University of Alberta

Mechanisms of cabbage seedpod weevil, *Ceutorhynchus obstrictus*, resistance associated with novel germplasm derived from *Sinapis alba* x *Brassica napus*

by

James Allen Tansey

A thesis submitted to the Faculty of Graduate Studies and Research

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Plant Science

Department of Agricultural, Food and Nutritional Sciences

Fall, 2009

Edmonton, Alberta

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled ‘Mechanisms of cabbage seedpod weevil, *Ceutorhynchus obstrictus* resistance associated with novel germplasm derived from *Sinapis alba* x *Brassica napus*’ submitted by James A. Tansey in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Agricultural, Food and Nutritional Science.

Examining Committee

_____

Dr. Lloyd M. Dosdall, Agricultural, Food and Nutritional Science

_____

Dr. Andrew Keddie, Biological Sciences

_____

Dr. Maya Evenden, Biological Sciences

_____

Dr. Habibur Rahman, Agricultural, Food and Nutritional Science

_____

Dr. Robert J. Lamb, Research Scientist, Cereal Research Centre, Agriculture and Agri-Food Canada. Winnipeg, Manitoba; Adjunct Professor, University of Manitoba, Entomology

Abstract

The cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae), is an important pest of brassicaceous oilseed crops, especially canola (*Brassica napus* L. and *Brassica rapa* L.) in North America and Europe. Application of foliar insecticide is the only method currently employed to control *C. obstrictus* populations; because this approach is environmentally unsustainable, alternatives including host plant resistance have been explored.

White mustard, *Sinapis alba* L., is resistant to *C. obstrictus* and was chosen as a potential source of resistance for *B. napus* oilseed. Interspecific crosses of *S. alba* x *B. napus* have produced several lines that are resistant to *C. obstrictus* feeding and oviposition and yield fewer, lighter-weight weevil larvae that take longer to develop. I investigated potential mechanisms of this resistance, including assessing differences in visual and olfactory cues among resistant and susceptible genotypes, and antixenosis and antibiosis. Determining effects of visual cues associated with host plant resistance required investigation of weevil vision. Deployment strategies for resistant germplasm were assessed to evaluate incorporation of susceptible refugia to promote long-term durability of resistance traits.

Results reported in Chapter 2 indicate that the *C. obstrictus* visual system is apparently trichromatic and incorporates receptors with response maxima near 350, 450, and 550 nm. Modelling indicated that UV light alone reduced weevil responses but the interaction of yellow and UV light increased responses at a threshold reflectance level of UV. Results reported in Chapter 3 indicated that

differences in yellow and UV reflectance among host plant flowers influence host selection in *C. obstrictus*. Results described in Chapter 4 determine differential attraction to the odours of *S. alba* and *B. napus* and among resistant and susceptible accessions. Inferences of the identities of glucosinolates found in varying amounts among susceptible and resistant genotypes suggested that 2-phenylethyl glucosinolate influenced attractiveness. Results described in Chapter 5 indicate differences in adult feeding and oviposition preferences among resistant and susceptible genotypes. Oocyte development, larval biomass and larval development time varied among weevils feeding on resistant and susceptible genotypes. Based on results of Chapter 4, 1-methoxy-3-indolylmethyl glucosinolate was implicated as contributing to antixenosis and antibiosis resistance. Results reported in Chapter 6 describe effects of mixed plots of resistant and susceptible genotypes on weevil spatial distribution and oviposition. These results are consistent with associational resistance and attributed to reduced apparency of susceptible plants in mixtures and antixenosis resistance associated with resistant germplasm.

Acknowledgements

I would like to first thank Drs. Lloyd Dosdall and Andrew Keddie for taking me on as a student and providing an opportunity and resources for me to develop my research skills and further my interest in insect-plant interactions. Dr. Maya Evenden was also a member of my supervisory committee and integral to the successful completion of this project. My interactions with my supervisory committee were always positive and I am most grateful for them. Their expert advice, helpful comments and willingness to let me tap their great knowledge of subjects applicable and peripheral to this project and relevant to my growth as an entomologist and researcher were invaluable.

Sincere thanks to Drs. Laima S. Kott, Scott D. Noble and Ron S. Fletcher for their collaborations. Without the hard work of the field and laboratory crews in Lethbridge and Edmonton, the scale of this project would have been greatly diminished. I must thank (in alphabetical order) Ross Adams, Nancy Cowle, Matt Fisher, Brett Hoffman, Jordana Hudak, Christina Gretzinger, Michael Gretzinger, Analea Mauro, Cameron Stevenson, Crystal Tebbie, Matt Treadwell and Nate Vos for their capable technical assistance at all times and for their patience with occasional long hours in harsh conditions. I would also like to sincerely thank Dr. Héctor Cárcamo of Agriculture and Agri-Food Canada, Lethbridge Research Centre for allowing us access to his lab and to exceptional facilities within the Centre. Other notable members of Agriculture and Agri-Food Canada, Lethbridge who have provided indispensible advice and assistance include Dr. Kevin Floate, Mr. Tom Kveder and Mr. Daniel Ethier.

My parents, Richard ‘Dick’ Tansey and Suzanne Lavallée are ultimately responsible for my participation in this project and they, and my brother Paul Tansey and extended family and friends deserve great credit for their support of this and all of my endeavours. Special thanks go to Jennifer Kranabetter. Long summers away from home made for good work but were never my favourite part of this project. Thanks for your patience, interest, support and sense of humour. Thanks to Rollie, Mary, Jeff and Paul Lavallée, Carmen Hankinson, Dale Hrdlicka, Judy Tansey, Joanne Nordmark Tansey, Yolande Lavallée and Monty Schneider, Mitch, Laura and Mark Lavallée, Leslie Hankinson and Susanne Schultz, Jennifer Hankinson Owen and Darren Owen, Rob and Liz Kranabetter, Donna Tansey (and the jars of grasshoppers), and Terry Sargent. Thanks too to Roy B. Malloy, Ethel Savard, and Tika and Oscar Kranabetter.

I would also like to thank the University of Alberta and, in particular, the Department of Agricultural, Food and Nutritional Sciences for providing a stimulating workplace and professional home for myself and this project. I learned much from casual interactions and enjoyed them immensely. My time at the University of Alberta has also allowed me to interact with several remarkable individuals including members of my candidacy examining committee: Drs. Edward Bork and Steven Strelkov. Notables also include several graduate students of this department and the Department of Biological Sciences. Thanks to Jeremy Hummel, Ravindran Subramaniam, Adam Blake, Amanda Van Haga, Artem Abdukakharov, Caroline Whitehouse, Jason Dombroskie, Joelle Lemmen, Kelley Dunfield, Lisa Lumley, Marla Schwarzfeld, Rana Sarfraz , Tyler Wist,

Wes Hunting, Dr. Thomas Simonsen, Chris Miluch, Mark McPherson and Philip Batista for their contributions to this experience. Also, thanks to Freddie Brent Young, Michael Cripps and Dr. James ‘Ding’ Johnson for their contributions to another related experience.

Funding for this project was provided by the University of Alberta, the Canola Council of Canada and grants to LMD from the Natural Sciences and Engineering Research Council of Canada and the Alberta Agricultural Research Institute and I am grateful for their support of this project.

Table of Contents

List of Tables

List of Figures

List of Symbols, Nomenclature, or Abbreviations

m	metre
cm	centimetre
km	kilometre
nm	nanometre
ha	hectare
kg	kilogram
d	day(s)
hr	hour(s)
L:D	ratio of light to dark
ca.	approximately
var.	variety
cv.	cultivar
λ	wavelength
λ_{max}	wavelength response maxima
UV	350 nm reflectance
B	450 nm reflectance
G	550 nm reflectance
Y	580 nm reflectance
UVB	interaction of UV and B
UVG	interaction of UV and G
UVY	interaction of UV and Y
BG	interaction of B and G
UV^2	quadratic function associated with UV
WS	weevil score (mean weevil larvae per pod)
SI	sampling interval
rh	relative humidity
R	resistant
S	susceptible
MS	moderately susceptible
I_a	main SADIE index

J_a	subsidiary SADIE index
δ	SADIE distance index
X	SADIE association index
°C	degrees Celsius

Chapter 1

Introduction

Ceutorhynchus obstrictus (Marsham), the cabbage seedpod weevil

Life history

Ceutorhynchus obstrictus (Coleoptera: Curculionidae) is univoltine and overwinters as an adult in the ground below leaf litter, usually in shelterbelts, grassy berms and windrows (Dmoch 1965; Ulmer and Dosdall 2006a; J. Tansey unpublished data). In western Canada, spring emergence begins when mean air temperature reaches 9 to 12°C and peaks when ground temperatures reach 15°C at depths of 5 cm (Ulmer and Dosdall 2006a). Newly emerged adults initially feed on a wide variety of brassicaceous plants near their overwintering sites before undertaking oviposition host-seeking migrations (Bonnemaison 1957).

Early season 'food' hosts in western Canada include wild mustard (*Sinapis arvensis* L.), hoary cress (*Lepidium draba* L.), field pennycress (*Thlaspi arvense* L.), flixweed (*Descurania sophia* (L.) Webb), shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.), radish (*Raphanus* spp.) and volunteer canola (*Brassica napus* L. and *Brassica rapa* L.) (Dmoch 1965; Dosdall and Moisey 2004). Most of these plants provide sustenance for overwintered weevils, resources for ovarian development and opportunities to aggregate and mate (Fox and Dosdall 2003). Adults typically feed for two to three weeks before mating (Williams and Free 1978). Diets containing racemes and post-diapause feeding duration influence egg development (Ni et al. 1990). Although *B. napus* racemes are preferred, some ovarian development can occur on green host-plant material (Ni et al. 1990). Fox and Dosdall (2003) reported differences in oocyte development associated with

different brassicaceous host plants. Ambient temperature also affects oocyte development (Ni et al. 1990).

Oviposition occurs in developing siliques of *B. napus, B. rapa, Raphanus* spp. and to a lesser extent *S. arvensis*; these species are considered 'true' hosts (Dmoch 1965). Weevils will oviposit and can complete development in low numbers on marginally suitable host plants like *S. alba* if confined to these plants (Dosdall and Kott 2006), but typically discriminate among potential host populations (Moyes and Raybould 2001; Kalischuk and Dosdall 2004). Assessment of host quality by females prior to oviposition is associated with a stepwise behavioural regimen: pod exploration, egg cavity formation by inserting the rostrum, turning to place the ovipositor above the cavity, deposition of a single egg, ovipositor retraction, pod brushing and pod abandonment (Kozlowski et al. 1983). Ulmer and Dosdall (2006a) showed that early stages of oviposition behaviour were slower on the relatively resistant host plants, *Brassica tournefortii* Gouan and *B. juncea* (L.) Czern., than the relatively susceptible *Brassica carinata* Braun, *B. napus*, and *B. rapa.*

Pod brushing involves deposition of an epideictic substance and is characterised by brushing the region of the pod near the oviposition site with the female's eighth abdominal tergite (Ferguson and Williams 1991). Epideictic substances, also called spacing pheromones, stimulate migration from a resource (Ryan 2002). Application of epideictic substances reduces repeated oviposition by an individual and conspecifics into limited larval food sources (Prokopy et al. 1984). Deposition of this substance results in reduced repeated oviposition on

that pod by conspecific females (Ferguson et al. 1999). This substance has been characterised as a mixture of iso- and *n*-alkanes, dimethylalkanes, alkenes, fatty acids, 15-nonacosanone, 15-nonacosanol, and cholesterol; deterrent properties were found to be associated with the polar fraction (Mudd et al. 1997). Deterrence promotes uniform distribution of progeny and efficient resource partitioning among larvae, an adaptive strategy given the limited carrying capacity of pods in brassicaceous plants (Mudd et al. 1997). Deposition of this substance may also influence spatial distribution of adults. However, the behavioural effects of oviposition-deterrent pheromone last only about 2 h (Ferguson and Williams 1991). Other means for females to detect developing larvae in pods likely exist but have not yet been discovered.

Larvae hatch in 6 to 10 days (Bonnemaison 1957) and feed on developing seeds, consuming five to six seeds during three instars (Dmoch 1965). Larvae feed for 14 to 21 days (Bonnemaison 1957). When feeding is complete, mature larvae chew through pod walls and drop to the soil where they burrow in and pupate. Pupation occurs in earthen cells 1 to 2 cm beneath the soil surface; these cells are approximately 6 mm in length (Dosdall and McFarlane 2004). Development from egg to adult requires 31 to 58 d in spring canola in western Canada (Dosdall and Moisey 2004).

The overwintering generation of adults emerge to feed on late flowers and pods of brassicaceous plants to acquire resources for overwintering (Dmoch 1965). Bonnemaison (1957) indicated that for successful overwintering, weevils must consume a large quantity of food in a relatively short time. Bartlet et al.

(1993) suggested that the reduced responses they observed to olfactory stimulants from field-collected specimens of the overwintering generation were associated with reduced nutritional requirements of weevils that were satiated and were preparing for diapause. Large numbers of *C. obstrictus* adults have been observed feeding on cortical and vascular tissues of cut *B. napus* stems in early September near Lethbridge, AB (J. Tansey, unpublished data). This observation suggests the importance of pre-diapause feeding.

Ceutorhynchus obstrictus: Taxonomic status

The cabbage seedpod weevil is currently considered a member of the Curculionidae subfamily Ceutorhynchinae Gistel, 1856 (Colonnelli 2004). However, Zherikhin and Egorov (1990) place the Ceutorhynchinae in the Baridinae and consider the Conoderinae, Ceutorhynchinae, Trigonocolinae and Orobitinae as tribes in this subfamily. Characters that support these groupings include: “a transverse carina at the hind margin of the pronotum, a strongly curved submarginal fold at the interior surface of the elytra, a total fusion of metepisternum and metepimeron, a strong median carina on the inside of the metathorax (an apparently unique feature in Curculionoidea) and a number of agreements in wing venation” (Oberprieler et al. 2007). Although some species in this grouping have a separate metepisternum and metepimeron, a large ascending mesepimera and a similar pygidium are shared among all members (Oberprieler et al. 2007). Baridinae, according to this concept, comprise approximately 8000 species; about one-half are in the Baridini. The Baridini are particularly diverse in

the Neotropics and larvae of these weevils develop in the fruits and stems of a large assemblage of angiosperms. Conoderini are often woodborers and hosts of this group include gymnosperms and monocotyledonous angiosperms; hosts of Ceutorhynchini include Brassicaceae and Polygonaceae (Oberprieler et al. 2007). Zherikhin and Gratshev (1995) considered the Conoderinae, Ceutorhynchinae, Trigonocolinae and Orobitinae as subfamilies in a separate family, the Baridiidae. Korotyaev (2008) considers the Ceutorhynchinae a valid subfamily and supports Thompson's (1992) opinion that this is one of the most clearly defined taxa of Curculionidae (Phanerognatha) although he supports their grouping with the Conoderinae (= Zygopinae) and Baridinae. Particular structures of the weevil vestiture do not support combining ceutorhynchines and other taxa in one subfamily or separation from the Curculionidae (Lyal et al. 2006). Colonnelli (2004) reported 1,316 species in the Ceutorhynchinae.

Characters that differentiate Ceutorhynchinae include relatively small size (1.2 to 7.0 mm long), and broad and convex ventral and often dorsal profile (Korotyaev 2008). Temperate species have elytra that are only slightly longer than their width, but in tropical species, elytra may be wider than long. These body proportions are associated with well developed indirect flight musculature suggesting Ceutorhynchinae as a highly advanced taxon (Korotyaev 2008). In addition, apices of the mesepimera are visible dorsally and project between posterior angles of pronotum and elytral humeri. The moderately long rostrum may be held tightly to the body and placed between coxae in dead or otherwise highly relaxed individuals; the meso- and metasternum are relatively deeply

depressed, but do not form the high keels characteristic of Cryptorhynchinae (Korotyaev 2008). The hind femora tend to be thickened, and hind tibiae are shortened with apices outcurved; these traits are associated with the highly derived ability of many members of this group to jump (Korotyaev 2008).

Adults of the tribe Ceutorhynchini are characterised by a scaled, usually unreflective body vestiture (Marvaldi and Lanteri 2005). When postocular lobes are present they partially cover the eye. Rudimentary or no uncus is associated with hind tibiae and the elytra do not cover the pygidium (Marvaldi and Lanteri 2005). Most larvae of this group bore in the stems or crowns of herbaceous plants although some are foliage feeders on aquatic plants (Marvaldi and Lanteri 2005).

The genus *Ceutorhynchus* Germar 1824 has over 300 species associated nearly exclusively with Brassicaceae and represents one of the predominant groups in the Holarctic fauna (Colonnelli 2004). However, there are approximately five times the numbers of Palaearctic as Nearctic *Ceutorhynchus* species (Korotyaev 2008). Some members have near cosmopolitan distributions (Colonnelli 2004). Adults are characterised by a moderately convex pronotum with curved or sinuous sides; when tubercles of the pronotum are present, they are distinct from the sides. Elytra are ovoid rather than cordiform. In addition to Brassicaceae, other host plants include Linaceae, Resedaceae, Tropeolaceae and Cannabinaceae (Colonnelli 2004). This genus has not been divided into subgenera (Korotyaev 2008).

Ceutorhynchus obstrictus is indigenous to the western Palaearctic and feeds exclusively on Brassicaceae (Colonnelli 2004). The nomenclature of this species

is complicated by historical misidentification and synonymy (Colonnelli 1993). Thomson (1859) designated *Curculio assimilis* Paykull 1792, the junior homonym of *Curculio assimilis* Fabricius 1775, as the type series of *Ceutorhynchus.* This designation was validated by the International Commission on Zoological Nomenclature (ICZN 1989) and the specific designation *Curculio assimilis* Fabricius 1775 was suppressed. Of the five specimens in the Paykull collection, one corresponds to *Ceutorhynchus syrites* Germar 1824 and the rest to *Ceutorhynchus pleurostigma* Marsham 1802. Thus the synonym *Ceutorhynchus assimilis* Paykull 1792 [= *C. pleurostigma* (Marsham 1802) syn. n.] was established. Specimens of types *Curculio pleurostigma* Marsham 1802 were examined by Colonnelli (1993) and corresponded well to subsequent interpretation and to types for *Curculio obstrictus* Marsham 1802. According to Colonnelli (1990), *Ceutorhynchus assimilis* Paykull 1775 must be called *C. obstrictus* Marsham 1802. Voucher specimens of *C. obstrictus* adults from my study have been placed in the University of Alberta's Strickland Museum of Entomology, Edmonton, AB, Canada.

Diagnostic characters

Adults of *C. obstrictus* are characterised by having round grey bodies 2 to 4 mm in length with bodies and legs covered with fine white scales. The proboscis is long and curved, and antennae are relatively small and bent (Laffin 2005). Adult *C. obstrictus* can be differentiated from its sympatric (in North America) congener *Ceutorhynchus neglectus* Blatchley by larger size, grey body colour and

leg colour; *C. neglectus* are 1 to 2 mm in length, have dark bodies covered in white scales and red-brown legs (Blatchley and Leng 1916).

There are three larval instars; although labial and maxillary development and development of labial and maxillary sensillae is greater in second and terminal instars and mean head capsule width varies among instars (first = 0.23 mm; second = 0.37 mm; third = 0.54 mm), other features are consistent among all instars (Dosdall and McFarlane 2004). Common features include: a stout, slightly curved, apodous scarabaeiform body with a free dark brown head as long as wide, stemmata and evenly convex, not-prominent two-segmented antennae; the epicranial suture is visible the entire length of the head and the frontal sutures are U-shaped and anteriorly complete; there are two pairs each of dorsal and ventral, one pair of lateral and three pairs of minute posterior epicranial setae; the clypeus has two pairs of setae; the anterior margin of the labrum is trilobed and the labrum has three pairs of setae and short subparallel labral rods; the epipharynx has three anterolateral setae, four anteromedian setae, and four median spines; each mandible has two apical teeth and one subapical tooth; the labial palpus is two-segmented and the posterior margin of labium is distinctly trilobed (Dosdall and McFarlane 2004). Pupae are approximately 4 mm long and are initially primarily white with pigmentation associated with developing compound eyes and sparse dark setae over the entire body (Dosdall and McFarlane 2004). Eggs are smooth, opaque white, and approximately 0.5 mm long and 0.3 mm wide (Dosdall and McFarlane 2004).

Origins, distribution and predicted spread of North American populations

Ceutorhynchus obstrictus is native to Europe and is common in brassicaceous oilseed production regions throughout Europe and Eurasia (Hill 1987). The first reported discovery in North America was in south-western British Columbia in 1931 (McLeod 1953, 1962). Weevils were likely initially transferred from European source populations as contaminants of Brassicaceous seed stocks (McLeod 1953). Since its accidental introduction to North America, it has dispersed or was accidentally distributed throughout continental U.S.A. (McCaffrey 1992). *Ceutorhynchus obstrictus* was first reported near Lethbridge, Alberta in 1995 (Cárcamo et al. 2001) and later in Québec and Ontario (Brodeur et al. 2001; Mason et al. 2004). Source populations of eastern and western North American populations differ: western North American populations were likely introduced from western or northern Europe, whereas north-eastern North American populations were introduced separately from Scandinavia or Russia (Laffin et al. 2005). Densities remained relatively low in Alberta from 1995 to 1998 but increased to outbreak levels in approximately 100 000 ha in the Lethbridge region from 1998 to 2001 (Dosdall et al. 2002).

