvae of these butterflies, and others which lay their eggs in clusters, have been shown to gain several advantages from the resulting larval aggregations including feeding facilitation, increased growth rates, decreased development time and reduced predation and parasitism (Lawrence, 1990; Denno and Benrey, 1997; Clark and Faeth, 1997). Similar to the bertha armyworm, larvae of these species generally spend the first few instars cryptically colored then become darkly colored and conspicuous in later instars (Lawrence, 1990; Clark and Faeth, 1997). The larvae of these species are often distasteful and the dark conspicuous coloration may warn predators as well as help the larvae thermoregulate. Although bertha armyworm are not truly gregarious, larvae do exhibit a highly concentrated distribution which may offer some of the same advantages observed among other species which feed in aggregations. Larval density was shown to have a profound effect on several aspects of larval physiology in a related species, the cabbage moth, *Mamestra brassicae* (Goulson and Cory, 1995). Increased larval density leads to faster development, lower susceptibility to disease, increased weight gain and

darker color. It would be of interest to determine if the size of larval aggregations affects bertha armyworm larvae in a manner similar to that in larvae of other Lepidoptera that have similar oviposition and larval feeding habits.

Gravid bertha armyworm females in the present study were much more attracted to the eggs of other conspecific females than they were to their own eggs. If aggregation is beneficial to larvae, it would be advantageous for gravid females to be more attracted by a different female's eggs than their own. This behavior would increase the potential size of larval aggregations beyond that which a single female could produce. Another female's eggs may also indicate that a plant has been evaluated and accepted as a suitable host plant by a conspecific female which may be an acceptance cue for gravid females.

Gravid females also showed a preference for host plants treated with a methanol egg wash, indicating that the basis for the attraction of bertha armyworm females to conspecific eggs is at least in part chemically based. Because bertha armyworm oviposit at night and eggs are usually laid on the underside of host plant leaves, a chemical attractant may be the most effective means of indicating the presence of eggs. A comparable, but opposite, phenomenon has been observed in *Pieris* spp. (Klijnstra, 1986; Klijnstra and Schoonhoven, 1987; Schoonhoven, 1990). These authors found that females were deterred by the presence of conspecific eggs and that a leaf remained unattractive even after conspecific eggs had been removed (Schoonhoven, 1990). In choice tests similar to those in the present study, it was determined that *Pieris* spp. eggs contained an oviposition deterring pheromone that appeared to be non-volatile. The oviposition-deterring pheromone in *Pieris* spp. is thought to be produced in the accessory glands,

which also produce the egg adhesive, and is released during egg deposition (Schoonhoven, 1990). It appears that there may be an oviposition-attracting pheromone produced by bertha armyworm which acts as an attractant to gravid females rather than as a deterrent as it does for *Pieris* spp. Further study to determine the chemical nature of the attractant and its thresholds would be useful, especially if there were the possibility of using such a pheromone as a means of attracting gravid females.

The bertha armyworm oviposition study has provided information on the most susceptible *Brassica* growth stages and cultivars. This information could assist in alerting producers to economically important bertha armyworm infestations earlier in the insect's development. Knowledge of the preferred location for oviposition may also facilitate development of more efficient scouting protocols. Further investigation of the attraction of gravid females to conspecific eggs, and its affect on larval performance, could lead to a better understanding of bertha armyworm and related pest species. Study of this phenomenon may also provide a means of trapping gravid females.

In the diamondback moth host-plant suitability studies, larvae of all instars thrived on all the plant lines tested from five crucifer species. With the exception of the glossy *B. rapa* line, diamondback moth larvae did not express significant differences in feeding preference or appreciable differences in larval growth and development among the crucifers tested.

Diamondback moth laid more eggs on *Brassica rapa* plants expressing the glossy leaf wax characteristic than on waxy plants, though the difference was not significant. This trend is consistent with oviposition preferences involving glossy lines of other *Brassica*

species (Lin et al., 1984; Justus et al., 2000). However, when first-instar larvae were given a choice between glossy and waxy *B. rapa* plants, larvae showed a preference for waxy *B. rapa* plants. Based on the results of the no-choice experiment, the difference in larval numbers on the two lines is not due to mortality. Thus, the feeding preference appears to reflect increased larval dispersal and movement rates on the glossy *B. rapa* line, similar to those observed on glossy lines of cabbage (Eigenbrode and Shelton, 1990; Eigenbrode et al., 1991a).