Ceutorhynchus obstrictus is dispersing north and east from southern Alberta at approximately 55 km per year; the weevil reached western Saskatchewan by 2000 (Dosdall et al. 2002). Dosdall et al. (2002) used the CLIMEX™ dynamic simulation model (Sutherst et al. 1999) to develop ecoclimatic indices (EI) and so predict patterns of geographic distribution of *C. obstrictus*. These evaluations were conducted incorporating current growing

conditions, particularly long-term meteorological data, current geographic distributions and known ecological requirements of *C. obstrictus*. Ecoclimatic Index values are obtained by combining a growth index (GI) with stress indices that suggest unsuitable conditions. Olfert and Weiss (2006) applied a modification of this model to predict *C. obstrictus* geographic distributions under conditions predicted to occur in Canada by the mid to late 21st century (Brlacich et al. 1997; Cohen and Miller 2001). Their final EI values were categorized as 'unfavourable' (0 < 10), 'suitable' (10 < 20), 'favourable' (20 < 30) and 'very favourable' (> 30). According to their results, a 3°C increase in mean annual temperature will increase the proportion of Canadian landmass favourable to *C. obstrictus* to 19.7 percent. Approximately 5.7 percent of this area is currently favourable to *C. obstrictus* (Dosdall et al. 2002). Canola production regions in southern Manitoba may support greater *C. obstrictus* populations than those seen in Alberta and Saskatchewan; (EI) values were greater for Manitoba than for the other Canadian Prairie Provinces (Dosdall et al. 2002).

Increases of 0 to 2°C in annual mean temperatures are projected for Alberta by the 2020's; increases of 2 to 3°C and 3 to 5°C are projected for the 2050's by median (HadCM3 B2(b)) and warmer and drier (CCSRNIES A1FI) scenarios, respectively (Barrow and Yu 2005 and references therein). Assessments of the effects of climatic factors, particularly ambient temperature and humidity on *C. obstrictus* flight and thus dispersal have been undertaken; results indicate that flight height and dispersal distances are correlated with ambient temperatures and negatively correlated with ambient relative humidity

(Tansey et al. 2008b). Wind speeds may also influence *C. obstrictus* flight height and dispersal; flight behaviour is greatest below 1.5m sec^{-1} (Kjaer-Pedersen 1992). Potential effects of the warming trend in North America (Barrow and Yu 2005) could include more rapid dispersal rates to suitable regions than currently anticipated. These regions include a northerly expansion of 400 to 600 km from current regions suitable for invasion by *C. obstrictus* in North America (Olfert and Weiss 2006).

It is thought that invasion of western Canadian canola agroecosystems by *C. obstrictus* has followed the sequence characteristic of invasive species including initial introduction followed by establishment in the new habitat, and subsequent range expansion (Andow et al. 1990). This may not be the case. Examination of distributions of *C. obstrictus* in Saskatchewan from 2002 to 2007 indicates that, rather than a pattern of establishment and subsequent range expansion, local populations found at a site may not be detected in following years. Invasion of new regions is not necessarily followed by establishment. Dosdall et al. (2009) attributed this elasticity in local population densities in part to dry conditions. Inadequate moisture could influence translocation to developing pods and so provide a less favourable environment for developing larvae; soils can crust when dry and so reduce the ability of mature larvae to enter for pupation. Also, *C. obstrictus* adults preferentially imbibe water rather than feed when removed from overwintering diapause (J. Tansey, unpublished data), suggesting the importance of adequate springtime moisture.

Economic importance of *C. obstrictus*

The cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae), is an invasive alien Brassicaceae oligophage and an important pest of brassicaceous oilseed crops in North America particularly canola, *B. napus* and *B. rapa*, and brown mustard, *B. juncea* (McCaffrey 1992; Buntin et al. 1995; Cárcamo et al. 2001; Dosdall et al. 2001). In addition to *C. obstrictus*, *C. neglectus*, *Ceutorhynchus americanus* Buchanan, *Ceutorhynchus oregonensis* Dietz, *Ceutorhynchus subpubescens* LeConte and *Ceutorhynchus rapae* Gyllenhal are also present in brassicaceous oilseed crops in western North America although none are responsible for the magnitude of economic damage attributed to *C. obstrictus* (Hoffman 1954; Anderson 1997; Dosdall et al. 1999; Dosdall et al. 2007a).

Canola is an important seasonal crop in North America, particularly the western prairie provinces of Canada and north-central and south-eastern states of the United States of America (McCaffrey 1992). Canada exports approximately 70 percent of its 11.3 million acres (10-year average) of canola production; in 2007 to 2008 Canada exported 1.3 million tonnes of canola oil, 1.9 million tonnes of seed meal, and 4.2 million tonnes of seed (Statistics Canada 2009). The total value of these exports exceeded 3 billion Canadian dollars (BCAD); annual estimates of the contribution of canola production to the Canadian economy exceed 13 BCAD (Canola Council of Canada 2008, 2009a). Canada is set to increase canola production by 65 percent by 2015 including a 44 percent increase in seed exports comprising anticipated exports to Japan of 2.0 million tonnes,

Mexico of 1.5 million tonnes, the United States of 1.0 million tonnes, and Pakistan and China of 2.0 million tonnes (Reuters 2007).

The financial cost of *C. obstrictus* to Alberta canola production was recently estimated at $5 000 000 per year (Colautti et al. 2006). An estimated 180,000 ha of *B. napus* and *B. rapa* canola and brown mustard (*B. juncea*) crops required insecticide application to control *C. obstrictus* populations in western Canada from 1999 to 2005; the estimated cost of applications was 4.5 million (CAD) (Western Committee on Crop Pests 2001, 2002, 2003; L. Dosdall unpublished data).

Feeding by both *C. obstrictus* adults and larvae are associated with crop damage and represent potential economic losses. Although brassicaceous oilseed plants normally abort up to 60 percent of flower buds, and flowers and can compensate for damage by retaining buds or flowers (Pechan and Morgan 1985; Lamb 1989 and references therein), increases in early-season losses of flowers and buds have been attributed to *C. obstrictus* adult feeding (Dosdall et al. 2001). Adult feeding on developing racemes results in a distinctive pattern of blanching and desiccation that has been termed ‘bud blast’ (Dosdall et al. 2001). Oviposition occurs in developing pods of brassicaceous host plants; although individual eggs are more common (Alford et al. 1991), up to 10 eggs per pod may be deposited by individual females (J. Tansey, unpublished data). Total fecundity of females can exceed 240 eggs (Bonnemaison 1957). Females chew circular holes in the walls of developing pods to oviposit next to seeds; larvae feed on developing seeds, consuming five to six during three instars (Dmoch 1965).

Larval feeding is also associated with reduced grain test weights (Buntin et al. 1998). Although plants can compensate for seed damage by increasing numbers of pods (Tatchell 1983), estimated losses attributed to *C. obstrictus* larval feeding are 15 to 20 percent for North American spring canola (Dosdall et al. 2001) and 35 percent for winter rape (McCaffrey et al. 1986). For European brassicaceous oilseed crops, losses can exceed 18 percent (Alford et al. 2003; Williams 2004). Increased numbers of pods may also be a compensatory response to adult feeding (Lamb 1989). Although canola crops can tolerate 26 percent infestation rates without measurable yield loss, seed weights are reduced by 20.2, 38.1 and 52.2 percent, for one, two and three larvae per pod, respectively (Buntin 1999).

Larvae chew circular holes in the pod wall and emerge to fall to the ground when they reach a pre-pupal wandering stage (Dmoch 1965). Holes associated with oviposition, adult feeding and larval emergence can act as avenues for other arthropods and fungal pathogens to enter pods (Dosdall et al. 2001). Other arthropods include thrips (Thysanoptera), parasitoid wasps and, in Europe, the pod midge *Dasineura brassicae* (Winnertz) (Diptera: Cecidomyiidae) (Lamb 1986; Dosdall et al. 2001, 2002). Although secondary damage to seeds associated with thrips has not been quantified, galling associated with *D. brassicae* larvae can contribute significantly to losses by causing premature dehiscence and seed loss (Ankersmit 1956). Because *D. brassicae* has a relatively weak ovipositor, it relies on deformations and holes in pods caused by *C. obstrictus* and other insects such as *Lygus* spp. to gain access to seeds (Ankersmit

1956; Hughes and Evans 2003). Important fungal pathogens in Canada include *Sclerotinia sclerotiorum* (Lib.) de Bary, *Leptosphaeria maculans* (Desmaz.), and *Alternaria* spp.; fungal pathogens entering holes in pods are most problematic under humid conditions (Thomas 2003).

In addition to losses associated with seed loss and reduced seed quality, larval feeding can also cause premature shattering of infested pods, and can delay maturation or result in uneven maturation of brassicaceous oilseed crops (Tulisalo et al. 1976). Because losses to canola production associated with all active stages of *C. obstrictus* and combined effects of direct damage by weevils and indirect effects associated with other arthropods and pathogens and agronomic considerations have not yet been subject to rigorous testing, loss estimates (McCaffrey et al. 1986; Dosdall et al. 2001; Alford et al. 2003; Williams 2004) are likely conservative. Larval exit-hole data from studies in 2007 and 2008 from *B. napus* cv. Q2 monocultures and numbers of weevils per sweep at mid flowering (15 per sweep in 2007; 20 per sweep in 2008) allowed development of a preliminary model. It was assumed that one exit-hole resulted in a total loss of yield from that pod due to shattering. From these data, I developed a natural logarithmic model [($y = 0.145 \ln(x)$] to explain numbers of exit-holes associated with adult numbers mid-season (Figure 1.1). Results indicate a linear relationship in pod loss as *C. obstrictus* adult densities increase to 10 adults per sweep net sample, and subsequently more gradual increases in pod loss as densities increase to 90 and 100 adults per sweep.

Control strategies

Chemical control

Chemical control is effective and widely used for control of *C. obstrictus* populations in Europe and North America. In western Canada, the pyrethroid insecticides Matador® and Silencer ® (cyhalothrin-lambda), and Decis ® (deltamethrin) are registered for application as foliar sprays to control *C. obstrictus* populations (Government of Alberta 2009). Foliar application of either deltamethrin or cyhalothrin-lambda is highly effective for reducing *C. obstrictus* populations (Cárcamo et al. 2005). Although chlorpyrifos (an organophosphate) can also be effective for reducing *C. obstrictus* abundance in western Canada, Cárcamo et al. (2005) found that control with this compound could be highly variable. Insecticide application is most effective when 70 percent of plants in a crop have three to ten open flowers so as to protect developing pods from oviposition and flowers from adults (Dosdall et al. 2001). An important consideration for recommending spraying in early flower is to minimize insecticide impacts on non-target organisms. At moderate commodity prices, chemical control is recommended in western Canada at a nominal economic threshold of three to four adults per sweep sample based on ten 180^{o} sweep samples in at least five locations per field (Dosdall et al. 2001). This threshold was reduced to two adults per sweep as commodity prices increased from near $400 per tonne in the spring and summer of 2007 to over $750 per tonne in the spring and summer of 2008; prices are currently (June 2009) near $500 per tonne (Canola Council of Canada 2009b).

Although insecticide application is effective for reducing local *C. obstrictus* populations, re-colonisation from nearby *B. napus* fields is common. A dense population of *C. obstrictus* in a *B. napus* field near Lethbridge was eradicated with the insecticide cyhalothrin-lambda on 26 June 2001 (98-100 percent Abbotts Adjusted Mortality, 48 h after treatment). Within 10 d, weevil densities approached 20 to 30 percent of pre-application levels field-wide. The nearest site with a large number of Brassicaceae hosts was a commercial *B. napus* field approximately 2 km from the study site (L. Dosdall, unpublished data). There are also environmental and human health concerns associated with the application of these insecticides. These include high acute mammalian toxicity (rat acute oral LD 50 = 395 mg kg^{-1} for deltamethrin and 278 mg kg^{-1} for cyhalothrin-lambda); both active ingredients also cause severe eye and skin irritation and are highly toxic to fishes and other aquatic organisms, pollinating bees, parasitoids and predators (Government of Alberta 2009). Buntin (1999) indicated that chemical foliar application is not warranted until pod infestation rates reach 26 to 40 percent. Based on the model developed for this document (Figure 1.1), pod loss of 26 percent is reached at six weevils per sweep. Although this model is a preliminary approximation of the system, it suggests that the nominal threshold of three to four adult weevils per sweep is reasonable and thresholds should likely not be set far below this level. Tolerance of relatively diffuse *C. obstrictus* populations will reduce chemical input and minimize its effects on beneficial insects and potential effects on human health and financial costs.

Proposed options for chemical control also include insecticidal seed treatments. Seed treatments with several different compounds are effective for controlling brassicaceous oilseed seedling pests such as *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae) (e.g. Tansey et al. 2008c, 2009). Evaluations of the effectiveness of insecticidal seed treatments have also been conducted for *C. obstrictus.* Cárcamo et al. (2005) reported similar larval emergence from *B. napus* grown from lindane (a chlorinated hydrocarbon)-treated and untreated seed. However, comparisons of the neonicotinoid insecticides clothianidin and imidacloprid and lindane indicated that these neonicotinoid compounds negatively influence *C. obstrictus* larval development due to sustained systemic activity of these compounds (Dosdall, in press). Although seed treatment with imidacloprid was reported to be effective for reducing *C. obstrictus* damage in Washington State (Bragg 1999a, b), Cárcamo et al. (2005) found that none of imidacloprid, lindane, or acetamiprid (a neonicotinoid) were effective for reducing damage in southern Alberta.

Trap crops

Examinations of stimulo-deterrent diversion (push-pull) strategies (as per Miller and Cowles 1990) to manipulate local *C. obstrictus* populations have been undertaken in North America (Buntin 1998; Cárcamo et al. 2007) and Europe (Buechi 1990; Cook et al. 2004, 2006), and may represent a useful control strategy to minimize insecticide usage. Trap crops are usually more attractive to species or are at a more attractive growth stage than the main crop (Shelton and

Badenes-Perez 2006). Attractiveness can be influenced by visual and/or chemical cues (e.g. Prokopy and Owens 1983). Greater attractiveness of a host plant species or growth stage has the effect of concentrating pest populations so that they are underrepresented in the main crop until it is past a vulnerable stage or may be eradicated with insecticides (Cook et al. 2006). Buntin (1998) found that 4.9 m wide perimeters of fall-seeded spring canola (trap crop) around 0.35 ha conventional winter canola plots (main crop) were moderately effective for reducing weevil numbers in the main crop. The spring canola flowered two to three weeks earlier and concentrated weevils. However, the main crop still sustained yield loss despite application of insecticide to the trap crop. Cárcamo et al. (2007) reported that trap crops consisting of 20- to 30-m-wide borders of *B. rapa* around 1600 m by 1600 m plots were effective for reducing *C. obstrictus* damage. The *B. rapa* trap crop flowered approximately one week earlier than the main crop and effectively concentrated *C. obstrictus* populations; these were sprayed with insecticide. Although the area sprayed was greatly reduced, *C. obstrictus* control was comparable to application of insecticides to the whole crop (Cárcamo et al. 2007).

Natural enemies

Natural enemies have less effect on *C. obstrictus* populations in their extended North American geographic range than in Europe (Harmon and McCaffrey 1996; Williams 2003; Dosdall et al. 2009). In Europe, *C. obstrictus* populations are subject to control by several species of parasitoids especially *Trichomalus*

perfectus (Walker) (Hymenoptera: Pteromalidae) and *Mesopolobus morys* (Walker) (Hymenoptera: Pteromalidae); rates of 80-90 percent parasitism of *C. obstrictus* local populations in Europe have been attributed to these two species (Buntin 1998; Murchie and Williams 1998; Williams 2003). Because of these high parasitism rates, classical biological control has been viewed as a potentially effective strategy to reduce *C. obstrictus* populations in North America (Muller 2006).

The first North American releases of potential classical biological control agents for *C. obstrictus* were made in 1949; three pteromalids, identified at the time as *Trichomalus fasciatus* (Thomson), *Xenocrepis pura* Mayr, and *Habrocytus* sp. were released in British Columbia (McLeod 1953). Problems with synonymy and misidentification became apparent after their release. Examination of voucher specimens preserved from the original releases determined that the correct identities of the species released were *T. perfectus*, *M. morys* and *Stenomalina gracilis* Walker (Hymenoptera: Pteromalidae), respectively (Gibson et al. 2005). *Trichomalus lucidus* (Walker), *Mesopolobus moryoides* Gibson and *Pteromalus puparum* (Linnaeus) (Hymenoptera: Pteromalidae) have also been reared from *Brassica* spp. pods in British Columbia although initial identification of these specimens placed these as *T. fasciatus*, *X. pura*, and *Habrocytus* sp. , respectively; *T. lucidus* from these samples was also misidentified as *P. puparum* (Gibson et al. 2006).

Several native and naturalised parasitoids have also expanded their host ranges to exploit naturalized *C. obstrictus* populations in North America; these

include *T. lucidus* and *M. moryoides* (Dosdall et al. 2009). Surveys of western Canadian *C. obstrictus* populations indicated that *T. lucidus, Chlorocytus* sp., *Pteromalus* sp. (Hymenoptera: Pteromalidae), and *Necremnus tidius* Walker (Hymenoptera: Eulophidae) are the most common parasitoids of *C. obstrictus* in this region (Dosdall et al. 2007b, 2009). *Trichomalus lucidus* and *N. tidius* have a Holarctic distributions but the origins of *Chlorocytus* sp., and *Pteromalus* sp. are currently unknown and both species require careful taxonomic evaluations to determine their status as native or introduced to North America (Dosdall et al. 2009). However, the effectiveness of these parasitoids to suppress *C. obstrictus* populations appears limited. Although parasitism rates associated with each species can fluctuate greatly, average combined parasitism attributable to these species is typically less than 15 percent (Dosdall et al. 2009). Efforts to control *C. obstrictus* populations may benefit from the introduction of European Chalcidoidea.

The larval ectoparasitoids *T. perfectus* and *M. morys* are currently being examined for suitability for release as classical biological control agents for *C. obstrictus* in North America (Kuhlman et al. 2006). However, some of the population fluctuations observed for naturalized parasitoids have been attributed to competition among Chalcidoidea for *C. obstrictus* larvae (Dosdall et al. 2009). This competition may limit the establishment of introduced biocontrol agents. Evaluation of the competitive interactions among these Chalcidoidea species is required.

Establishment of an effective classical biological control program for *C. obstrictus* requires accurate identity of potential agents and host specificity testing (Gillespie et al. 2006). Non-target hosts that may be threatened by potential *C. obstrictus* biological control agents include potential and established biological control agents for invasive plants species (Kuhlmann et al. 2006). Threatened weed biocontrol agents may include the stem feeding *Hadroplontus litura* (Fabricius) released on Canada thistle, *Cirsium arvense* (L.) Scopoli [Asteraceae] (McClay et al. 2002a), *Microplontus edentulus* (Schultze) released on scentless chamomile *Tripleurospermum perforatum* (Mérat) Laínz (=*Matricaria perforata* Mérat) (McClay et al. 2002b), and the seed feeding *Ceutorhynchus turbatus* (Schultze), the foliar feeding *Ceutorhynchus cardariae* Korotyaev, and *Ceutorhynchus merkli* Korotyaev, currently being considered for release on hoary cress, *L. draba* (=*Cardaria draba*) [Asteraceae] (Cripps et al. 2006). *Trichomalus perfectus* has been reared from *C. cardariae* (F. Muller and M. Cripps, unpublished data). Native North American weevils may also be threatened by introduced and redistributed parasitoids with Nearctic and Holarctic distributions (Dosdall et al. 2009). Both *N. tidius* and *T. lucidus* can exploit *C. neglectus* as a host (Dosdall et al. 2007b). Likely increases in population sizes of parasitoids associated with increasing *C. obstrictus* populations and competition between *C. obstrictus* and *C. neglectus* may combine to reduce populations of the indigenous weevil (Dosdall et al. 2007b).

Development of resistant germplasm

Development of insect-resistant genotypes may reduce our current reliance on chemical insecticides (Dosdall et al. 2001). Intergeneric hybridization is an effective means of incorporating desired traits into a cultivated crop species by broadening its genetic base (Brown et al. 1997). White mustard, *Sinapis alba* L. [2n = 24, SaSa genome (Hemmingway 1976)], is currently grown for use as a condiment and has many desirable agronomic traits. This species has a relatively high seed yield (Gareau et al. 1990), is drought and high temperature tolerant (Downey et al. 1975), and is resistant to pod shattering (Kadkol et al. 1984). Some *S. alba* genotypes are highly resistant to infection by fungal pathogens like *Alternaria* spp. (Brun et al. 1987). *Sinapis alba* is also resistant or tolerant to several insect species that frequently attack *B. napus* including *Phyllotreta* spp. flea beetles (Coleoptera: Chrysomelidae) (e.g. Lamb 1980), the root maggots *Delia radicum* (L.) and *Delia floralis* (Fallén) (Dosdall et al. 1994), and *C. obstrictus* (Doucette 1947; Kalischuk and Dosdall 2004). Kalischuk and Dosdall (2004) conducted comparisons of *C. obstrictus* feeding punctures, eggs, larvae and larval exit-holes and new generation adult feeding among seven brassicaceous species including *B. napus* and *S. alba*. They found that although late-season adults will feed upon all of the species tested, *S. alba* was resistant to direct damage associated with *C. obstrictus* larvae and sustained fewer larvae. Although *S. alba* is more resistant to these insects than *B. napus,* most genotypes are unsuitable for oil production due to poor oil and meal quality associated with intermediate levels of erucic acid and high glucosinolate content in seeds (Brown

et al. 1997 and references therein). Intergeneric hybrid progenies of *B. napus* x *S. alba* were examined as a way to combine the desirable traits of *B. napus* and *S. alba* and produce a *B. napus* plant with canola seed quality and insect resistance (Brown et al. 1997; Kott and Dosdall 2006).

Although introgression can be an effective means to introduce desired traits to crops, there are reproductive barriers among member of the Brassicaceae that restrict the transfer of genetic material among member species. Methods examined to overcome barriers among members of the Brassicaceae include protoplast fusion (Primard et al. 1988), *in vitro* ovule fertilization (Zenkteller 1990), ovary culture (Chevre et al. 1994) and embryo rescue (Ripley and Arnison 1990). Embryo rescue has been used successfully for more difficult crosses including *S. alba* x *B. napus* (Brown et al. 1997; Kott and Dosdall 2004). By this technique, embryos are excised from ovaries or ovules before they are aborted and reared on artificial media (Sharma et al. 1996). Intergeneric lines for the current study were developed by crossing *S. alba* cv. Kirby and *B. napus* cv; F1 hybrids were produced using an embryo rescue technique (Ripley and Arnison 1990). These were backcrossed with *B. napus* for three generations (Kott and Dosdall 2004). Doubled haploids (563) were propagated from 12 of these lines, using the *in vitro* method of Fletcher et al. (1998). Of these, 230 lines were selected for having canola-quality seed glucosinolate content (Kott and Dosdall 2004).

Putative introgression of resistance from *S alba* x *B. napus* has produced several lines that have proven resistant to *C. obstrictus* in field trails and laboratory experiments (Dosdall and Kott 2006). Development times of *C.*

obstrictus larvae are significantly delayed and overall production of larvae is reduced in resistant lines. Although mechanisms of *C. obstrictus* resistance are still unclear, they likely include both antixenosis and antibiosis. They may also include variable visual and olfactory factors that influence host selection (Tansey et al. 2008a).

Interactions with host plants

Herbivorous insects can demonstrate great selectivity among visual and olfactory cues used for host location (Prokopy and Owens 1983). Visser (1986) indicated that glucosinolates and their volatile hydrolysis products, which are relatively specific to Brassicaceae, should act as host association cues to specialists of this group. Host location in many herbivorous insects is thought to follow a stepwise process: directed flight in response to olfactory cues including upwind anemotaxis, behavioural responses to reflected spectral properties at relatively close ranges and chemical and tactile cues at intimate ranges (Kennedy 1965; Finch and Collier 2000).

Glucosinolates

Although glucosinolates are characteristic of the Brassicaceae, 500 species of non-brassicaceous plants also produce at least one of 120 known glucosinolates; these include several species in the Capparaceae and Caricaceae and at least one genus in the Euphorbiaceae (*Drypetes*; syn. *Putranjiva*) (Fahey et al. 2001 and references therein).

Glucosinolates are glycosides comprised of glucose bonded to another non-sugar group by an S-glycosidic bond (thioglycoside) resulting in S-D-thioglucose and sulfonated oxime moieties (Halkier and Gershenzon 2006 and references therein). These are generally water soluble anions balanced in nature by cations (usually potassium) (Halkier and Gershenzon 2006 and references therein). Glucosinolates are characterised by associated R- groups and more than 120 different R- groups have been characterised; these fall into three main categories: 1) Aliphatic - with side groups derived from aliphatic amino acids (most commonly methionine), 2) Aromatic - derived from phenylalanine or tyrosine, and 3) Indolyl = heterocyclic - derived from tryptophan (Fahey et al. 2001; Halkier and Gershenzon 2006). Although as many as 34 individual glucosinolates have been detected in *Arabidopsis thaliana* L. (Kliebenstein et. al. 2001), most members of the Brassicaceae have fewer than six major glucosinolates with a few others in trace amounts (Rask et. al. 2001 and references therein). Different glucosinolates are expressed at different developmental stages and in different tissues (e.g. Porter et al. 1991).

Glucosinolates are typically not volatile so the effect on olfactory responses of herbivores and, in part, defensive function of glucosinolates are realized through the action of myrosinases (β-thioglucosidase); these enzymes facilitate irreversible hydrolysis of the thioglucosidic bond and liberation of the D-glucose and thiohydroximate-O-sulphonate (aglycone) moieties (Rask et al. 2001 and references therein). Little is known about substrate specificity of myrosinases (e.g. Reed et. al. 1993). The unstable aglycone liberates sulfate and

rearranges nonenzymatically to yield bioactive epithionitriles, nitriles, thiocyanates and isothiocyanates; a mixture of products is typically formed with low pH favoring nitrile formation, and neutral or high pH favoring isothiocyanates (Halkier and Gershenzon 2006 and references therein). Approximately 20 genes code for myrosinases in *B. napus*; these include three subfamilies of genes: MA, MB, and MC (Falk et al. 1995). A myrosinase in *B. napus* roots not associated with any of the three described subfamilies has also been detected (Rask et al. 2000).

Many of the products of the aglycone rearrangement are also toxic to plants, thus glucosinolates are compartmentalized in 'sulfur rich' cells between phloem and epidermis (Rask et al. 2000) and myrosinase in adjacent 'myrosin' cells (Andréasson et al. 2001). Detoxification of glucosinolate hydrolysis products, particularly isothiocyanates *in planta* is not well understood (Wittstock and Gershenzon 2002). Compartmentalization means that volatile hydrolysis products of glucosinolates are generally produced only when tissue and so myrosin and sulphur rich cells are damaged (Rask et al. 2000; Andréasson et al. 2001). However, allyl, 3-butenyl, 4-pentenyl, and 2-phenylethyl isothiocyanates have been detected in headspace volatiles of undamaged *B. napus* (Blight et al. 1995).

Glucosinolate profiles vary greatly among Brassicaceae including among commercial varieties and this variability may influence herbivore responses. Examinations of *S. alba*, *B. napus, B. rapa*, and *B. juncea* indicated differences in the proportions of 3-butenyl, 4-pentenyl, 2-phenylethyl and *p*-hydroxybenzyl

glucosinolates (McCloskey and Isman 1993); sinalbin (*p*-hydroxybenzyl glucosinolate) was prevalent in *S. alba* but absent from the other species. McCaffrey et al. (1999) also detected differences in the glucosinolate profiles of *B. napus*, *S. alba* and an intergeneric *S. alba* x *B. napus* hybrid accession. Sinalbin was not detected from seeds, leaves or pods of *B. napus* but was present in all *S. alba* tissues examined and in the leaves and seeds of the hybrid; 3-butenyl and 4-pentenyl glucosinolates were present in pods of *B. napus* and the hybrid but not in *S. alba* (McCaffrey et al. (1999). Ulmer and Dosdall (2006b) also found, in a comparison of the seed and pod glucosinolate profiles of two *S. alba* (cvs. L-GS and AC Pennant) and two *B. rapa* accessions (cvs. Echo and Boreal), *B. carinata* Braun (cv. Dodolla), *B. juncea* (cv. H-Butenyl), and *B. napus* (cv. AC Excel) that a detectable level (0.05 μmol g^{-1}) of sinalbin was limited to *S. alba* (cv. AC Pennant). However, 3-butenyl glucosinolate was also detected in the seed of all accessions tested and in the pods of all but *S. alba* (cv. AC Pennant) and *B. napus* (cv. AC Excel); 4-pentenyl glucosinolate was detected in the seed of all but *B. carinata* and *S. alba* and the pods of *B. rapa*. Amounts of 3-butenyl and 4-pentenyl glucosinolate were much higher in *B. rapa* (cv. Echo and cv. Boreal) and *B. juncea* than any other accessions tested. Assessments of *B. rapa* as a trap crop for control of *C. obstrictus* in *B. napus* has shown that it is more attractive than the main crop (Cárcamo et al. 2007); greater attraction may be associated with levels of 3-butenyl glucosinolate hydrolysis products (Moyes et al. 2000; Moyes and Raybould 2001). Importantly, *B. rapa* flowers about a week earlier than *B.*

napus oilseeds (Thomas 2003) so differential attraction of *C. obstrictus* in the field is also likely influenced by visual cues.

Differing responses of insect herbivores to *S. alba*, *B. napus*, and *B. rapa* have been attributed to *p*-hydroxybenzyl glucosinolate content (Bodnaryk 1991). However, *p*-hydroxybenzyl isothiocyanate is the major hydrolysis product of this glucosinolate and is highly unstable; its half-life is approximately 6 min at *S. alba* physiological pH (Borek and Morra 2005; Vaughn and Berhow 2005). A quinone is formed from the breakdown of *p*-hydroxybenzyl isothiocyanate; this compound hydrolyzes to SCN^- . Given the short half-life of *p*-hydroxybenzyl isothiocyanate, it is unlikely to influence long-distance olfactory responses in *C. obstrictus*. Differences in the attractiveness of *S. alba*, *B. napus*, and *B. rapa* are likely associated with amounts and perhaps proportions of stimulatory kairomones including 3-butenyl and 2-phenylethyl isothiocyanate (as per McCaffrey et al. 1999).

Chemical differences among *S. alba* x *B. napus* genotypes

Several tissues of the genotypes examined in this study have been subject to high performance liquid chromatography (HPLC) analysis (Shaw 2008). A peak was associated with an as yet uncharacterised compound (retention time 21.4 ± 0.03 min), associated with upper cauline leaves of resistant and susceptible *S. alba* x *B. napus* lines, that was determined though myrosinase degradation to be a glucosinolate. Peak height was inversely correlated with weevil infestation scores (the mean numbers of larvae per pod from genotypes in replicated field trials)

and, on average, the peak was 3.5 times larger in resistant than susceptible lines (Shaw 2008). Shaw (2008) also detected differences among resistant and susceptible *S. alba* x *B. napus* lines in the amounts of another as yet unidentified glucosinolate (retention time 20.5 ± 0.01 min) associated with seeds of immature pods; its peak height was correlated with weevil infestation scores and, on average, the peak was 3.5 times greater in susceptible than resistant lines (Shaw 2008). Determining the identities of these compounds will help determine their roles in interactions among *C. obstrictus* and these novel genotypes. Estimates of the identities of these compounds are made for this study and addressed in Chapter 4. Potential roles and implications of these compounds in relation to resistance are addressed in Chapters 4 and 5.

Vision in *C. obstrictus*

At relatively close ranges, spectral quality of light reflected by a potential host plant is the predominant cue associated with detection and alightment in many herbivorous insects (Prokopy and Owens 1983). Directed flight of herbivorous insects in response to visual cues has been proposed (Moericke 1952). Visual systems with receptors with response maxima ($\lambda_{max)}$ near 350 nm (UV), 450 nm (blue) and 550 nm (green) are most common among the insects examined to date (Briscoe and Chittka 2001). Yellow (λ = 560-590 nm) also influences many herbivorous insects as a 'supernormal' stimulus (Prokopy and Owens 1983). The set of UV, blue, and green photoreceptors is ancestral to Insecta; lineages have added or lost receptors due to selective pressures (Chittka 1996). A modeled

hypothetical system of spectral receptor types with λ_{max} = 340, 440 and 560 nm was found to be near optimal for distinguishing floral colours from background and among co-occurring species (Chittka 1996). A system with these responses maxima is typical of the Apidae (Hymenoptera) (Chittka 1996). Visually, flowers can differ in size, shape, symmetry, depth, spatial frequency, spatial orientation and height; all these traits may require differentiation and can influence the responses of anthophilous insects (Dafni et al. 1997).

Although improved population monitoring and design of *C. obstrictus*-resistant host plants would benefit from assessments of responses of these weevils to specific frequencies of reflected light, few previous studies have investigated their visual system. Exceptions include assessments of trap colours: Smart et al. (1997) found that yellow traps were attractive to *C. obstrictus* but black, white, and green traps were not. Buechi (1990) found that yellow, light green, and white traps were attractive to *C. obstrictus*. Effects of specific frequencies of light including those in the ultraviolet range have not yet been conducted for *C. obstrictus*. However, in an examination of the responses of *Anthonomus pomorum* L. (Coleoptera: Curculionidae), Hausmann et al. (2004) detected an affinity of females for UV, green, and blue light and concluded that these weevils have a trichromatic visual system; they also suggested that visual cues are an important component of *A. pomorum* host plant location and selection. Investigations of the *C. obstrictus* visual system are essential to better understand the importance of visual cues to *C. obstrictus* host selection. Improved understanding of weevil vision will allow the development of more efficient

trapping systems to monitor and possibly influence *C. obstrictus* populations. Resistance breeding may also benefit from a better understanding of the importance and specific responses of *C. obstrictus* to visual cues; plants that express suboptimal visual cues could be less subject to attack.

Behavioural evidence is necessary to assess potential effects of specific wavelengths on responses like host selection and results of an investigation into the behavioural responses of *C. obstrictus* to components of the hypothesized UV-blue-green system are presented in Chapter 2. Effects of the potential 'supernormal' stimulus yellow are also assessed as are interactions among frequencies. A large naturalized *C. obstrictus* local population was offered traps that reflected various amounts of each component of the hypothesised visual system. Captures of *C. obstrictus* males, females, and gravid females associated with each trap colour over two growing seasons were compared. These evaluations allowed a greater insight into visual cues associated with host selection in *C. obstrictus.* Effects of weevil ontogeny on responses were also tested.

Visual cues and host selection

Ceutorhynchus obstrictus is a Brassicaceae oligophage and adults are capable of exploiting various members of this plant family at different points of their phenology (Dmoch 1965). Plant species chosen as oviposition sites are more limited than those exploited as early-season 'food' hosts (as per Dmoch 1965). Differences in the amounts of specific frequencies of light reflected from flowers

and foliage are likely an effective means of discriminating hosts for *C. obstrictus.* Differences in the spectral quality associated with different potential host plants occur. Examinations of cultivar-specific *B. rapa* floral UV colour proportions (the proportion of light below and above 365 nm) have indicated differences; these have been implicated in the preferences of anthophilous insects, particularly pollinators (Yoshioka et al. 2005). Differences in amounts of 350 and 580 nm light reflected from *S. alba* and *B. napus* flowers have been detected; *S. alba* flowers reflect more 350 nm light and *B. napus* reflect more 550 nm light (Tansey et al. 2008a). These differences may influence attractiveness of the differentially acceptable host plants. Frearsen et al. (2006) demonstrated decreased *C. obstrictus* attack on apetalous *B. napus* (and so lacking the distinct yellow colouration typical of flowers). Their results suggest both the importance of floral visual cues for host location and the potential role of yellow colouration in allowing *C. obstrictus* to differentiate flowers from foliage. However, *B. napus* petals also produce odours (Cook et al. 2005), so the exclusive influence of spectral quality on these results cannot be explicitly concluded.

Yellow is a relatively ubiquitous flower colour, is attractive to many anthophilous insects and considered a means to discriminate foliage from non-foliage (Prokopy and Owens 1983); perception of this colour also contributes to discrimination among tissues. Chittka (1996) also indicated that a system of spectral receptor types with λ_{max} = 340, 440 and 560 nm was near optimal for discrimination of flowers from foliage and among green foliage. Different amounts of 350 nm reflected from *S. alba* and *B. napus* foliage have also been

detected; *S. alba* reflect less of this frequency from foliage than *B. napus* (Tansey et al. 2008a). *Ceutorhynchus obstrictus* host choices may also be influenced by the contrast at specific frequencies between flowers and foliage.

A specific predetermined template of stimuli has been implicated in insect responses to objects; stimuli that do not conform are ignored (Laughlin 1981; Wehner 1981). Although *C. obstrictus* can complete development in *S. alba* siliques, it will preferentially oviposit in *B. napus*, given choices (Kalischuk and Dosdall 2004). Differing amounts of floral 550 nm and 580 nm reflectance may be a means to distinguish these hosts, and in light of the antibiotic properties of *S. alba* to *C. obstrictus* (e.g. Dosdall and Kott 2006), suggests the adaptive significance of visual cues to host selection. Strauss et al. (2004) detected differences in the levels of induced glucosinolates among *Raphanus sativus* L. (Brassicaceae) petal colour variants (yellow, white; anthocyanin-containing bronze and pink). Apparency theory (Feeny 1976; Rhoades and Cates 1976) predicts reduced colonization of less apparent lines and plants that are more heavily defended by qualitative toxins should be less apparent.

Results of assessments of *C. obstrictus* responses to visual cues associated with *B. napus, S. alba* and resistant and susceptible *S. alba* x *B. napus* genotypes are presented in Chapter 3. Weevil responses to visual cues associated with each genotype, genotypic floral and foliar reflectance properties and relationships of reflectance properties and weevil responses are assessed. These results are related to results of field resistance trials of these genotypes (Shaw 2008). Results of this

study suggest courses for breeding strategies and deployment of resistant germplasm.

Olfactory cues

Ceutorhynchus obstrictus is attracted to volatile compounds associated with *B. napus* (Free and Williams 1978; Bartlet et al. 1993). Mixes of 3-butenyl, 4-pentenyl and 2-phenylethyl isothiocyanates are attractive to *C. obstrictus* in the late spring (Smart et al. 1997). Each of these compounds was attractive when used to bait field traps (Smart and Blight 1997). However, electroantennogram assessments of these compounds have been somewhat inconsistent. Only 2-phenylethyl isothiocyanate elicited strong responses in one study (Blight et al. 1995), and 3-butenyl and 4-pentenyl isothiocyanates elicited the strongest electroantennogram responses in another (Evans and Allen-Williams 1992). Smart and Blight (1997) found that 3-butenyl, 4-pentenyl and 2-phenylethyl isothiocyanates are attractive to *C. obstrictus*. *Ceutorhynchus napi* (Gyllenhal) and *Ceutorhynchus pallidactylus* (Marsham) are also attracted to 2-phenylethyl isothiocyanate (Walczak et al. 1998). No 3-butenyl, 4-pentenyl or 2-phenylethyl glucosinolates were detected in an examination of *S. alba* foliage (McCloskey and Isman 1993). The amounts of 3-butenyl glucosinolate and so amounts of hydrolysis products of this compound are related to *C. obstrictus* oviposition, and facilitate location and discrimination of host populations (Moyes et al. 2000; Moyes and Raybould 2001).

Although stimulatory kairomones are likely important for host location and discrimination among potential hosts, the effective range of olfactory cues is limited; these rarely elicit behavioural responses in most herbivores insects at ranges greater than several metres (Finch 1980; Reddy et al. 2004). Finch (1980) calculated that *D. radicum* (syn. = *D. brassicae*) (Diptera: Anthomyiidae) should not be able to detect host odours at distances greater than 4.6 m. Calculations were based on the electrophysiological threshold concentrations of green-leaf volatiles and considered a wind speed of 0.45 m sec^{-1} (Finch 1980). *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is considered an 'olfactory searcher' and primarily employs odours to locate host plants (Reddy et al. 2004; Bukovinszky et al. 2005). It is suggested that female *P. xylostella* respond to host odours encountered while flying low (Pivnick et al. 1994). *Plutella xylostella* will respond to host plant odours at 1.3 m in the laboratory (Reddy et al. 2004). *Phyllotreta cruciferae* (Goeze) and *Phyllotreta striolata* (Fabricius) (Coleoptera: Chrysomelidae) are attracted to allyl isothiocyanates (Pivnick et al. 1992). Although small plants are only weakly attractive (Lamb and Palaniswamy 1990), these flea beetles will respond to allyl isothiocyanate lures 1.5 m from a *B. napus* oilseed crop (Soroka et al. 2005). Insect larvae also respond to host odours; *Pieris rapae* (L.) (Lepidoptera: Pieridae) larvae responded to host odours at several centimetres (Jones 1977). Although reliance on olfaction for host discrimination is likely low, relatively passively mobile insects also discriminate among potential hosts. *Brevicoryne brassicae* (L.) (Homoptera: Aphididae) is a relatively weak flyer so is dependent on winds for long distance dispersal (Compton 2002).

Aphids use visual cues to discriminate landscape elements as they descend (Kennedy et al. 1959). They then make short 'attack' flights to neighbouring plants (e.g. Compton 2002). Although there is evidence that these aphids respond to host odours by descending in the air column, most studies suggest that host plant recognition occurs only after landing (Petterson 1973) and long distance orientation to olfactory cues is limited (Bukovinsky et al. 2005). However, *Brassica napus* extracts are attractive to *C. obstrictus* at distances greater than 20 m (Evans and Allen-Williams 1993).