There were no significant differences in diamondback moth survival among the *B. rapa* lines tested in a no-choice situation. However, the no-choice experiment was carried out in a growth cabinet where the impact of environmental factors on mortality is greatly reduced. Under field conditions, resistance to diamondback moth in glossy *B. oleracea* depends largely on environmental factors, including predation, desiccation and drowning (Eigenbrode et al., 1991a: Eigenbrode et al., 1995). The absence of these external factors in the present study with *B. rapa* lines may have had an effect similar to that observed in greenhouse experiments with *B. oleracea* (Eigenbrode et al., 1995). Consistent with previous work involving *B. oleracea*, fourth-instar larvae fed equally on all the plant lines tested and did not show the same preference for 'Waxy' relative to the 'Glossy' *B. rapa* as did first-instar larvae (Eigenbrode et al., 1990). Thus, fourth-instar feeding preferences may not reflect the resistance of glossy plants to diamondback moth.

As in *B. oleracea*, the relative resistance of glossy *B. rapa* lines to diamondback moth appears to have a behavioural basis and is expressed against first-instar larvae (Eigenbrode et al., 1990). Evaluation of diamondback moth survival on glossy and waxy

lines of *B. rapa* in the field, in the presence of natural enemies and other environmental factors, is necessary to confirm these findings.

Plant-based resistance to bertha armyworm in crucifer crops may be achievable based on plant chemistry, specifically foliar glucosinolates. Elevated levels of specific glucosinolates, which are achievable in living plants, significantly reduce larval feeding and survival and may also have an impact on host-plant suitability for oviposition. However, glucosinolates, including sinigrin, which show the most promise for such resistance are attractive to many insect pests which specialize on cruciferous plants. Important pest insects of crucifer crops on the Canadian prairies, such as flea beetle, *Phyllotreta* spp. (Hicks, 1974; Pivnick et al., 1992), cabbage root fly, *Delia radicum* (Nottingham, 1988; Braven et al., 1996) and diamondback moth (Reed, 1989; Pivnick et al., 1990b) are attracted by glucosinolates. Increased levels of glucosinolates in the foliage of crucifer crops may increase the risk of crop damage by these pests. Despite this threat, bertha armyworm outbreaks are cyclic and relatively predictable based on changing population levels. Thus, in years of projected bertha armyworm outbreaks, the risk of crop damage to high glucosinolate crops by crucifer specialists may be sufficiently offset by reduced bertha armyworm damage to warrant implementation.

Plant-based resistance based on crucifer plant chemistry does not appear to be a viable option for control of the diamondback moth, which is well adapted to a wide range of cruciferous plants. However, certain physical characteristics, such as the glossy leaf wax characteristic, may offer crucifer crops some protection from this insect. This mechanism of resistance needs to be further investigated with respect to diamondback moth and other

crop pests on the Canadian prairies. The glossy leaf wax characteristic has been associated with risks such as increased attractiveness to other insect pests, including flea beetle (Eigenbrode et al., 2000). Thus, the effectiveness of plant-based resistance strategies against diamondback moth, based on leaf wax characteristics, will have to be further studied on the Canadian prairies and weighed against the risks, based on the population levels of relevant pest insects.

7. Summary and Conclusions

7.1. Summary

Crucifer host-plant suitability for bertha armyworm larval feeding was tested using plant lines from five different crucifer species in choice and no-choice experiments. The larval feeding experiments suggest that the lines which contain high foliar levels of the glucosinolates sinigrin and sinalbin are less preferred by feeding larvae and, when larvae are forced to consume the high glucosinolate lines, their growth and development are significantly slowed. These findings are consistent with those of previous studies and offer further evidence that specific glucosinolates provide crucifer plants some protection against bertha armyworm larval feeding. Thus, there appears to be potential for larval feeding resistance based on plant chemistry.

Different crucifer cultivars and growth stages were also examined for attractiveness for bertha armyworm oviposition. There are significant differences in oviposition attractiveness among the cultivars tested. Consistent with the findings of the larval feeding studies, *B. napus* plants are significantly more preferred for oviposition than *B. juncea* plants. However, *S. alba* plants are most attractive for ovipositing females despite being relatively poor host plants for the larval stage. Full-flower plants are significantly more attractive to ovipositing females than either pre-flower or pod plants. Most eggs are laid in the upper portion of the crop canopy and most oviposition occurs on the underside of leaves.