Initial responses to host odours by herbivorous insects include positive anemotaxis: an upwind movement in response to olfactory cues. Although directed flight in response to olfactory cues has not yet been documented for *C. obstrictus*, adults orient upwind toward *B. napus* foliage and flowers and their extracts in olfactometer studies (Bartlet et al. 1997). *Ceutorhynchus obstrictus* have also been documented moving upwind in response to host odours in wind-tunnel tests (Evans and Allen-Williams 1998). Upwind orientation is maintained when the odour source is removed (Kjaer-Pedersen 1992). Gravid *D. radicum* also respond to host odours with positive anemotaxis. Mean flight distance of these flies is approximately 0.5 m while in the odour plume; if the odour source is lost or crosses flight direction, downwind and circling flight begins until the plume is re-entered (Hawkes et al. 1978). Directed flight in response to odour sources has also been demonstrated for female *Mamestra brassicae* L. (Lepidoptera: Noctuidae) (Rojas and Wyatt 1999) and *P. xylostella* (Couty et al. 2006). Plume characteristics influence forager flight patterns. *Delia radicum* respond to diffuse

odours with fast straight flights and to discrete plumes with slower flight and changes in direction (Nottingham and Croaker 1985). Changes in odour concentration may alter flight track angle (Nottingham and Croaker 1987). The best strategy for locating odour sources is upwind flight in response to initial perception and maintaining position or casting if odour is lost (David et al. 1982). *Delia radicum* response distance to host odours increased with reduced wind speed (Finch 1980). *Delia radicum* responded to *Brassica oleracea* L. plots from 15 m in light winds (Hawkes 1974). Wind speed also influences responses of *C. obstrictus*; male responses to leaf extracts decrease when wind speed increases from 0.446 to 0.682 m sec^{-1}; female responses decrease from 0.682 to 1.062 m sec^{-1} (Evans and Allen-William 1998).

Results of laboratory olfactometer assessments of responses of *C. obstrictus* to odours of whole plants, racemes and cauline leaves of *C. obstrictus*-resistant and -susceptible *S. alba* x *B. napus* genotypes and the parental genotypes, *B. napus* and *S. alba*, are discussed in Chapter 4. Both overwintered- and fall-generation weevils were tested. I also investigated potential identities of the uncharacterised glucosinolates detected by Shaw (2008) and found to be related to weevil infestation. Effects of detected polymorphisms on attractiveness of volatile cues associated with specific host genotypes are addressed. Based on these results, potential deployment strategies for novel germplasm and courses for resistance breeding are suggested.

Antixenosis resistance

Mechanisms of resistance reported for *S. alba* and several novel *S. alba* x *B. napus* genotypes also include non-preference or antixenosis modes as defined by Painter (1951) and Kogan and Ortman (1978). Kogan and Ortman (1978) proposed the term antixenosis as a preplacement for Reginald Painter's (1951) 'non-preference'. The term applies to the expression of traits (for example, chemical) by one organism that dissuade its exploitation by another organism; Kogan and Ortman (1978) proposed the term to place emphasis on plant traits rather than those of the potential herbivore. Antixenosis resistance associated with *C. obstrictus* and potential host plants results in fewer eggs deposited by *C. obstrictus* in resistant plants (McCaffrey et al. 1999; Dosdall and Kott 2006). Differences in oviposition and feeding preferences have been attributed in part to varying amounts of the same attractive glucosinolate hydrolysis products that influence olfactory responses (McCaffrey et al. 1999).

Effects of deterrent compounds likely also account for differences in weevil feeding and oviposition preferences. Differences in susceptibilities of *S. alba*, *B. napus*, and *B. rapa* to insect herbivores have been attributed to *p*-hydroxybenzyl glucosinolate (Bodnaryk 1991). However, *C. obstrictus* oviposition and feeding behaviour were examined for *S. alba* varieties that differed greatly in *p*-hydroxybenzyl glucosinolate content; plants of the low *p*-hydroxybenyl glucosinolate variety were not preferentially chosen as oviposition sites by *C. obstrictus* and both demonstrated antixenosis resistance (Ulmer and Dosdall 2006b). Ulmer and Dosdall (2006b) concluded that antixenosis resistance

to *C. obstrictus* demonstrated by both varieties could not be attributed to *p*-hydroxybenzyl concentration. Although detectable levels of *p*-hydroxybenzyl glucosinolate were not reported from introgressed *S. alba* x *B. napus* genotypes tested by Dosdall and Kott (2006), several of these genotypes expressed antixenosis resistance to *C. obstrictus*. A factor other than *p*-hydroxybenzyl concentration is involved in antixenosis resistance to *C. obstrictus* in *S. alba* and the novel *S. alba* x *B. napus* germplasm tested by Dosdall and Kott (2006).

High alkenyl-low indolyl *B. napus* varieties are colonized less by *C. obstrictus* than high alkenyl-low indolyl varieties (Cook et al. 2006). *Brassica rapa* was comparably attractive as a high alkenyl-low indolyl *B. napus* variety in linear track olfactometer tests; these were more attractive to *C. obstrictus* than a high indolyl-low alkenyl glucosinolate *B. napus* variety (Cook et al. 2006). Although much of the difference in colonization demonstrated between high and low indolyl varieties could be attributed to greater amounts of alkenyl glucosinolate hydrolysis products and their influence on olfactory responses, indolyl glucosinolates may have also contributed to the reported differences in olfactory responses reported by Cook et al. (2006) and antixenosis reported by Dosdall and Kott (2006).

Hydrolysis products of 1-methoxy-3-indolylmethyl glucosinolate include indole-isothiocyanates, indole-cyanides, indolyl-3-carbinol, thiocyanate and possibly phytoalexins and auxins (Cole 1976; Mithen 1992; Mewis et al. 2002). Indole isothiocyanates, particularly 1-methoxy-3-indolylmethyl isothiocyanate, reduce oviposition by the Brassicaceae oligophage *Hellula undalis* (Fabricius)

(Lepidoptera: Pyralidae) (Mewis et al. 2002). Although the slight volatility of indole-cyanides reduces the likelihood of effecting long-distance olfactory responses of *C. obstrictus*, responses at more intimate ranges may reduce attractiveness and contribute to antixenosis.

Differences in adult *C. obstrictus* feeding and oviposition preferences among various *B. napus* varieties and between *B. napus* and *S. alba* may also be associated with induced qualitative defences. Induction of 1-methoxy-3-indolylmethyl glucosinolate has been detected in *B. napus* in response to *P. cruciferae* feeding (Bodnaryk 1992) and exogenous application of methyl jasmonate and jasmonic acid (Bodnaryk 1994; Doughty et al. 1995). Systemic 17-fold increases in 1-methoxy-3-indolylmethyl glucosinolate content were detected in a *B. napus* oilseed variety in response to *D. floralis* (Diptera: Anthomyiidae) attack (Birch et al. 1992). Induction of indole glucosinolates can result in reduced herbivore feeding: induction of indolyl glucosinolates reduced *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae) feeding on *B. napus* cotyledons (Bartlet et al. 1999).

Antibiosis resistance

Antibiosis resistance is also an important mechanism of resistance (Painter 1951): these are cases where the interaction of two organisms is harmful to one of the participants. Antibiosis resistance has been demonstrated for novel *S. alba* x *B. napus* germplasm (McCaffrey et al. 1999; Dosdall and Kott 2006). Although differences in oviposition and feeding preferences have been attributed to

differing levels of stimulatory kairomones (McCaffrey et al. 1999), mechanisms of antibiosis resistance remain unclear. Prolonged *C. obstrictus* larval development times and reduced larval weights in *S. alba* and *S. alba* x *B. napus* hosts have been attributed to *p*-hydroxybenzyl glucosinolate content (McCaffrey et al. 1999). Differences in susceptibilities of *S. alba*, *B. napus*, and *B. rapa* to several herbivores have also been attributed to differences in *p*-hydroxybenzyl glucosinolate content (Bodnaryk 1991). The effects of *p*-hydroxybenzyl glucosinolate and/or hydrolysis products of this compound as a repellent, antifeedant, and/or toxic constituent remain unclear.

Antibiosis resistance was demonstrated for *S. alba* var. L-GS; this variety was associated with reduced *C. obstrictus* oviposition, longer larval development times and reduced larval weights relative to *B. napus* (Ulmer and Dosdall 2006b). However, *p*-hydroxybenzyl glucosinolate was not detected in any of the tissues examined for this variety. It seems likely that another factor must be influencing larval development in this *S. alba* variety and likely in resistant introgressed *S. alba* x *B. napus* lines tested by McCaffrey et al. (1999) and Dosdall and Kott (2006). Hopkins et al. (1998) detected 1-methoxy-3-indolylmethyl glucosinolate in all tissues of three cultivars of *S. alba.*

The potential effects of greater constitutive or induced 1-methoxy-3-indolylmethyl glucosinolate levels may also contribute to reductions in larval weights and prolonged development times demonstrated by Dosdall and Kott (2006). Prolonged *P. rapae* development times were associated with *B. oleracea* expressing greater levels of 1-methoxy-3-indolylmethyl glucosinolate (Gols et al.

2008). Increases in 1-methoxy-3-indolylmethyl glucosinolate levels of more than 10-fold (from < 0.01 to 0.10 mg g^{-1}) in response to mechanical damage in *S. alba* have been reported (Koritsas et al. 1991). It is important to note that development times in *S. alba* AC Pennant (a high *p*-hydroxybenzyl glucosinolate variety) were greater and larval weights were less than for *S. alba* L-GS (Ulmer and Dosdall 2006b). There may be an effect of *p*-hydroxybenzyl glucosinolate on larval development and/or an interaction of *p*-hydroxybenzyl and 1-methoxy-3-indolylmethyl glucosinolates.

Results of examinations of laboratory and greenhouse assessments of antixenosis and antibiosis resistance in novel *S. alba* x *B. napus* genotypes are presented in Chapter 5. *Ceutorhynchus obstrictus* feeding and oviposition preferences among resistant and susceptible novel *S. alba* x *B. napus* germplasm and the parental genotypes, *B. napus* and *S. alba*, were assessed. Larval development times and weights were also assessed for novel and parental germplasm. Assessments of oocyte development of females on resistant and susceptible novel germplasm, parental genotypes and an early-season food host, *Thlaspi arvense* L., were also conducted. *Thlaspi arvense* is a purported early-season food host of *C. obstrictus* (Dmoch 1965), but demonstrates profound antixenosis resistance to the flea beetle, *P. cruciferae* (Palaniswamy et al. 1997; Gavloski et al. 2000). The effects of polymorphisms detected among these genotypes (Shaw 2008) on feeding and oviposition preferences, larval development and oocyte development are also inferred. Deployment strategies

for resistant genotypes and potential strategies for future resistance breeding are discussed.

Deployment of resistant germplasm

Deployment strategies for resistant genotypes require examination. Based on previous studies of introgressed *S. alba* x *B. napus* genotypes (McCaffrey et al. 1999; Dosdall and Kott 2006), antibiosis resistance is anticipated in the genotypes tested in this study. Toxic cultivars are not evolutionarily sustainable in monoculture (Bernal et al. 2004); therefore means to minimize the selective pressures associated with antibiosis resistance are required. Differences in olfactory cues and visual apparency (as per Feeny 1976) may initially contribute to resistance of novel *S. alba* x *B. napus* genotypes in the field. However, herbivore search efficiency has been shown to evolve to correspond to parental oviposition choices and not be influenced by apparency (Parmesan 1991). Because all *S. alba* x *B. napus* genotypes tested by Dosdall and Kott (2006) and the highly weevil-resistant *S. alba* can support *C. obstrictus* larval development (McCaffrey et al. 1999; Dosdall and Kott 2006), it is likely that all genotypes tested in this study can be considered conditionally suitable hosts for *C. obstrictus* larvae. Larval development on these genotypes will facilitate modification of search efficiency. The widespread adoption of resistant germplasm depends in large part on the durability of resistant traits (Rausher 2001). Because of these considerations, incorporation of susceptible refugia has been proposed as means of preserving resistant traits for these genotypes (Tansey et al. 2008a).

Examples of incorporation of susceptible refugia are best known from deployment of transgenic corn (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.) that express *Bacillus thuringiensis* Berliner (*Bt*) δ- endotoxins. A 'high-dose-refuge' (HDR) strategy to reduce selective pressures associated with crops that produce (*Bt*) toxins is recommended by regulatory authorities; the refuge component of this strategy involved planting non-toxic cultivars that are accessible to and can be used by pests for development in close proximity to these crops (Gould 1998; USEPA 2001). This strategy was implemented with the advent of crop genotypes toxic enough to achieve complete mortality of pest populations (Vacher et al. 2003). By this strategy, complete mortality of susceptible homozygotes and majority mortality of heterozygotes occurs after consumption of *Bt* crops; the likelihood that remaining heterozygotes will mate with susceptible homozygotes from susceptible refugia increases and thus reduces the frequency of resistant homozygotes (Gould 1998). The efficiency of this strategy depends in large part on a relatively low frequency of resistance alleles, the functional recessive nature of resistant alleles and random mating among populations (Bourguet et al. 2000; Carrière et al. 2001). Relatively large refuges are required to prevent emergence of resistance in pests (Carrière et al. 2001). Strategies for incorporating lower levels of toxicity have not been well studied (Gould 1998).

Vacher et al. (2003) employed a population genetic model of tobacco budworm, *Heliothis virescens* L. (Lepidoptera: Noctuidae), mortality and gene flow in mixed resistant-susceptible cotton agroecosystems. They assessed the

effects of toxicity associated with transgenic plants and spatial structure and size of refugia on a scale of kilometres. Their results indicated that the HDR strategy is adequate for delaying emergence of resistance traits in this pest but suboptimal for preventing its emergence. Their results also indicated that a strategy that incorporates transgenic plants with low toxicity coupled with refugia occupying approximately 25 percent of the production area in strips separated by approximately 20 km was more effective for maintaining the efficacy of transgenic traits than current HDR practices (Vacher et al. 2003). Comparable results were obtained for modeled 400 km^2 and 600 km^2 cultivated regions (Vacher et al. 2003).

Several insect pests have evolved resistance to *Bt* in the laboratory and field populations of *P. xylostella* and greenhouse populations of *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) have developed resistance to *Bt* sprays (Tabashnik 1994; Tabashnik et al. 2002; Janmaat and Myers 2003). Some field populations of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) have developed resistance to Cry1Ac in Arkansas and Mississippi, seven to eight years after commercial introduction of *Bt* cotton (Ali et al. 2006; Tabashnik et al 2008).

Although several of the *S. alba* x *B. napus* genotypes tested by Dosdall and Kott (2006) demonstrate antibiosis resistance to *C. obstrictus*, none of the genotypes tested including *S. alba* were associated with high adult mortality. Thus, although tests of deployment strategies incorporating spatial parameters that approximate agroecosystems requires testing and modelling, resistant genotypes tested in this study can likely be considered low toxicity and should allow

sustained expression of resistance traits with relatively small refugia (as per Vacher et al. 2003).

An important consideration in the deployment of resistant germplasm is the incorporation and spatial arrangements of susceptible refugia. These considerations assume that virulence in the insect population is recessive and rare; functional recessiveness can be conferred by the HDR strategy, ensuring great mortality of heterozygotes and reducing the frequency of virulence alleles (Rausher 2001). Sufficient frequency of avirulence alleles associated with refugia can contribute to prevention or delay of emergence of homozygous virulence alleles (Gould 1986; Rausher 2001). Random genetic exchange between virulent and avirulent members of pest populations contributes to delays in the emergence of virulence (Comins 1977).

Appropriate strategies for deployment depend in large part on the mobility of pests (Rausher 2001). For pests like *H. virescens*, mobility of larvae is relatively high and may result in dilution of toxic properties associated with resistant plants by movement away from resistant hosts and greater frequency of heterozygotes than if resistant and susceptible hosts are blocked (Rausher 2001). In cases where juvenile insects are limited to individual plants such as *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), interspersed refugia are appropriate because larvae are immobile and therefore exposed to antibiosis associated with resistant plants for their entire development (Smith et al. 2007). For *S. mosellana*, mating likely occurs before dispersal (Pivnick and Labbé 1992), requiring homozygous avirulent mates in close proximity to virulent homozygotes; resistant

plants should be interspersed with susceptible lines (Smith et al. 2007). *Ceutorhynchus obstrictus* larval development is also limited to individual plants, but mating occurs after overwintering and may be some distance from plants that supported larval development (e.g. Dmoch 1965). This aspect of *C. obstrictus* life history places fewer limitations on deployment strategies for resistant germplasm but does not supersede the need for susceptible refugia.

Incorporating susceptible refugia with resistant genotypes may have benefits for crop protection beyond preservation of resistance traits. Arthropod pest outbreaks occur more frequently in monocultures than polycultures (Elton 1958; Pimentel 1961). Inverse relationships between plant diversity and individual herbivore numbers have been documented (Risch et al. 1983). Polycultures of plants with variable resistance traits were typical of traditional and subsistence systems for millennia; these cropping strategies help to stabilize yields and reduce the effects of pests. For example, polycultures of olive, grape and wheat were incorporated by Mediterranean Bronze Age (ca. 2000 BC) farmers to increase production (Renfrew 1972). Polycultures are associated with greater emigration and reduced immigration of specialist herbivores than monocultures (Kareiva 1983; Elmstrom et al. 1988).

Although agroecosystem diversification usually involves the incorporation of different plant species, varietal mixtures incorporated as intercrops, trap crops and border crops can also influence herbivorous insect populations. Latin American cassava, *Manihot esculenta* Crantz (Euphorbiaceae), farmers have traditionally used varietal mixtures to reduce herbivore loads (Lozano et al. 1980).

Mixed populations of glabrous and hirsute cotton genotypes incur less damage by the fleahopper, *Pseudatomoscelis seriatus* Reuter (Hemiptera: Miridae), and yield more than either line grown alone (Benedict et al. 1986). Although evaluations of the effects of genotypic mixes on insect populations are relatively rare, those that have been conducted indicate reduced impacts of insect pests. The contributions of genetic or taxonomic diversity to reductions in herbivore feeding are consistent with associational resistance (Tahvanainen and Root 1972).

Mechanisms linked to associational resistance (inverse relationship of herbivore occurrence and/or damage with increased agroecosystem diversity) include reductions in the apparency (as per Feeny 1976) of susceptible genotypes as well as increased competition among potentially pestiferous herbivorous species and improved natural enemy diversity and density (Root 1973; Risch et al. 1983; Baliddawa 1985; Andow 1990). Plant apparency influences host location and discrimination (Feeny 1976) and is the most likely factor influenced by simultaneous, sympatric assemblages of mixed genotypes. Apparency can be influenced by both visual and olfactory cues (Feeny 1976). Susceptible genotypes may be obscured when in close proximity to genotypes with less attractive visual and olfactory cues. Movement of *C. obstrictus* from overwintering sites to crops is influenced by host-plant odour (Evans and Allen-Williams 1993). The odours of plant neighbours, rather than of individual plants, were suggested to be the primary factor influencing attraction of *C. obstrictus* to *B. oleracea* and *Brassica nigra* (L.) local populations and thus oviposition (Moyes and Raybould 2001).

Emigration of weevils from sites due to antixenosis resistance could also contribute to associational resistance. Barbosa et al. (2007) conducted a large meta-analysis of associational resistance and associational susceptibility. Their analysis incorporated habitat (managed or unmanaged), palatability of potential host plant neighbours, taxonomic similarity of plant neighbours, herbivore feeding guild, feeding type and host range, and plot size and arrangement. Results of their review and analysis indicated the importance of herbivore abundance or plant damage as predictors; however, associational resistance was consistently associated with unpalatable (antixenotic) neighbours and associational susceptibility with palatable neighbours (Barbosa et al. 2007). Somewhat surprisingly, their results also indicated that associational resistance was not strongly influenced by herbivore host range and that the influence of taxonomic similarity among neighbours was variable (Barbosa et al. 2007).

It should be noted that within-site genotypic diversity may actually promote the growth of some insect populations. Tansey (2001) demonstrated a positive correlation between within-site diversity of *Euphorbia esula* L. with *Aphthona nigriscutis* Foudras (Coleoptera: Chrysomelidae) population size. It was suggested that host preferences of these beetles contributed to aggregated distributions, thus allowing them to avoid Allee or 'underpopulation' effects: inability of widely dispersed (on the scale of an insect) members of populations to find mates, avoid predators or overcome host defences (Allee 1931). The predilection of a species to aggregate likely influences responses to within-site host variability. Aggregations of *Aphthona* spp. can be very dense and large;

densities of 2500 m^{-1} *Aphthona lacertosa* (Rosh.) are not uncommon (Van Hezewijk and Bourchier 2005). Although *C. obstrictus* also assumes aggregated distributions, particularly on early flowers of canola crops (Dosdall et al. 2006), their highly localised densities, by my estimation, do not approach those of the *Aphthona* spp. examined to date (Van Hezewijk and Bourchier 2005; J. Tansey, unpublished data). Although conspecific chemical cues are produced by female *C. obstrictus* (Evans and Bergeron 1994) and male *A. nigriscutis* (Tansey et al. 2005) and likely influence conspecific spatial distribution in these species, the effects of epideictic substances associated with *C. obstrictus* oviposition certainly augment this insect's propensity for aggregated distributions mid-season.

Results of a small field plot study examining the effects of mixing *C. obstrictus*-susceptible and -resistant genotypes in various proportions and comparison of these seeding arrangements with resistant or susceptible monocultures are presented in Chapter 6. Mixes were achieved by planting alternating strips of resistant and susceptible genotypes; resistance or susceptibility was based on assessments by Shaw (2008). Plots were established in a region inhabited by a large, naturalized *C. obstrictus* population. This small plot study was used to test the hypothesis that mixtures of resistant and susceptible genotypes deter *C. obstrictus* attack on susceptible germplasm (associational resistance). Adult weevils were trapped and assessed weekly over two growing seasons and their spatial distribution determined in each plot. Oviposition associated with each mixture of resistant and susceptible genotypes was also assessed annually. Potential mechanisms of associational resistance are

discussed in light of results of Chapters 2 to 5 and suggestions regarding deployment strategies for resistant genotypes that may best exploit these factors are made.

Figure

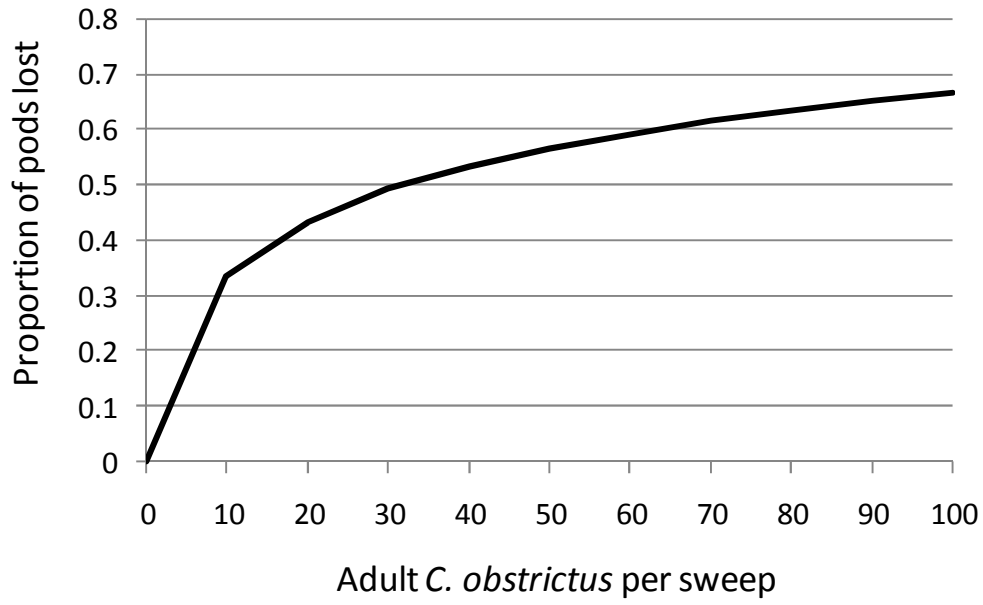

Figure 1.1. Model representing proportion of *Brassica napus* var. Q2 pods from small plot monocultures lost associated with numbers of adult *C. obstrictus* per sweep mid-season.

References

Alford, D.V., D.A. Cooper, and I.H. Williams. 1991. Insect Pests of Oilseed Rape. HGCA, London. HGCA Oilseeds Research Review No. OS1.

Alford, D.V., C. Nilsson, and B. Ulber. 2003. Insect pests of oilseed rape crops, pp. 9–41. *In*: D.V. Alford (ed). Biocontrol of Oilseed Rape Pests. Blackwell Science.

Ali, M.I., R.G. Luttrell, and S.Y. Young III. 2006. Susceptibilities of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) populations to Cry1Ac insecticidal protein. Journal of Economic Entomology 99: 164–175.

Allee, W.C. 1931. Animal Aggregations. A Study in General Sociology. University of Chicago Press, Chicago.

Anderson, R. S. 1997. Weevils (Coleoptera: Curculionoidea, excluding Scolytinae and Platypodinae) of the Yukon, pp. 523–662. *In*: Danks, H.V., and J.A. Downes (eds.). Insects of the Yukon. Biological Survey of Canada (Terrestrial Arthropods), Ottawa, Ontario.

Andréasson, E., L.B. Jorgensen, A.S. Hoglund, L. Rask, and J. Meijer. 2001. Different myrosinase and idioblast distribution in *Arabidopsis* and *Brassica napus*. Plant Physiology 127: 1750–63

Andow, D. A. 1990. Population dynamics of an insect herbivore in simple and diverse habitats. Ecology 71: 1006–1017.

Andow, D.A., P.M. Kareiva, S.A. Levin, and A. Okubo. 1990. Spread of invading organisms. Landscape Ecology 4: 177-188.

Ankersmit, G.W. 1956. The biology and control of the colza seed pod weevil (*Ceutorhynchus assimilis* Payk.) and the brassica pod midge (*Dasyneura brassicae* Winn.). Institut Voor Plantziektenkundig Onderzoek Wageningen Mededelingen 132: 1–59.

Baliddawa, C.W. 1985. Plant species diversity and crop pest control. Insect Science and its Application 6: 479–487.

Barbosa, P., I. Kaplan, Z. Szendrei, A. Szczepaniec, J. Hines, and H. Martinson. 2007. Associational resistance and susceptibility: Having right or wrong neighbours. Proceedings of the Joint Meetings of the Ecological Society of America and the Society for Ecological Restoration. 5-10 August, San Jose, California.

Barrow, E., and G. Yu. 2005. Climate Scenarios for Alberta. A Report Prepared for the Prairie Adaptation Research Collaborative (PARC) in co-operation with Alberta Environment. University of Regina, Saskatchewan http://wwwparcca/research_pub_scenarioshtm.
Accessed 15 December 2007.

Bartlet, E., M.M. Blight, A.J. Hick, and I.H. Williams. 1993. The responses of the cabbage seed weevil (*Ceutorhynchus assimilis*) to the odour of oilseed rape *(Brassica napus)* and some volatile isothiocyanates. Entomologia Experimentalis et Applicata 68: 295-302.

Bartlet, E., M.M. Blight, P. Lank, and I.H. Williams. 1997. The responses of the cabbage seed weevil *Ceutorhynchus assimilis* to volatile compounds from

oilseed rape in a linear track olfactometer. Entomologia Experimentalis et Applicata 85: 257-262.

Bartlet E., G. Kiddle, I. Williams, and R. Wallsgrove. 1999. Wound induced increases in the glucosinolate content of oilseed rape and their effect on subsequent herbivory by a crucifer specialist. Entomologia Experimentalis et Applicata 91: 163–167.

Benedict, J.H., M.F. Treacy, and J.C. Segers. 1986. Role of mixed plantings of susceptible and resistant phenotypes on yield and fleahopper behavior, pp. 486–488. *In* Proceedings of the Beltwide Cotton Conference, Las Vegas, NV. 4-9 Jan. 1986. National Cotton Council of America. Memphis, TN.

Bernal, J.S., J. Prsifka, M. Sétamou, and K.M. Heinz. 2004. Transgenic insecticidal cultivars in integrated pest management: Challenges and opportunities, pp. 123-145. *In*: Koul, O., G.S. Dhaliwal, and G.W. Cuperus (eds.). Integrated Pest Management: Potential, Constraints and Challenges. CABI Publishing, Oxon, United Kingdom.

Birch, A.N.E., D.W. Griffiths, R.J. Hopkins, W.H. MacFarlane Smith, and R.G. McKinlay. 1992. Glucosinolate responses of swede, kale, forage and oilseed rape to root damage by turnip root fly (*Delia floralis*) larvae. Journal of the Science of Food and Agriculture 60: 1-9.

Blatchley, W.S., and C.W. Leng 1916. Rhynchophora or Weevils of North Eastern America. 682 pp.

Blight, M.M., J.A. Pickett, L.J. Wadhams, and C. M. Woodcock. 1995. Antennal perception of oilseed rape *Brassica napus* (Brassicaceae) volatiles by the

cabbage seed weevil, *Ceutorhynchus assimilis* (Coleoptera: Curculionidae). Journal of Chemical Ecology 21: 1649–1664.

Bodnaryk, R.P. 1991. Developmental profile of sinalbin (*p*-hydroxybenzyl glucosinolate) in mustard seedlings, *Sinapis alba* L., and its relationship to insect resistance. Journal of Chemical Ecology 17: 1543–1556.

Bodnaryk, R.P. 1992. Effects of wounding on glucosinolates in the cotyledons of oilseed rape and mustard. Phytochemistry 31: 2671–2677.

Bodnaryk, R.P. 1994. Potent effect of jasmonates on indole glucosinolates in oilseed rape and mustard. Phytochemistry 35: 301–305.

Bonnemaison, L. 1957. Le charançon des siliques (*Ceutorhynchus assimilis* Payk.) biologie et méthode de lutte. Annales des Épiphytes 4: 387–543.

Borek, V., and M.J. Morra. 2005. Ionic thiocyanate (SCN-) production from 4-hydroxybenzyl glucosinolate contained in *Sinapis alba* seed meal. Journal of Agricultural and Food Chemistry 53: 8650-8654.

Bourguet, D., A. Genissel, and M. Raymond. 2000. Insecticide resistance and dominance levels. Journal of Economic Entomology 93: 1588-1595.

Bragg, D.E. 1999a. Comparisons of insecticide strategies in spring canola. Arthropod Management Tests 24: 205.

Bragg, D.E. 1999b. Soft chemical research in spring canola. Arthropod Management Tests 24: 206.

Briscoe, A.D., and L. Chittka. 2001. The evolution of colour vision in insects. Annual Review of Entomology 46: 471–510.

Brlacich, M., C. Bryant, B. Veehof, and A. Beauchesne. 1997. Implications of global climatic change for Canadian agriculture: A review and appraisal of research from 1984–1997, pp. 220–256. *In*: Canada Country Study: Climate Impacts and Adaptation. Environment Canada, Toronto, ON, Canada.

Brodeur, J., L. Leclerc, M. Fournier, and M. Roy. 2001. Cabbage seedpod weevil (Coleoptera: Curculionidae) a new pest of canola in northeastern North America. The Canadian Entomologist 133: 709–711.

Brown, J., A.P. Brown, J.B. Davis, and D.A. Erickson. 1997. Intergeneric hybridization between *Sinapis alba* and *Brassica napus.* Euphytica 93: 163–168.

Brun, H., J. Plessis, and M. Renard. 1987. Resistance of some crucifers to *Alternaria brassicae* (Berk.) Sacc. Proceedings of the International Rapeseed Congress. May 1987. Poznan, Poland.

Bukovinszky, T., R.P.J. Potting, Y. Clough, J.C. van Lenteren, and L.E.M. Vet. 2005. The role of pre- and post-alighting detection mechanisms in the responses to patch size by specialist herbivores. Oikos 109: 435– 446.

Buechi, R. 1990. Investigations on the use of turnip rape as a trap plant to control oilseed rape pests. IOBC/wprs Bulletin 13: 32–39.

Buntin, G.D. 1998. Cabbage seedpod weevil (*Ceutorhynchus assimilis* Paykull) management by trap cropping and its effect on parasitism by *Trichomalus perfectus* (Walker) in oilseed rape. Crop Protection 17: 299–305.

Buntin, G.D. 1999. Damage loss assessment and control of the cabbage seedpod weevil (Coleoptera: Curculionidae) in winter canola using insecticides. Journal of Economic Entomology 92: 220-227.

Buntin, G. D., J.P. McCaffrey, P.L. Raymer, and J. Romero. 1995. Quality and germination of rapeseed and canola seed damaged by adult cabbage seedpod weevil, *Ceutorhynchus assimilis* (Paykull) [Coleoptera: Curculionidae]. The Canadian Journal of Plant Science 75: 539-541.

Canola Council of Canada. 2008. Canola socio-economic value report. http://www.canola-council.org/uploads/Canola_in_Canada_Socio_Economic_Value_Report_January_08.pdf. accessed 10 June, 2009.

Canola Council of Canada. 2009a. Market links. http://www.canola-council.org/market_links.aspx. Accessed 15 June 2009.

Canola Council of Canada. 2009b. Canola Insect Thresholds Update Summer 2008. http://www.canola-council.org/agronomic_publications.aspx. Accessed 10 May 2009.

Cárcamo, H.A., L. Dosdall, M. Dolinski, O. Olfert, and J.R. Byers. 2001. The cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae) - a review. Journal of the Entomological Society of British Columbia 98: 201-210.

Cárcamo, H.A., L.M. Dosdall, D. Johnson, an O. Olfert. 2005. Evaluation of foliar and seed treatments for control of the cabbage seedpod weevil (Coleoptera: Curculionidae) in canola. The Canadian Entomologist 137: 476-487.

Cárcamo, H.A., R. Dunn, L.M. Dosdall, and O. Olfert. 2007. Managing cabbage seedpod weevil in canola using a trap crop – a commercial field-scale study in western Canada. Crop Protection 26: 1325-1334.

Carrière, Y., T.J. Dennehy, B. Pedersen, S. Haller, C. Ellers-Kirk, L. Antilla, Y.B. Liu, E. Willott, and B.E. Tabashnik. 2001. Large-scale management of insect resistance to transgenic cotton in Arizona: Can transgenic insecticidal crops be sustained? Journal of Economic Entomology 94: 315-325.

Chevre, A.M., F. Eber, E. Margale, and M.C. Kerlan. 1994. Comparison of somatic and sexual *Brassica napus – Sinapis alba* hybrids and their progeny by cytogenetic studies and molecular characterization. Genome 37: 367–374.

Chittka, L. 1996. Optimal sets of colour receptors and colour opponent systems for coding of natural objects in insect vision. Journal of Theoretical Biology 181: 179–196.

Cohen S., and K. Miller. 2001. North America, pp.737–800. *In*: McCarthy, J.J., O.F. Canziani, N.A. Leary, D.J. Dokken, and K.S. White (eds.). Climate Change 2001: Impacts, Adaptation, and Vulnerability. Cambridge University Press, Cambridge, UK.

Cole, R.A. 1976. Isothiocyanates, nitriles, and thiocyanates as products of autolysis of glucosinolates in Cruciferae. Phytochemistry 15: 759-762.

Colonnelli, E. 1990. Curculionidae Ceutorhynchinae from the Canaries and Macronesia (Coleoptera). Vieracea 18: 317-337.

Colonnelli, E. 1993. The Ceutorhynchinae types of I.C. Fabricius and G. von Paykull (Coleoptera: Curculionidae). Koleopterologische Rundschau 63: 299-310.

Colonnelli, E. 2004. Catalogue of Ceutorhynchinae of the World with a Key to Genera. Argania, Barcelona.

Compton, S. G. 2002. Sailing with the wind: Dispersal by small flying insects, pp. 113–133. *In*: Bullock, M.M., R.E. Kenward, and R.S. Hails (eds). Dispersal Ecology. Blackwell Publishing, Oxford, UK.

Cole, R.A. 1976. Isothiocyanates, nitriles, and thiocyanates as products of autolysis of glucosinolates in Cruciferae. Phytochemistry 15: 759-762.

Colautti, R.I., S.A. Bailey, C.D.A. van Overdijk, K. Amundsen, and H. MacIsaac. 2006. Characterised and projected costs of nonindigenous species in Canada. Biological Invasions 8: 45-59.

Cook, S.M., N.P. Watts, F. Hunter, L.E. Smart, and I.H. Williams. 2004. Effects of a turnip rape trap crop on the spatial distribution of *Meligethes aeneus* and *Ceutorhynchus assimilis* in oilseed rape. IOBC wprs Bulletin 27: 199–206.

Cook, S.M., J.S. Sandoz, A.P. Martin, D.A. Murray, G.M Poppy, and I.H. Williams. 2005. Could learning of pollen odours by honey bees (*Apis mellifera*) play a role in their foraging behaviour? Physiological Entomology 30: 164–174.

Cook, S.M., L.E. Smart, J.L. Martin, D.A. Murray, N.P. Watts, and I.H. Williams. 2006. Exploitation of host plant preferences in pest management strategies for oilseed rape (*Brassica napus*). Entomologia Experimentalis et Applicata 119: 221–229.

Couty, A., H. Van Emden, J.N. Perry, J.I.M. Hardie, J.A. Pickett, and L. J. Wadhams. 2006. The roles of olfaction and vision in host-plant finding by the diamondback moth, *Plutella xylostella*. Physiological Entomology 31: 134 – 145.

Cripps, M.G., H.L. Hinz, J. L. Mckenney, B.L. Harmon, F.W. Merickel, and M. Schwarzlaender. 2006. Comparative survey of the phytophagous arthropod faunas associated with *Lepidium draba* in Europe and the western United States, and the potential for biological weed control. Biocontrol Science and Technology 16: 1007-1030.

Dafni, A., M. Lehrer, and P. G. Kevan. 1997. Spatial flower parameters and insect spatial vision. Biological Reviews 72: 239-282.

David, C.T, J.S. Kennedy, A.R. Ludlow, J.N. Perry, and C. Wall. 1982. A reappraisal of insect flight towards a distant point source of wind-borne odor. Journal of Chemical Ecology 8: 1207-1215.

Dmoch, J. 1965. The dynamics of a population of the cabbage seedpod weevil (*Ceutorhynchus assimilis* Payk.) and the development of winter rape. Part I. Ekologiya Polska. Seria A 13: 249–287.

Dosdall, L.M. Responses of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae), to seed treatments of canola (*Brassica napus* L.) with the neonicotinoid compounds clothianidin and imidacloprid. Pest Management Science, in press.

Dosdall, L. M., and L.S. Kott. 2006. Introgression of resistance to cabbage seedpod weevil to canola from yellow mustard. Crop Science 46: 2437-2445.

Dosdall, L.M., and M.A. McFarlane. 2004. Morphology of the pre-imaginal life stages of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae). The Coleopterists' Bulletin 58: 45–52.

Dosdall, L.M., and D.W.A. Moisey. 2004. Developmental biology of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae), in spring canola, *Brassica napus*, in western Canada. Annals of the Entomological Society of America 97: 458-465.

Dosdall, L.M., M.J. Herbut, and N.T. Cowle. 1994. Susceptibilities of species and cultivars of canola and mustard to infestation by root maggots (*Delia* spp.) (Diptera: Anthomyiidae). The Canadian Entomologist 126: 251-260.

Dosdall, L.M., M.A. McFarlane, and P. Palaniswamy. 1999. Biology and larval morphology of *Ceutorhynchus neglectus* Blatchley (Coleoptera:

Curculionidae), a minor pest of canola (Brassicaceae) in western Canada. The Canadian Entomologist 131: 231–242.

Dosdall, L. M., D. Moisey, H. Cárcamo, and R. Dunn. 2001. Cabbage seedpod weevil fact sheet. Alberta Agriculture, Food and Rural Development, Edmonton, AB, Canada.

Dosdall, L.M., R.M. Weiss, O. Olfert, and H.A. Cárcamo. 2002. Temporal and geographical distribution patterns of cabbage seedpod weevil (Coleoptera: Curculionidae) in canola. The Canadian Entomologist 134: 403-418.

Dosdall, L.M., B.J. Ulmer, G.A.P. Gibson, and H.A. Cárcamo. 2006. The spatio-temporal distribution dynamics of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae), and its larval parasitoids in canola in western Canada. Biocontrol Science and Technology 16: 987-1006.

Dosdall, L.M., B.J. Ulmer, and P. Bouchard. 2007a. Life history, larval morphology, and Nearctic distribution of *Ceutorhynchus subpubescens* (Coleoptera: Curculionidae). Annals of the Entomological Society of America 100: 178-186.

Dosdall, L.M., G.A.P. Gibson, O. Olfert, B.A. Keddie, and B.J. Ulmer. 2007b. Contributions to the life history, host range, and distribution of *Necremnus tidius* (Walker) (Hymenoptera: Eulophidae). Annals of the Entomological Society of America 100: 861–868.

Dosdall, L.M., G.A.P. Gibson, O.O. Olfert, and P.G. Mason. 2009. Responses of Chalcidoidea (Hymenoptera) parasitoids to invasions of the cabbage

seedpod weevil (Coleoptera: Curculionidae) in western Canada. Biological Invasions 11:109–125.

Doucette, C.F. 1947. Host plants of the cabbage seedpod weevil. Journal of Economic Entomology 40: 838-840.

Doughty, K.J., G.A. Kiddle, B.J. Pye, R.M. Wallsgrove, and J.A. Pickett. 1995. Selective induction of glucosinolates in oilseed rape leaves by methyl jasmonate. Phytochemistry 38: 347-350.

Downey, R.K., G.R. Stringham, D.I. McGregor and S. Steffanson. 1975. Breeding rapeseed and mustard crops, pp. 157–183. *In*: Harapiak, J.T. (ed.). Oilseed and Pulse Crops in Western Canada. Western Cooperative Fertilizers Ltd., Calgary, Alberta, Canada.

Elmstrom, K.M., D.A. Andow, and W.W. Barclay. 1988. Flea beetle movement in a broccoli monoculture and diculture. Environmental Entomology 17: 299–305.

Elton, C. S. 1958. The Ecology of Invasions by Animals and Plants. London: Methuen.

Evans, K.A., and L.J. Allen-Williams. 1992. Electroantennogram responses of the cabbage seed weevil, *Ceutorhynchus assimilis,* to oilseed rape, *Brassica napus* ssp. *oleifera,* volatiles. Journal of Chemical Ecology 18: 1641-1659.

Evans, K.A., and L.J. Allen-Williams. 1993. Distant olfactory response of the cabbage seed weevil, *Ceutorhynchus assimilis,* to oilseed rape odour in the field. Physiological Entomology 18: 251-256.