Ovipositing bertha armyworm are attracted to conspecific eggs. Females lay significantly more eggs on leaves which already have another female's eggs, or have been treated with a methanol wash of another female's eggs, than they do on a control leaf. This trend was also observed in the field experiments.

The diamondback moth larval feeding experiments indicate that this insect, which specializes on crucifers, is well adapted to a wide range of crucifer host-plants. Diamondback moth larval feeding preferences, growth and development are not significantly different across a wide range of physically and chemically distinct crucifer hosts. There is, however, evidence that leaf wax characteristics may affect the relative resistance of *Brassica* plants to diamondback moth. The glossy line of *B. rapa* was relatively resistant to first-instar larval feeding relative to the waxy lines in the present study. These findings are consistent with previous work involving *B. oleracea* in which glossy lines are resistant to first-instar larval feeding relative to the waxy wild-type lines.

7.2. Conclusions

Crucifer plants which contain high foliar levels of certain glucosinolates, such as sinigrin and sinalbin, deter larval feeding and if consumed as a sole food source retard larval growth and development. The high sinigrin *B. juncea* lines and the high glucosinolate *S. alba* line were relatively resistant to larval feeding compared to the other crucifer lines tested. It is likely that elevated levels of these glucosinolates in the foliage of crucifer field crops would reduce larval growth, development and survival, and possibly reduce the fecundity of surviving adult females. These effects could be of great benefit in

an integrated pest management program and would help keep population levels below the action threshold. However, in outbreak years larval feeding on these crops would result in damage that would require an alternative method of control.

Host-plant resistance as a defence strategy against bertha armyworm must include development of plant lines less attractive to gravid females as it is these which ultimately determine the plants/crops infested. There are significant differences in bertha armyworm oviposition preference among the different crucifer species tested, though, the reasons for these differences are unclear. Consistent with the larval studies, *B. juncea* plants are less preferred than *B. napus* plants for oviposition. It is possible that ovipositing females possess sensilla sensitive to sinigrin similar to those found in the larvae and are deterred by those plants which contain it, though this requires study. *Sinapis alba* plants are preferred for oviposition over all other plants tested despite being a relatively poor host-plant for larval development. This apparent contradiction may be a result of females being unable to detect deterrents or being stimulated by other attributes of the plant to the extent that the deterrents are overridden. Such attributes may include trichome density, though further investigation is required.

Flowering plants are the most preferred growth stage for oviposition. The flowering stage may attract ovipositing females because of the nectar food source. Plants at this stage may also provide the most suitable crop canopy for egg laying and development. Flowering plants also provide larvae with the largest potential food source. Further work is necessary to determine if it is the flowers themselves which attract females or whether attraction is due to other attributes of these plants. Females lay most eggs in

the upper part of the crop canopy on the underside of leaves. Placement in the crop canopy may be related to the micro-climate the eggs require for optimal development or may be a result of a nearby nectar food source. Laying on the underside of the leaves may offer the eggs greater protection from environmental factors such as weather, predators and parasitoids.

Information on the most susceptible *Brassica* cultivars and growth stages should assist in alerting producers to economically important bertha armyworm infestations earlier in the insect's development. Establishing the preferred location for oviposition should also facilitate development of more efficient scouting protocols.

Gravid bertha armyworm are attracted to conspecific eggs. The attraction to conspecific eggs is interesting biologically and somewhat unique; however, it may also have implications for applied research. Females show a preference for oviposition sites near previously laid eggs in the field and in laboratory experiments. This attraction is stronger for eggs laid by a different female than it is for eggs laid by the same female, which should allow for larger egg aggregations. The reason for the attraction to conspecific eggs is likely reflected in larval performance which may be enhanced in larger groups, though further study is needed. The results of the methanol egg wash experiment indicate that the nature of the attraction is chemical. If the chemical attractant could be isolated and synthesized it may be possible to attract and trap gravid females.

Diamondback moth larvae appear to be well adapted to a wide range of physically and chemically distinct crucifer cultivars. Diamondback moth larvae from first- to fourth-instar do not express significant feeding preferences or differing developmental rates

among the crucifer lines tested. However, the glossy leaf wax characteristic does appear to provide the 'Glossy' *B. rapa* line with relative resistance to first-instar larvae relative to waxy wild-type cultivars. Further work involving field experiments should be undertaken to determine if the relative resistance to diamondback moth larvae observed in glossy *B. oleracea* is also expressed by glossy *B. rapa* lines.

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