Evans, K.A., and L.J. Allen-Williams. 1998. Response of cabbage seed weevil (*Ceutorhynchus assimilis*) to baits of extracted and synthetic host-plant odor. Journal of Chemical Ecology 24: 2101-2114.

Evans, K.A., and J. Bergeron. 1994. Behavioral and electrophysiological response of cabbage seed weevils (*Ceutorhynchus assimilis*) to conspecific odor. Journal of Chemical Ecology 20: 979-989.

Fahey, J.W., A.T. Zalcmann, and P. Talalay. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56: 5-51.

Falk, A., B. Ek, and L. Rask. 1995. Characterization of a new myrosinase in *Brassica napus*. Plant Molecular Biology 27: 863–874 .

Feeny, P. P. 1976. Plant apparency and chemical defence. Recent Advances in Phytochemistry 10: 1-40.

Ferguson, A.W., and I.H. Williams. 1991. Deposition and longevity of oviposition-deterring pheromone in the cabbage seed weevil. Physiological Entomology 16: 27-33.

Ferguson, A.W., J. Ziesmann, M.M. Blight, I. H. Williams, L. J. Wadhams, S.J. Clark, C.M. Woodcock, and A. Mudd. 1999. Perception of oviposition-deterring pheromone by cabbage seed weevil (*Ceutorhynchus assimilis*). Journal of Chemical Ecology 25: 1655-1670.

Finch, S. 1980. Chemical attraction of plant-feeding insects to plants. Applied Biology 5: 67-143.

Finch, S. and R.H. Collier. 2000. Host-plant selection by insects - a theory based on 'appropriate/inappropriate landings' by pest insects of cruciferous plants. Entomologia Experimentalis et Applicata 96: 91–102.

Fletcher, R., J. Coventry, and L.S. Kott. 1998. Doubled haploid technology for spring and winter *Brassica napus*. University of Guelph Technical Bulletin. Guelph, Ontario.

Fox, A.S., and L.M. Dosdall. 2003. Reproductive biology of *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae) on wild and cultivated Brassicaceae in southern Alberta. Journal of Entomological Science 38: 365-376.

Frearson, D.J.T., A.W. Ferguson, N. Laugier, and I.H. Williams. 2006. The influence of petals on numbers of pests and their parasitoids in oilseed rape. *In* Proceedings of the International Symposium on Integrated Pest Management in Oilseed Rape. 3-5 April 2006. Paulinerkirche, Göttingen, Germany.

Free, J.B., and I.H. Williams. 1978. The responses of the pollen beetle, *Meligethes aeneus*, and the seed weevil, *Ceutorhynchus assimilis*, to oil-seed rape, *Brassica napus*, and other plants. Journal of Applied Ecology 15: 761–774.

Gareau, R.M., D.L. Auld, and M. Heikkinen. 1990. Evaluation of seven species of oilseed as spring planted crops for the Pacific Northwest. Progress Report 227, University of Idaho, Agriculture Experimental Station.

Gavloski, J.E., U. Ekuere, A. Keddie, L. Dosdall, and L .Kott. 2000. Identification and evaluation of flea beetle (*Phyllotreta cruciferae*)

resistance within Brassicaceae. The Canadian Journal of Plant Science 80: 881-887.

Gibson, G.A.P., H. Baur, B. Ulmer, L. Dosdall, and F. J. Muller. 2005. On the misidentification of the chalcid (Hymenoptera: Chalcidoidea) parasitoids of the cabbage seedpod weevil (Coleoptera: Curculionidae) in North America. The Canadian Entomologist 137: 381-403.

Gibson, G.A.P., D.R. Gillespie, and L.M. Dosdall. 2006. The species of Chalcidoidea (Hymenoptera) introduced to North America for biological control of the cabbage seedpod weevil, and the first recovery of *Stenomalina gracilis* (Chalcidoidea: Pteromalidae). The Canadian Entomologist 138: 285-291.

Gillespie, D.R., P.G. Mason, L.M. Dosdall, P. Bouchard, and G.A.P. Gibson 2006. Importance of long-term research in classical biological control: An analytical review of a release against the cabbage seedpod weevil in North America. Journal of Applied Entomology 130: 401–409.

Gols, R., R. Wagenaar, T. Bukovinszky, N. M. van Dam, M. Dicke, J.M. Bullock, and J.A. Harvey. 2008. Genetic variation in defense chemistry in wild cabbages affects herbivores and their endoparasitoids. Ecology 89: 1616-1626.

Gould, F. 1986. Simulation models for predicting durability of insect-resistant germ plasm: Hessian fly (Diptera: Cecidomyiidae)-resistant winter wheat. Environmental Entomology 15: 11–23.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: Integrating pest genetics and ecology. Annual Review of Entomology 47: 701–722.

Government of Alberta. 2009. Decision making tools. http://www.agric.gov.ab.ca/app21/ldcalc. accessed 10 June, 2009.

Halkier, B.A., and J. Gershenzon. 2006. Biology and biochemistry of glucosinolates. Annual Review of Plant Biology 57: 303–33.

Harmon, B. L. and J. P. McCaffrey. 1996. Parasitism of adult *Ceutorhynchus assimilis* (Coleoptera; Curculionidae) by *Microctonus melanopus* (Hymenoptera: Braconidae) in northern Idaho and eastern Washington. Journal of Agricultural Entomology 14: 55-59.

Hausmann, C., J. Samietz, and S. Dorn. 2004. Visual orientation of overwintered *Anthonomus pomorum* (Coleoptera: Curculionidae). Environmental Entomology 33: 1410–1415.

Hawkes, C. 1974. Dispersal of adult cabbage root fly (*Erioischia brassicae* (Bouché)) in relation to a brassica crop. Journal of Applied Ecology 11: 84-93.

Hemmingway, J.S. 1976. Mustards: *Brassica* spp. and *Sinapis alba*. *In*: Simmonds, N.W.(Ed.), Evolution of Crop Plants. Longman Inc. NY.

Hill, D.S. 1987. Agricultural Insect Pests of Temperate Regions and Their Control. Cambridge University Press, Cambridge.

Hoffman, A. 1954. Faune de France. 59. Coléoptères Curculionidés (Première partie), pp. 487-1208. Fédération Française des Sociétés Sciences Naturelles. P. Lechavalier, Paris.

Hopkins, R.J., B. Ekbom, and L. Henkow. 1998. Glucosinolate content and susceptibility for insect attack of three populations of *Sinapis alba*. Journal of Chemical Ecology 24: 1203–1216.

Hughes, J.M., and K.A. Evans. 2003. Lygid bug damage as a pod access mechanism for *Dasineura brassicae* (Dipt., Cecidomyiidae) oviposition. Journal of Applied Entomology 127: 116-118.

ICZN. 1989. International Commission on Zoological Nomenclature. Opinion 1529. *Ceutorhynchus* Germar, 1824, *Rhinoncus* Shoenherr, 1825 and *Curculio assimilis* Paykull, 1792 (Insecta, Diptera [sic!]): conserved, and *Curculio assimilis* Paykull, 1792 and *Curculio pericarpius* Linnaeus, 1758 designated as the type species of *Ceutorhynchus* and *Rhinoncus* respectively. Bulletin of Zoological Nomenclature 46: 71-73.

Janmaat, A.F., and J. Myers. 2003. Rapid evolution and the cost of resistance to *Bacillus thuringiensis* in greenhouse populations of cabbage loopers, *Trichoplusia ni*. Proceedings of the Royal Society of London. B 270: 2263–2270.

Jones, R. E. 1977. Search behaviour: A study of three caterpillar species. Behaviour 60: 237-259.

Kadkol, G.P., R.H. MacMillan, R.P. Burrow, and G.M. Halloran.1984. Evaluation of *Brassica* genotypes for resistance to shatter. Development of a laboratory test. Euphytica 33: 63–73.

Kalischuk, A.R., and L.M. Dosdall. 2004. Susceptibilities of seven Brassicaceae species to infestation by the cabbage seedpod weevil (Coleoptera: Curculionidae). The Canadian Entomologist 136: 265-276.

Kareiva, P. M. 1983. Influence of vegetation texture on herbivore populations: resource concentration and herbivore movement, pp. 259–289. *In* Denno, R. F., and M. S. McClure (eds). Variable plants and herbivores in natural and managed systems. Academic Press, New York.

Kennedy, J.S. 1965. Mechanisms of host plant selection. Annals of Applied Biology 56: 317 – 322.

Kennedy, J.S., C.O. Booth, and W.J.S. Kershaw. 1959. Host finding by aphids in the field. II *Aphis fabae* Scop. (Gynoparae) and *Brevicoryne brassicae* L. Annals of Applied Biology 47: 424-444.

Kjær-Pedersen, C. 1992. Flight behaviour of the cabbage seedpod weevil. Entomologia Experimentalis et Applicata 62: 61-66.

Kliebenstein, D.J., J. Gershenzon, and T. Mitchell-Olds. 2001. Comparative quantitative trait loci mapping of aliphatic, indolic and benzylic glucosinolate production in *Arabidopsis thaliana* leaves and seeds. Genetics 159: 359–70.

Kogan, M., and E.F. Ortman. 1978. Antixenosis - A new term proposed to define Painter's 'non preference' modality of resistance. Bulletin of the Entomological Society of America 24: 175–176.

Koritsas, V.M., J. A. Lewis, and G. R. Fenwick. 1991. Glucosinolate responses of oilseed rape, mustard and kale to mechanical wounding and infestation by

cabbage stem flea beetle (*Psylliodes chrysocephala*). Annals of Applied Biology 118: 209-221.

Korotyaev, B.A. 2008. Geographical distribution of the weevil subfamily Ceutorhynchinae (Coleoptera, Curculionidae). Entomological Review 88: 928–947.

Kott, L.S., and L.M. Dosdall. 2004. Introgression of root maggot resistance (*Delia* spp.) derived from *Sinapis alba* L. into *Brassica napus* L. Brassica 6: 55–62.

Kozlowski, M.W., S. Lux, and J. Dmoch. 1983. Oviposition behavior and pod marking in the cabbage seed weevil, *Ceutorhynchus assimilis*. Entomologia Experimentalis et Applicata 34: 277-282.

Kuhlmann, U., P.G. Mason, H.L. Hinz, B. Blossey, R.A. De Clerck-Floate, L.M. Dosdall, J.P. McCaffrey, M. Schwarzlaender, O. Olfert, J. Brodeur, A. Gassmann, A.S. McClay, and R.N. Wiedenmann. 2006. Avoiding conflicts between insect and weed biological control: selection of non-target species to assess host specificity of cabbage seedpod weevil parasitoids. Journal of Applied Entomology 130: 129–141.

Laffin, R.D. 2005. Species page - *Ceutorhynchus obstrictus*. University of Alberta, Strickland Museum. http://www.entomology.ualberta.ca/searching_species_details.php?s=5440. Accessed 1 June 2009.

Laffin, R.D., L.M. Dosdall, and F.A.H. Sperling. 2005. Population structure of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera:

Curculionidae): Origins of North America introductions. Environmental Entomology 34: 504-510.

Lamb, R.J. 1980. Hairs protect pods of mustard (*Brassica hirta* cv Gisilba) from flea beetle feeding damage. The Canadian Journal of Plant Science 60: 1439–1440.

Lamb, R.J. 1989. Entomology of oilseed *Brassica* crops. Annual Review of Entomology 34: 211-229.

Lamb, R.J., and P. Palaniswamy. 1990. Host discrimination by a crucifer-feeding flea beetle *Phyllotreta striolata* (F.) (Coleoptera: Chrysomelidae). The Canadian Entomologist 122: 817-824.

Laughlin, S. 1981. Neural principles in the peripheral visual systems of invertebrates, pp. 133-280. *In*: Autrum, H. (ed.). Handbook of Sensory Physiology, Vol. 7, Sect. 6B. Springer, Berlin/New York.

Lozano, J., D. Byrne, and A. Bellotti. 1980. Cassava/ecosystem relationships and their influence on breeding strategy. Tropical Pest Management 26: 180-187.

Lyal, C.H.C., D.A. Douglas, and S.J. Hine. 2006. Morphology and systematic significance of sclerolepidia in the Weevils (Coleoptera; Curculionoidea). Systematics and Biodiversity 4: 203–241.

Marvaldi, A.E., and A.A. Lanteri. 2005. Key to higher taxa of South American weevils based on adult characters (Coleoptera, Curculionoidea). Revista Chilena de Historia Natural 78: 65-87.

Mason, P.G., T. Baute, O. Olfert, and M. Roy. 2004. Cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae) in Ontario and Quebec. Proceedings of the Entomological Society of Ontario 134: 107–113.

McCaffrey, J. P. 1992. Review of the U.S. canola pest complex: cabbage seedpod weevil, pp. 140-143. *In* Proceedings, 1992 U.S. Canola Conference, 5-6 March 1992. Ameri-Can Pedigreed Seed Company, Memphis, TN.

McCaffrey, J. P., L.E. O'Keefe, and H.W. Homan. 1986. Cabbage seedpod weevil control in winter rapeseed. University of Idaho, College of Agriculture, Cooperative Extension Service, Agricultural Experiment Station, CIC Series No. 782.

McCaffrey, J.P., B.L. Harmon, J. Brown, A.P. Brown, and J.B. Davis. 1999. Assessment of *Sinapis alba*, *Brassica napus* and *S. alba* x *B. napus* hybrids for resistance to cabbage seedpod weevil, *Ceutorhynchus assimilis* (Coleoptera: Curculionidae). Journal of Agricultural Science 132: 289–295.

McClay, A.S., R.S. Bourchier, R.A. Butts, and D.P. Peschken. 2002a. *Cirsium arvense* (L.) Scopoli, Canada thistle (Asteraceae), pp. 318–330. *In*: Mason, P.G., and J.T. Huber (eds.). Biological Control Programmes in Canada, 1981– 2000. CABI Publishing, Wallingford, Oxon, UK.

McClay, A.S., R.S. Bourchier, R.A. Butts, and D.P. Peschken. 2002b. *Matricaria perforata* Mérat, scentless chamomile (Asteraceae). pp. 395-402. *In*: Biological control programmes in Canada, 1981-2000. Mason, P.G., J.T. Huber (eds.). CABI Publishing, Wallingford, Oxon, UK.

McCloskey, C., and M.B. Isman. 1993. Influence of foliar glucosinolates in oilseed rape and mustard on feeding and growth of the bertha armyworm, *Mamestra configurata* Walker. Journal of Chemical Ecology 19: 249-266.

McLeod, J.H. 1953. Notes on the cabbage seedpod weevil, *Ceutorhynchus assimilis* (Payk.) (Coleoptera: Curculionidae), and its parasites. Proceedings of the Entomological Society of British Columbia 49: 11-18.

McLeod, J. H. 1962. Cabbage seedpod weevil - *Ceutorhynchus assimilis* (Payk.) Curculionidae, pp. 5-6. *In*: J. H. McLeod, B. M. McGugan, and H. C. Coppel (eds.). A Review of the Biological Control Attempts Against Insects and Weeds in Canada. Commonwealth Institute of Biological Control, Trinidad.

Mewis, I., C. Ulrichs, and W.H. Schnitzler. 2002. Possible role of glucosinolates and their hydrolysis products in oviposition and host-plant finding by cabbage webworm, *Hellula undalis*. Entomologia Experimentalis et Applicata 105: 129–139.

Miller, J.R., and R.S. Cowles. 1990. Stimulo-deterrent diversion: a concept and its possible application to onion maggot control. Journal of Chemical Ecology 16: 3197–3212.

Mithen, R. 1992. Leaf glucosinolate profiles and their relationship to pest and disease resistance in oilseed rape. Euphytica 63: 71–83.

Moericke, V. 1952. Farben als Landereize für geflügelten Blattläusen (Aphidoidea). Zeitschrift für Naturforschung 7: 304–324.

Moyes C.L., and A.F. Raybould. 2001. The role of spatial scale and intraspecific variation in secondary chemistry in host plant location by *Ceutorhynchus assimilis* (Coleoptera: Curculionidae). Proceedings of the Royal Society of London 268: 1567-1573.

Moyes, C.L., H.A. Collin, G. Britton, and A.F. Raybould. 2000. Glucosinolates and differential herbivory in wild populations of *Brassica oleracea*. Journal of Chemical Ecology 26: 2625-2641.

Mudd, A., A.W. Ferguson, M.M. Blight, I.H. Williams, P. Scubla, M. Solinas, and S.J. Clark. 1997. Extraction, isolation, and composition of oviposition-deterring secretion of cabbage seed weevil, *Ceutorhynchus assimilis.* Journal of Chemical Ecology 23: 227-2240.

Muller, F.M. 2006. Ensuring the Safety of Classical Biological Control for Cabbage Seedpod Weevil in Canada: Assessment of the Ecological Host Range of Candidate Ectoparasitoids in Europe and Clarification of their Taxonomic Status. Unpublished Ph.D. thesis. Faculty of Agricultural Sciences, Georg-August-University, Göttingen, Germany. 133 pp.

Murchie, A.K., and I.H. Williams. 1998. A bibliography of the parasitoids of the cabbage seed weevil (*Ceutorhynchus assimilis* Payk.). IOBC wprs Bulletin 21: 163-169.

Ni, X.Z., J.P. McCaffrey, R.L. Stoltz, and B.L. Harmon. 1990. Effects of postdiapause adult diet and temperature on oogenesis of the cabbage seedpod weevil (Coleoptera: Curculionidae). Journal of Economic Entomology 83: 2246–2251.

Nottingham, S.E., and T.H. Coaker. 1985. The olfactory responses of cabbage root fly *Delia radicum* to the host plant volatile allylisothiocyanate. Entomologia Experimentalis et Applicata 39: 307-316.

Oberprieler, R.G., A.E. Marvaldi, and R.S. Anderson. 2007. Weevils, weevils, weevils everywhere. Zootaxa 1668: 491–520.

Olfert, O., and R.M. Weiss. 2006. Impact of climate change on potential distributions and relative abundances of *Oulema melanopus, Meligethes viridescens* and *Ceutorhynchus obstrictus* in Canada. Agriculture, Ecosystems and Environment 113: 295–301.

Painter, R.H. 1951. Insect Resistance in Crop Plants. University of Kansas, Lawrence, KS.

Palaniswamy, P., R.J. Lamb, and R.P. Bodnaryk. 1997. Antibiosis of preferred and non-preferred host-plants for the flea beetle, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae). The Canadian Entomologist 129: 43-49.

Parmesan, C. 1991. Evidence against plant "apparency" as a constraint on evolution of insect search efficiency (Lepidoptera: Nymphalidae). Journal of Insect Behavior 4: 417-430.

Pechan, P.A., and D.G. Morgan. 1985. Defoliation and its effects on pod and seed development in oil seed rape (*Brassica napus* L.). Journal of Experimental Botany 36: 458-68.

Petterson, J. 1973. Olfactory reactions of *Brevicoryne brassicae* (L.). Swedish Journal of Agricultural Research 3: 95-103.

Pimentel, D. 1961. Species diversity and insect population outbreaks. Annals of the Entomological Society of America 54: 76-86.

Pivnick, K.A., R.J. Lamb, and D. Reed. 1992. Response of flea beetles, *Phyllotreta* spp., to mustard oils and nitriles in field trapping experiments. Journal of Chemical Ecology 18: 863-873.

Pivnick, K.A., and E. Labbé. 1992 Emergence and calling rhythms, and mating behaviour of the orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae). The Canadian Entomologist 124: 501–507.

Primard, C., F. Vedel, C. Mathieu, G. Palletier, and A.M. Chevre. 1988. Interspecific hybridization between *Brassica napus* and *Brassica hirta* (*Sinapis alba* L.). Theoretical and Applied Genetics 75: 546–552.

Porter, A.J.R., A.M. Morton, G. Kiddle, K.J. Doughty, and R.M. Wallsgrove. 1991. Variation in the glucosinolate content of oilseed rape (*Brassica napus* L.) leaves. I, Effect of leaf age and position. Annals of Applied Biology 118: 461-467.

Prokopy, R.J., and E.D. Owens. 1983. Visual detection of plants by herbivorous insects. Annual Review of Entomology 28: 337-364.

Prokopy, R. J., B.D. Roitberg, and A.L. Averill. 1984. Resource partitioning, pp. 301-330. *In* Bell, W.J., and R.T. Cardé (eds.). Chemical Ecology of Insects. Chapman and Hall, London.

Rask, L., E. Andreasson, B. Ekbom, S. Eriksson, B. Pontoppidan, and J. Meijer. 2000. Myrosinase: Gene family evolution and herbivore defense in Brassicaceae. Plant Molecular Biology 42: 93–113

Rausher, M.D. 2001. Co-evolution and plant resistance to natural enemies. Nature 411: 857–863.

Reddy, G.V.P., E. Tabone, and M.T. Smith. 2004. Mediation of host selection and oviposition behavior in the diamondback moth *Plutella xylostella* and its predator *Chrysoperla carnea* by chemical cues from cole crops. Biological Control 29: 270-277.

Reed, D.W., L. Davin, J.C. Jain, V. Deluca, L. Nelson, and E.W. Underhill. 1993. Purification and properties of udp-glucose-thiohydroximate glucosyltransferase from *Brassica napus* L. seedlings. Archives of Biochemistry and Biophysics 305: 526-532.

Renfrew, C. 1972. The Emergence of Civilization: The Cyclades and the Aegean in the Third Millennium B. C. Methuen, London.

Ripley, V.L., and P.G. Arnison. 1990. Hybridization of *Sinapis alba* L. and *Brassica napus* L. via embryo rescue. Plant Breeding 104: 26–33.

Reuters. 2007. Canadian Canola Industry: Canada to boost canola production 65 pct by 2015. http://www.canola-council.org/canola_boost.aspx. accessed 10 June, 2009.

Rhoades, D.F., and R.G. Cates. 1976. Toward a general theory of plant antiherbivore chemistry. Recent Advances in Phytochemistry 10: 168–213.

Risch, S. J., D.A. Andow, and M.A. Altieri. 1983. Agroecosystem diversity and pest control: Data, tentative conclusions, and new research directions. Environmental Entomology 12: 625-629.

Rojas, J.C., and T.D. Wyatt. 1999. Role of visual cues and interaction with host odour during the host-finding behaviour of the cabbage moth. Entomologia Experimentalis et Applicata 91: 59–65.

Root, R.B. 1973. Organization of a plant-arthropod association in simple and diverse habitats: The fauna of collards (Brassica oleracea). Ecological Monographs 43: 95-124.

Ryan, M.F. 2002. Insect Chemoreception: Fundamental and Applied. Kluwer Academic Publishers, The Netherlands. 303 pp.

Sharma, D.R., R. Kaur, and K. Kumar. 1996. Embryo rescue in plants - A review. Euphytica 89: 325–337.

Shaw, E. 2008. The Detection of Biochemical Markers for Cabbage Seedpod Weevil (*Ceutorhynchus obstrictus*) Resistance in *Brassica napus* L. x *Sinapis alba* L. Germplasm. M.Sc. Thesis, University of Guelph, Guelph, ON. 155 pp.

Shelton, A.M., and F.R. Badenes-Perez. 2006. Concepts and applications of trap cropping in pest management. Annual Review of Entomology 51: 285–308.

Smart, L.E., and M.M. Blight. 1997. Field discrimination of oilseed rape, *Brassica napus* volatiles by cabbage seed weevil, *Ceutorhynchus assimilis*. Journal of Chemical Ecology 23: 2555-2567.

Smart, L. E., M.M. Blight, and A.J. Hick. 1997. The effect of visual cues and a mixture of isothiocyanates on trap capture of cabbage seed weevil, *Ceutorhynchus assimilis.* Journal of Chemical Ecology 23: 889-902.

Soroka, J.J., R.J. Bartelt, B.W. Zilkowski, and A.A. Cossé. 2005. Responses of the flea beetle *Phyllotreta cruciferae* to synthetic aggregation pheromone components and host plant volatiles in field trials. Journal of Chemical Ecology 31: 1829–1843.

Statistics Canada. 2009. Cereal and Oilseed Review. http://www.statcan.gc.ca/bsolc/olc-cel/olc-cel?catno=22-007-XIB&lang=eng. Accessed 18 June 2009.

Strauss, S.Y., R.E. Irwin, and V.M. Lambrix. 2004. Optimal defence theory and flower petal colour predict variation in the secondary chemistry of wild radish. Ecology 92: 132–141.

Sutherst, R.W., G.F. Maywald, T. Yonow, and P.M. Stevens. 1999. CLIMEX. Predicting the Effects of Climate on Plants and Animals. User Guide CSIRO Publishing, Melbourne, 88 pp.

Tabashnik, B. E. 1994. Evolution of resistance to *Bacillus thuringiensis*. Annual Review of Entomology 39: 47–49.

Tabashnik, B.E., Y.B. Liu, T.J. Dennehy, M.A. Sims, M.S. Sisterson, R.W. Biggs, and Y. Carrière. 2002. Inheritance of resistance to Bt toxin Cry1Ac in a field-derived strain of pink bollworm (Lepidoptera: Gelechiidae). Journal of Economic Entomology 95: 1018-1026.

Tabashnik, B.E, A.J. Gassmann, D.W Crowder, and Y. Carrière. 2008. Insect resistance to *Bt* crops: Evidence versus theory. Nature Biotechnology 26: 199-202.

Tahvanainen, J. O., and R. B. Root. 1972. The influence of vegetational diversity on the population ecology of a specialized herbivore, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). Oecologia 10: 321–346.

Tansey, J.A. 2001. Leafy spurge (*Euphorbia* spp. near *esula*) variability and its effects upon *Aphthona nigriscutis* (Coleoptera: Chrysomelidae) feeding preferences and congregation behaviour. M.Sc. Thesis, University of Alberta, Edmonton, Canada. 185 pp.

Tansey, J.A., A.S. McClay, D.E. Cole, and B.A Keddie. 2005. Evidence for the influence of conspecific chemical cues on *Aphthona nigriscutis* (Coleoptera: Chrysomelidae) behaviour and distribution. BioControl 50: 343–358.

Tansey, J.A., S.D. Noble, L.M. Dosdall, and B.A. Keddie. 2008a. Visual cues influence host choices of the cabbage seedpod weevil. Proceedings of the Entomological Society of Alberta, 56th annual meeting. Edmonton, Alberta. 6-8 November 2008.

Tansey, J.A., L. Dosdall, A. Keddie, and O. Olfert. 2008b. Cabbage seedpod weevil flight behaviour is influenced by climatic factors. Joint Annual Meeting of the Entomological Society of Canada and the Entomological Society of Ontario, 19-22 October 2008, Ottawa, ON.

Tansey, J.A., L.M. Dosdall, B.A. Keddie, and R.M. Sarfraz. 2008c. Differences in *Phyllotreta cruciferae* and *Phyllotreta striolata* (Coleoptera:

Chrysomelidae) responses to neonicotinoid seed treatments. Journal of Economic Entomology 101: 159-167.

Tansey, J.A., L.M. Dosdall, and B.A. Keddie. 2009. *Phyllotreta cruciferae* and *Phyllotreta striolata* responses to insecticidal seed treatments with different modes of action. Journal of Applied Entomology 133: 201-209.

Tatchell, G.M. 1983. Compensation in spring-sown oil-seed rape (*Brassica napus* L.) plants in response to injury to their flower buds and pods. Journal of Agricultural Science (Cambridge) 101: 565-573.

Thomas, P. 2003. Canola Growers Manual. Canola Council of Canada, Winnipeg, MB

Thomson, C.G. 1859. Skandinaviens Coleoptera, synoptisk bearbetade. Vol. 1, Lund, pp. IV + 1-290.

Thompson, R.T. 1992. Observations on the morphology and classification of weevils (Coleoptera, Curculionoidea) with a key to major groups. Journal of Natural History 26: 835–891.

Tulisalo, U., S. Korpela, and A. Pohto. 1976. The yield loss caused by the seedpod weevil *Ceutorhynchus assimilis* Payk. (Col., Curculionidae) on summer turnip rape in cage experiments. Acta Entomologica Fennica 42: 98-102.

Ulmer, B.J., and L.M. Dosdall. 2006a. Spring emergence biology of the cabbage seedpod weevil (Coleoptera: Curculionidae). Annals of the Entomological Society of America 99: 64-69.

Ulmer, B.J., and L.M. Dosdall. 2006b. Glucosinolate profile and oviposition behaviour in relation to the susceptibilities of different Brassicaceae to the cabbage seedpod weevil. Entomologia Experimentalis et Applicata 121: 203-213.

USEPA. 2001. Bt cotton refuge requirements for the 2001 growing season. http://www.epa.gov/oppbppd1/biopesticides/otherdocs/bt_cotton_refuge_2001.htm. Accessed 19 June, 2009.

Vacher, C., D. Bourguet, F. Rousset, C. Chevillon, and M.E. Hochberg. 2003. Modelling the spatial configuration of refuges for a sustainable control of pests: A case study of Bt cotton. Journal of Evolutionary Biology 16: 378–387.

Van Hezewijk, B., and R. Bourchier. 2005. Is two company or a crowd: How does conspecific density affect the small-scale dispersal of a weed biocontrol agent? Biocontrol Science and Technology 15: 191-205.

Vaughn, S. F., and M.A. Berhow. 2005. Glucosinolate hydrolysis products from various plant sources: pH effects, isolation, and purification. Industrial Crops and Products 21: 193-202.

Visser, J.H. 1986. Host odor perception in phytophagous insects. Annual Review of Entomology 31: 121-144.

Walczak, B., M. Kelm, Z. Klukowski, L.E. Smart, A.W. Ferguson, and I.H. Williams. 1998. The effect of trap design and 2-phenylethyl isothiocyanate on catches of stem weevils (*Ceutorhynchus pallidactylus* Marsh. and *C. napi* Gyll.) in winter oilseed rape. OILB WPRS Bulletin 21: 141–146.

Wehner, R. 1981. Spatial vision in arthropods, pp. 287-616. *In*: Autrum, H. (ed.). 1981. Handbook of Sensory Physiology, Vol. 7, Sect. 6C. Springer, Berlin/New York.

Western Committee on Crop Pests. 2001. Minutes of the 41st Annual meeting. 15-16 October, Banff, AB.

Western Committee on Crop Pests. 2002. Minutes of the 42nd Annual meeting 31 October, Kelowna, BC.

Western Committee on Crop Pests. 2003. Minutes of the 43rd Annual meeting 4 October, Winnipeg, Manitoba.

Williams, I. H. 2003. Parasitoids of cabbage seed weevil, pp. 97-112. *In*: Alford, D.V. (ed.), Biocontrol of Oilseed Rape Pests. Blackwell Science, Oxford.

Williams, I.H. 2004. Advances in insect pest management of oilseed rape in Europe, pp. 181–208. *In*: Horowitz, A.R., and I. Ishaaya (eds.). Insect Pest Management. Springer-Verlag.

Williams, I.H., and J. B. Free. 1978. Feeding and mating behaviour of pollen beetles (*Meligethes aeneus* Fab.) and seed weevils (*Ceutorhynchus assimilis* Payk.) in oil-seed rape (*Brassica napus* L.). Journal of Agricultural Science (Cambridge) 91: 453-459.

Wittstock, U., and J. Gershenzon. 2002. Constitutive plant toxins and their role in plant defense against herbivores and pathogens. Current Opinions in Plant Biology 5: 300–307

Yoshioka, H., A. Horisaki, K. Kobayashi, Syafaruddin, S. Niikura, S. Ninomiya, and R. Ohsawa. 2005. Intraspecific variation in the ultraviolet colour proportion of flowers in *Brassica rapa* L. Plant Breeding 124: 551-556.

Zenkteller, M. 1990. *In vitro* fertilization of ovules of some species of Brassicaceae. Plant Breeding 105: 221–228.

Zherikhin, V.V., and A. Egorov. 1990. Weevils (Coleoptera, Curculionidae) of the U.S.S.R. Far East (A review of subfamilies with description of new taxa). Biological-Pedagogical Institute, Vladivostok. 164 pp.

Zherikhin, V.V., and V.G. Gratshev. 1995. A comparative study of the hind wing venation of the superfamily Curculionoidea, with phylogenetic implications, pp. 633–777. *In*: Pakaluk, J., and S.A. Ślipiński (eds.). Biology, Phylogeny and Classification of Coleoptera, Volume 2. Muzeum i Instytut Zoologii PAN, Warszawa, Poland. 533 pp.

Chapter 2

Visual responses of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham): evidence for trichromatic vision

Introduction

The cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (syn. *C. assimilis* (Paykull)) (Coleoptera: Curculionidae) is an important pest of brassicaceous oilseed crops in North America and Europe; attack on canola, *Brassica napus* L. and *Brassica rapa* L., and mustard, *Brassica juncea* (L.) Czern. is of particular concern (McCaffrey 1992; Buntin et al. 1995; Dosdall et al. 2001). This European weevil was first reported in south-western British Columbia in 1931 (McLeod 1962). It has since dispersed or was accidentally introduced, so its current range encompasses most of continental U.S.A. (McCaffrey 1992). In the Canadian prairies, *C. obstrictus* was first reported near Lethbridge, Alberta in 1995 (Cárcamo et al. 2001) and was recently found in Québec and Ontario (Brodeur et al. 2001; Mason et al. 2003). Western North American populations were likely introduced from source locations in western or northern Europe, whereas north-eastern North American populations were introduced separately from Scandinavia or Russia (Laffin et al. 2005). *Ceutorhynchus obstrictus* is dispersing north and east from southern Alberta at approximately 55 km per year; by 2000 the weevil reached western Saskatchewan and is predicted to eventually occupy all western Canadian canola production regions (Dosdall et al. 2002).

Ceutorhynchus obstrictus is univoltine and overwinters as an adult in the soil below leaf litter, usually in shelterbelts (Dmoch 1965; Ulmer and Dosdall 2006). In spring, newly emerged adults feed on brassicaceous plants near their overwintering sites for a few days before undergoing host-seeking migrations (Bonnemaison 1957). Most of these plants provide sustenance for newly emerged

overwintered weevils, resources for ovarian development and opportunities to aggregate and mate (Fox and Dosdall 2003). Oviposition occurs in developing siliques of *B. napus, B. rapa, Raphanus* spp. and to a lesser extent *Sinapis arvensis* L. (Dmoch 1965). Larvae feed on developing seeds, consuming five to six seeds during three instars (Dmoch 1965). Mature larvae chew through pod walls and drop to the soil where they burrow in and pupate. The next generation of adults emerges about 10 d later; development from egg to adult requires 31-58 d in spring canola in western Canada (Dosdall and Moisey 2004).

Herbivorous insects use visual and olfactory cues for host location and demonstrate great selectivity among visual cues (Prokopy and Owens 1983). Host location has been suggested to follow a stepwise process that incorporates directed flight in response to olfactory cues, visual responses to reflected spectral properties of potential host plants at relatively close range and chemical and tactile cues at intimate ranges (Kennedy 1965). Directed flight in response to visual cues has also been proposed (Moericke 1952). At relatively close ranges, spectral quality of the light reflected by a potential host plant is the predominant cue associated with detection and alightment in many herbivorous insects (Prokopy and Owens 1983).

Most insect visual systems examined to date have near-ultraviolet receptors (UV) with response maxima near 350 nm ($\lambda_{max} \approx 350$ nm), blue receptors ($\lambda_{max} \approx 450$ nm), and green receptors ($\lambda_{max} \approx 550$ nm) (Briscoe and Chittka 2001). Many herbivorous insects also respond to yellow ($\lambda = 560$-590 nm) as a 'supernormal' stimulus (Prokopy and Owens 1983). Chittka (1996)

suggested that this set of UV, blue, and green photoreceptors is ancestral to Insecta and that some lineages have since lost or added receptors as a result of different selection pressures. Chittka (1996) found that a modeled system of spectral receptor types with λ_{max} = 340, 440 and 560 nm, approximating the type typical of Apidae (Hymenoptera), was near optimal for distinguishing floral colours from background, flower colours among sympatric plant species and discrimination of green foliage.

Understanding the visual responses of *C. obstrictus* to light of specific wavelengths has important implications for improved population monitoring and for developing *C. obstrictus*-resistant plants, yet few previous studies have investigated the visual system of this species. In earlier studies, Smart et al. (1997) found that yellow traps captured many more *C. obstrictus* than black, white, and green traps; Buechi (1990) found that although yellow traps captured more weevils than other colours, light green and white traps also captured large numbers of *C. obstrictus*. Effects of UV reflection or interactions of UV reflection and human-visible frequencies on *C. obstrictus* behaviour have not yet been examined. Hausmann et al. (2004) found an affinity of female *Anthonomus pomorum* L. (Coleoptera: Curculionidae) for UV, green, and blue transmitted light in laboratory choice tests; they concluded that these weevils have a trichromatic visual system and use visual cues for host plant location.

Electrophysiological studies can detect perception of, and sensitivity to, specific frequencies of transmitted light, but they are limited in their ability to detect behavioural effects. Because insect responses to various wavelengths of

light can include discrimination and potential interactions of dominant wavelengths, behavioural evidence is necessary to assess potential effects of specific wavelengths on responses like host selection. The objective of this study was to investigate the behavioural responses of *C. obstrictus* to components of the hypothesized UV-blue-green system and the potential 'supernormal' stimulus, yellow, and assess interactions among frequencies. A large naturalized *C. obstrictus* local population was offered traps painted with black, yellow, green, and blue with colours mixed at various ratios with white paints that reflected different levels of UV. I sought to compare the behavioural responses of *C. obstrictus* males, females, and gravid females to the colours over the course of two field seasons, and so gain greater insight into the visual cues responsible for host selection in this species and assess potential effects of weevil ontogeny on responses.

Materials and Methods

Trap colours and spectrophotometric analysis

Painted bowl traps, half-filled with propylene glycol, were used to assess attractiveness of colours by comparing captures. The bowl dimensions were 15 cm in diameter and 9 cm deep, and they were set at a height of 1 m on metal poles. Traps were coated with blue, green, black and white paints (TiO_2 or $PbCO_3$). TiO_2 reflects less UV (350 nm) than $PbCO_3$ (Judd et al. 1988). UV-reflecting qualities of the paints were increased by mixing them with a UV-reflecting white pigment, $PbCO_3$, in a method similar to that of Moericke (1969)

and adopted by Judd et al. (1988). Some yellow bowl traps were left unpainted while the saturation series were obtained by mixing in TiO_2 or $PbCO_3$ at different ratios in all selected colours. For each colour, a series consisted of unmixed and mixed colours at a ratio of 3:1 or 1:3 with either TiO_2 or $PbCO_3$. This generated a series designated, for example, as follows: Yellow, Yellow: Ti (3:1), Yellow: Ti (1:3), Yellow: Pb (3:1), Yellow: Pb (1:3). Each colour (yellow, blue and green) was designated in an identical manner and will hereafter be referred to by this designation. Black and white (TiO_2 and $PbCO_3$) paints will hereafter be referred to as Black, Ti White and Pb White, respectively. Mixtures (1:1) of black and white (TiO_2 and $PbCO_3$) paints will hereafter be referred to as Ti Grey and Pb Grey, respectively. Similar proportions of Yellow, Green, Blue or Black and either TiO_2 or $PbCO_3$ appeared monochromatic to the human eye. Reflectance properties of individual tints (paints and mixtures) were assessed using a dual-beam spectrophotometer operating between 250 and 700 nm (Cary 5G UV-Vis-NIR, Varian, Inc., Mississauga, Ont.). Reflectance was assessed at 1 nm increments and corrected for proportion reflected by a 99% Spectralon reflectance standard (Labsphere, North Sutton, NH).

Trap captures

Bowl traps, set on metal posts at a height of 1 m (figure 2.1), were deployed near Lethbridge, Alberta (49° 41′ 39″ N, 112° 49′ 58.3″ W) near a 2.0 ha *B. napus* field in 2007 and an irrigated 10.7 ha *B. napus* field in 2008. Fields chosen in 2007 and 2008 were approximately 1.5 km apart. Both fields were seeded to *B. napus*

and the experimental plot area was surrounded by ≥ 10 m borders of *B. rapa.* Traps were set 10 m from a shelterbelt comprised mainly of cottonwood (*Populus angustifolia* James) and 4 m from the field's western edge in 2007 and approximately 2 m from that field's eastern edge in 2008. Individual tints were replicated four times; traps were separated by 1 m. Insects were gathered from bowl traps weekly from 1 June to 10 August 2007 and 30 May to 8 August 2008 and stored in 70% ethanol. Sampling intervals will hereafter be referred to as weeks 1 to 10. Preserved *C. obstrictus* were dissected to determine sex and gravid status of females. Gravid status was determined by the presence or absence of chorionated oocytes in the ovarioles or lateral oviducts (as per Fox and Dosdall 2003). All females with chorionated oocytes were assumed to be gravid.

All analyses were conducted with SAS version 9.1 (SAS Institute Inc. 2005). Weekly weevil counts from each bowl trap were analyzed using a repeated measures analysis with Poisson generalized estimating equations (proc GENMOD). Proportions of *C. obstrictus* males, total females and gravid females from each trap on each sampling interval were compared by repeated measures analysis with binomial generalized estimating equations (proc GENMOD). An exchangeable correlation structure was used for these and weekly weevil count analyses to account for correlation of counts from each trap. Pair-wise comparisons for weekly count and analyses of the proportion of males, total females and gravid females in catches were made using a Wald chi-square test (LSMEANS statement with DIFF option in proc GENMOD) (SAS Institute Inc. 2005). A Bonferroni adjustment in *p* values to 0.0025 was made to achieve an

overall criterion level of statistical significance of 0.05 for the 20 tint comparisons.

Relating reflectance properties to trap captures

Assessments of relationships between reflective properties of traps and weevil catches were conducted using multiple regression stepwise procedure analysis (proc REG with the Selection = Stepwise option). Proportions of males and total and gravid females were evaluated as a proportion of each group captured weekly. Capture data were transformed [$\log_{10}(x+0.1)$] to normalize residuals. Bonferroni adjustments were calculated by dividing the nominal alpha level (0.05) by the number of hypotheses. Modified alpha values were incorporated into the SLENTRY= α function (significance level to enter) and SLSTAY = α function (significance level to remain); only variables significant at modified alpha values were allowed to enter and stay in models. Models were developed to represent the responses of male, total female and gravid female *C. obstrictus* to amounts of ultraviolet (350 nm), blue (450 nm), green (530 nm) and yellow (580 nm) light reflected from traps as well as sampling interval (quantified as 1 to 10) and year. Tested factors also included variables created to represent interactions between main effects, quadratic relationships of main effects, between main effects and sampling interval, and between year and sampling interval.

Results

Overview of trap captures

Comparison of the numbers of weevils captured by all tints in 2007 and 2008 indicated significant differences between years (χ^2 = 60.46, *df* = 1, *P* < 0.0001); more *C. obstrictus* were captured in 2007 (6505) than in 2008 (1539). A significant effect of sampling interval was also apparent (χ^2 = 55.60, *df* = 9, *P* < 0.0001) as was a significant interaction of sampling interval and year (χ^2 = 41.20, *df* = 9, *P* < 0.0001). In 2007, significant decreases in *C. obstrictus* numbers occurred between weeks 2 and 3, 5 and 6, 7 and 8, and 9 and 10; significant increases occurred between weeks 3 and 4, 4 and 5, and 8 and 9 (*P* < 0.05 for all comparisons). In 2008, significant increases in *C. obstrictus* numbers occurred between weeks 1 and 2, and weeks 4 and 5; significant decreases occurred between weeks 2 and 3, 5 and 6, and 6 and 7 (*P* < 0.05 for all comparisons). Significantly more *C. obstrictus* were captured in weeks 1, 5, 6, 7, 8, 9 and 10 in 2007 than in 2008. More *C. obstrictus* were captured in weeks 2 and 3, 2008 than 2007. Similar numbers were captured week 4, 2007 and 2008.

Trap colour significantly influenced capture numbers (χ^2 = 41.27, *df* = 19, *P* = 0.0022) (Figure 2.2). Yellow traps captured greater numbers of *C. obstrictus* than any other colour (*P* < 0.0001 for all comparisons). Green and Yellow: Pb (3:1) traps captured similar numbers (χ^2 = 0.14, *df* = 1, *P* = 0.7044). These colours captured more *C. obstrictus* than Yellow: Ti (3:1) (*P* < 0.0001 for both comparisons). Yellow: Ti (3:1) traps captured more *C. obstrictus* than the remaining colours (*P* < 0.0001 for all comparisons). Green: Pb (3:1) and Green:

Ti (3:1) captured similar numbers of weevils (χ^2 = 0.07, *df* = 1, *P* = 0.7894) and significantly more than Yellow: Pb (1:3) (*P* < 0.0001 for both comparisons); these traps captured significantly more *C. obstrictus* than Yellow: Ti (1:3), Green: Ti (1:3), Green: Pb (1:3), Pb White, or Pb Grey (*P* < 0.001 for all comparisons). These traps captured more *C. obstrictus* than Ti Grey, Ti White, Black, Blue, Blue: Pb (3:1), Blue: Pb (1:3), Blue: Ti (3:1), or Blue: Ti (1:3) (*P* < 0.001 for all comparisons) (Figure 2.2). No significant interactions of trap colour and sampling interval (χ^2 = 80.0, *df* = 80, *P* = 0.4790), or trap colour and year (χ^2 = 26.98, *df* = 19, *P* = 0.1051) were apparent.

Proportions of males in traps

Similar proportions of males were captured in 2007 (48%) and 2008 (46%) (χ^2 = 0.27; *df* = 1; *P* = 0.6014). A significant effect of sampling interval was apparent (χ^2 = 41.51; *df* = 9; *P* < 0.0001). Significant decreases in proportions of males were observed weeks 3 to 4 and 6 to 7; significant increases were observed in weeks 4 to 5 and 7 to 8 (*P* < 0.05 for all comparisons). No significant linear trend was associated with proportions of males captured by date for 2007 and 2008 (*F* = 0.10; df =1, 9; *P* = 0.7543). A significant effect of trap colour was also apparent (χ^2= 30.50; *df* = 19; *P* = 0.0458) (Figure 2.3). Yellow traps captured greater proportions of males than Yellow: Pb (3:1) or Green traps (χ^2 = 21.31, *df* = 1, *P* < 0.0001, and χ^2 = 39.55; *df* = 1; *P* < 0.0001). Green and Yellow: Pb (3:1) captured similar proportions (χ^2 = 1.13, *df* = 1, *P* = 0.2878) and significantly more than Yellow: Ti (3:1) (*P* < 0.0001 for both comparisons). Yellow: Ti (3:1) captured

greater proportions of male weevils than Green: Pb (3:1) or Green: Ti (3:1) ($P <$ 0.0025 for both comparisons). Green: Pb (3:1) captured greater proportions than Yellow: Pb (1:3), Pb White, Green: Pb (1:3), Green: Ti (1:3), or Yellow: Ti (1:3) ($P < 0.001$ for all comparisons). Green: Ti (1:3), Yellow: Pb (1:3), Pb White, and Green: Ti (1:3) captured greater proportions of *C. obstrictus* males than Pb Grey, Ti Grey, Ti White, Black, Blue, Blue: Pb (3:1), Blue: Pb (1:3), Blue: Ti (3:1), and Blue: Ti (1:3) ($P < 0.0025$ for all comparisons) (Figure 2.3). No significant interaction of trap colour and year was apparent ($\chi^2 = 28.38$; $df = 19$; $P = 0.0764$). No interactions of year and sampling interval ($\chi^2 = 11.45$; $df = 9$; $P = 0.2464$), or sampling interval and trap colour ($\chi^2 = 80.0$; $df = 80$; $P = 0.4790$) were detected. Examination of the proportions of males captured by each colour as a proportion of total males captured also indicated significant differences among treatments ($\chi^2 = 30.20$, $df = 19$, $P = 0.0493$). Patterns among colours were similar to those demonstrated for males as a proportion of total captures. Yellow traps captured 28% of males (Figure 2.4).

Proportions of total females in traps

Similar proportions of females were captured in 2007 (52%) and 2008 (54%) ($\chi^2 = 0.41$; $df = 1$; $P = 0.5234$). A significant effect of sampling interval was also apparent ($\chi^2 = 33.33$, $df = 1$, $P < 0.0001$). Increases in proportions of total females in traps occurred between weeks 1 and 2, 4 and 5, 7 and 8, and 8 and 9; decreases occurred between weeks 5 and 6 ($P < 0.05$ for both comparisons). No significant linear trend was associated with proportions of total females captured

by date for 2007 and 2008 (F = 0.10; df =1, 9; P = 0.7543). A significant effect of trap colour was also apparent (χ^2 = 32.51; df = 19; P = 0.0274). Yellow traps captured greater proportions of female weevils than Yellow: Pb (3:1) (χ^2 = 408.25, df = 1, P < 0.0001) or Green traps (χ^2 = 35.34, df = 1, P < 0.0001). Green and Yellow: Pb (3:1) traps captured similar proportions of total females (X^2 = 2.80, df = 1, P = 0.0944) and significantly more than Yellow: Ti (3:1) (P < 0.0001 for both comparisons). Yellow: Ti (3:1) traps captured greater proportions of female weevils than traps of remaining colours (P < 0.0025 for all comparisons). Green: Pb (3:1) and Green: Ti (3:1) traps captured similar proportions of females (P > 0.0025); Green: Pb (3:1) captured greater proportions than Yellow: Pb (1:3), Green: Pb (1:3) and Green: Ti (1:3) (P < 0.0025 for both comparisons). Yellow: Pb (1:3), Green: Pb (1:3) and Green: Ti (1:3) traps captured similar proportions of females; Green: Pb (1:3), Green: Ti (1:3), Pb White Ti Grey, Blue: Ti (1:3), Yellow: Ti (1:3), Pb Grey, Blue: Pb (1:3), and Blue traps caught similar proportions of total females (P > 0.0025 for all comparisons). Traps of these colours captured greater proportions than Ti White or Blue: Ti (3:1) traps (P < 0.0025 for all comparisons) (Figure 2.3). No significant interaction of trap colour and year was apparent (χ^2 = 26.11; df = 19; P = 0.1273). No significant interactions of sampling interval and trap colour (χ^2 = 80.0, df = 81, P = 0.5105), or sampling interval and year (χ^2 = 12.71, df = 9, P = 0.1763) were detected. Examination of the proportions of females captured by traps of each colour as a proportion of total females captured also indicated significant differences among treatments (χ^2 = 37.57, df = 19, P = 0.0067). Patterns among colours were similar

to those demonstrated for females as a proportion of total captures. Yellow traps captured 43% of all females (Figure 2.4).

Proportions of gravid females in traps

Greater proportions of gravid females were captured in 2008 (19%) than 2007 (15%) (χ^2 = 32.07; *df* = 1; *P* < 0.0001). A significant effect of sampling interval was also apparent (χ^2 = 65.88, *df* = 1, *P* < 0.0001) as was an interaction of sampling interval and year (χ^2 = 29.04, *df* = 9, *P* = 0.0006). In 2007, increases in proportions of gravid females captured in traps occurred between weeks 2 and 3, 4 and 5, 7 and 8 and 9 and 10; decreases occurred between weeks 8 and 9 (*P* < 0.001 for all comparisons). In 2008, increases in proportions of gravid females captured in traps occurred between weeks 2 and 3, and 4 and 5; decreases occurred between weeks 1 and 2 (*P* < 0.001 for all comparisons). Although no significant linear trend was associated with proportions of gravid females captured by date for 2007 and 2008 (*F* =0.77; df =1, 9; *P* = 0.4045), greater proportions of gravid females were apparent late in the season. A significant effect of trap colour was also apparent (χ^2 = 51.92; *df* = 19; *P* = 0.0274) (Figure 2.3). Yellow traps captured greater proportions of gravid females than Yellow: Pb (3:1) or Green traps (*P* < 0.0001 for both comparisons). Green and Yellow: Pb (3:1) traps captured similar proportions of gravid females (*P* > 0.05). Among remaining traps, Yellow: Pb (1:3), Yellow: Ti (3:1), Green: Pb (3:1), and Green: Ti (3:1) captured similar proportions of gravid females, and significantly more than Yellow: Ti (1:3), Green: Pb (1:3), Green: Ti (1:3), and any blue, white, grey

or black traps ($P < 0.0001$ for all comparisons) (Figure 2.3). Although no significant interaction of trap colour and year was apparent ($\chi^2 = 20.76$; $df = 19$; $P = 0.35.02$), pair-wise comparisons indicated that Yellow: Ti (3:1), Yellow: Pb (3:1), Yellow, Green: Ti (3:1), Green, Ti Black, Pb Black, and Blue: Ti (1:3) traps caught greater proportions of gravid females in 2008 than 2007 (Figure 2.3). No significant interaction of sampling interval and trap colour ($\chi^2 = 80.0$, $df = 80$, $P = 0.4790$) was apparent. Examination of the proportions of gravid females captured by each colour as a proportion of total gravid females captured also indicated significant differences among treatments ($\chi^2 = 43.04$, $df = 19$, $P = 0.0013$). Patterns among colours were similar to those demonstrated for gravid females as a proportion of total captures. Yellow traps captured 61% of gravid females (Figure 2.4).

Relating reflectance properties to *C. obstrictus* trap catches

Reflectance properties of each trap colour are presented in Figure 2.5. In the following section, UV (350 nm), Blue (450 nm), Green (550 nm) and Yellow (580 nm) reflectance will be referred to as *UV*, *B*, *G*, and *Y*, respectively. The multiple regression stepwise-selection procedure examining relationships between total males per trap as a proportion of weekly total male captures and specific frequencies reflected from these traps during sampling periods 1-10, 2007 and 2008 indicated contributions to the final model by *G* ($F = 108.38$; df = 1, 1599; $P < 0.0001$), *B* ($F = 254.23.14$; df = 1, 1599; $P < 0.0001$), and the variables created to represent interactions of *UV* and *Y* ($F = 185.09$; df = 1, 1599; $P < 0.0001$), *B*

and *G* (F = 497.66; df = 1, 1599; $P < 0.0001$), and the quadratic function UV^2 (F = 56.57; df = 1, 1599; $P < 0.0001$). A significant interaction of sampling interval (*SI*) and *Y* (*YSI*) was also detected (F = 24.17; df = 1, 1599; $P < 0.0001$). A model developed to describe the relationship of reflectance of specific frequencies from traps and proportions of males in weekly catches is: $\log_{10}(x + 0.1) = -0.85229 + 0.41882\ (G) - 0.93095\ (B) + 8.80952\ (UVY) - 2.06857\ (BG) - 26.15332\ (UV^2) + 2.11239\ (B^2) - 0.00914\ (YSI)$ ($F_{7, 1599} = 167.44$; $R^2 = 0.4273$; $P < 0.0001$) (Table 1).

The multiple regression stepwise-selection procedure indicated contributions to the final model of the relationship between total females per trap as a proportion of weekly total female captures and trap reflectance properties by *G* (F = 74.64; df = 1, 1599; $P < 0.0001$), *B* (F = 476.42; df = 1, 1599; $P < 0.0001$), and the variables created to represent interactions of *UV* and *Y* (F = 327.32; df = 1, 1599; $P < 0.0001$), *B* and *G* (F = 57.86; df = 1, 1599; $P < 0.0001$), and the quadratics UV^2 (F = 89.63; df = 1, 1599; $P < 0.0001$), B^2 (F = 227.89; df = 1, 1599; $P < 0.0001$) and G^2 (F = 19.62; df = 1, 1599; $P = 0.0001$). A model developed for the relationship of reflectance of specific frequencies from traps and proportions of females caught is: $\log_{10}(x + 0.1) = -0.83681 + 0.91539\ (G) - 1.36029\ (B) + 12.188\ (UVY) - 1.80406\ (BG) - 36.18053\ (UV^2) + 2.33536\ (B^2) - 0.82901\ (G^2)$ ($F_{7, 1599} = 276.95$; $R^2 = 0.5491$; $P < 0.0001$) (Table 1).

The multiple regression stepwise-selection procedure examining relationships between corrected proportions of gravid females captured by and specific frequencies reflected from these traps indicated contributions to the final

model by *G* (F = 25.40; df = 1, 1599; $P < 0.0001$), *B* (F = 141.19; df = 1, 1599; $P < 0.0001$), and the variables created to represent interactions of *UV* and *Y* (F = 170.53; df = 1, 1599; $P < 0.0001$), *B* and *G* (F = 15.30; df = 1, 1599; $P < 0.0001$), and the quadratics UV^2 (F = 47.19; df = 1, 1599; $P < 0.0001$), B^2 (F = 66.74; df = 1, 1599; $P = 0.0001$) and G^2 (F = 12.59; df = 1, 1599; $P = 0.0001$). A significant effect of sampling interval (*SI*) was also detected (F = 34.57; df = 1, 1599; $P < 0.0001$). A model developed for the relationship of reflectance of specific frequencies from traps and proportions of weekly catches is: $\log_{10}(x + 0.1) = -0.92863 + 0.82662\ (G) - 1.12209\ (B) + 12.47426\ (UVY) - 1.39674\ (BG) - 35.45179\ (UV^2) + 1.87464\ (B^2) - 0.94033\ (G^2) + 0.00994\ (SI)$ ($F_{8, 1599} = 87.87$; $R^2 = 0.3064$; $P < 0.0001$) (Table 1).

Comparisons of modeled responses of males and females to the interaction of *UV* and *Y* in the absence of *G* or *B* indicated great differences. Both males and females responded to attractive effects of this interaction; both sexes were most attracted to increasing levels of *Y* and moderately low levels of *UV* (Figure 2.6). The greatest modeled total and gravid female proportions were associated with proportions of reflected *Y* of 1.0 and *UV* of ca. 0.18. Increases and decreases in the proportions of *UV* from a proportion of 0.18 reduced attractiveness. Proportions of reflected *UV* < 0.17 > reduced modeled male proportions. Great differences were apparent in modeled responses of total and gravid females to the proportions of reflected *Y* and *UV*: gravid females were most sensitive to this interaction with a modeled response of $\log_{10}(x + 0.1) = 0.27$ at $Y = 1.0$ and $UV = 0.18$. Modeled total female and male responses at these proportions of *Y* and *UV*

were $\log_{10}(x+0.1) = 0.18$ and $\log_{10}(x+0.1) = -0.21$, respectively. Responses to the interaction broadened at lower *Y* and *UV* levels for total and gravid females and males. At $Y = 0.5$, greatest female responses were at $UV <$ 0.07-0.10 >; greatest male responses were at $UV <$ 0.06 - 0.10 >. Neutral response was associated with levels of $UV = 0.45$ at $Y = 1.0$ for females and males. Greater negative effects on females than males was apparent at $Y = 1.0$ and $UV = 0.5$: $\log_{10}(x+0.1) = -3.79$ and $\log_{10}(x+0.1) = -3.08$, for total females and males, respectively. Reduced *Y* reflectance depressed levels of *UV* required to elicit neutral responses for total and gravid females and total males: 0.35, 0.37 and 0.36, respectively. The addition of the effect of *B* influenced models associated with both males and females. At proportions of reflected *Y* of 1.0 and *UV* of 0.18, moderate levels of *B* reflectance (0.33) reduced responses for gravid females, total females and males. In the absence of the attractive effects of the interaction of *Y* and *UV*, values of $B = 0.33$ reduced gravid female, total female and male modeled responses. Including moderate levels of *G* reflectance in models increased responses of total and gravid females and males. The attractive effects of *G* increased until 0.65 for total females and males and to 0.45 for gravid females; at proportions greater than these, modeled responses decreased. Proportions of *B* and *G* also interacted. At $B = 0.33$, $G = 0.33$, $Y = 1.0$ and $UV = 0.18$, responses of females were reduced in a similar manner to those associated with $B = 0.33$ in the absence of *G*. Males were increasingly attracted to the interaction of *B* and *G* with greater proportions of *B* and *G* reflected.

Discussion

Total captures of *C. obstrictus* adults were much greater in 2007 than 2008. Although local populations can undergo natural variability between years (Dosdall et al. 2008), differences in this study can likely be attributed to two factors: insecticide application and excessive irrigation on the 2008 site. Pyrethroid insecticide (Decis®: deltamethrin) was applied to an area adjacent to the 2008 study site in late June and this reduced the local *C. obstrictus* population. Excessive irrigation produced some pools of standing water in the field throughout the season and likely increased mortality of pupating *C. obstrictus.* The second, large emergence peak observed in 2007 and typical of *C. obstrictus* populations (Bonnemaison 1957; Dmoch 1965) did not occur in 2008 and explains larger proportions of gravid females late in the 2008 season.

Despite differences in capture numbers between years, consistent overall trends were apparent. Yellow traps captured the greatest numbers of *C. obstrictus* in both years. Green traps also captured many weevils albeit fewer than Yellow. These results are intermediate to those of Buechi (1990) and Smart et al. (1997): Smart et al. (1997) found that yellow traps were attractive but green traps were not; Buechi (1990) found that yellow, light green and white traps were attractive. I also found that a mixture of yellow and Pb White paints at (3:1) captured similar numbers of *C. obstrictus* as Green, and more than mixtures of Yellow and Ti White at (3:1). Mixtures of Yellow and Pb White captured more total and gravid females but not males than Yellow and Ti White at (1:3). Blue and mixes of Blue and Ti or Pb White, Black, Grey (both Pb and Ti) and Pb White caught similar

and low numbers of *C. obstrictus* in both years. These results suggest a positive interaction of Yellow and UV and a greater sensitivity to this interaction by females than males. Differences in total, and proportions of male, total female and gravid female *C. obstrictus* between monochromatic mixes of Green and Pb White and Ti White were not significant, suggesting little interaction of Green and UV.

Analysis of relationships of trap catches with reflectance properties indicated that significant linear trends in *C. obstrictus* responses were associated with 550 and 450 nm; quadratic effects were associated with 350 nm; and interactions of 580 and 350 nm were detected. Females were more sensitive to this interaction of Yellow and UV than males. Male *C. obstrictus* did not respond to trap colour as strongly as females, which could be attributable to greater reliance on and/or sensitivity to visual cues by females than males. Relatively broader responses of males than females and total females than gravid femalaes, as indicated by proportions of each group captured by Yellow traps and modeled responses, indicate greater discrimination among visual cues by females than males and by gravid females than total female *C. obstrictus*. An important consideration in this analysis is the evaluation of total and gravid females. Had analysis incorporated comparisons of non-gravid female and gravid female trap catches, differences associated with these groups would have been greater. The current analysis allowed comparisons of female and male responses but still indicates effects of gravid status. Differences between sexes in responses to visual cues have also been observed in some other insect species. Sivinski (1990)

determined that female *Anastrepha suspense* (Loew) (Diptera: Tephritidae) showed strong preferences among five colours of spherical fruit models and that males were less discriminating. Female *A. pomorum*, were found to be more responsive to UV, green, and blue light than males (Hausmann et al. 2004). Hausmann et al. (2004) attributed this difference to greater affinity of females to host plant-associated visual cues.

Significant effects of all components of the hypothesized trichromatic system as well as differences in the signs and magnitudes of coefficients associated with models suggest that *C. obstrictus* has trichromatic vision. However, without electroretinogram assessment, these values should be interpreted as approximate response maxima. Although visual systems of many insects have been reported to have $\lambda_{max} \approx$ 350 nm, 450, and 550 nm (Briscoe and Chittka 2001), this is the first documented evidence for trichromatic vision in the Ceutorhynchinae, and only the second for the Curculionidae (Hausmann et al. 2004). Chittka (1996) proposed that a set of UV, blue, and green photoreceptors is ancestral and traceable to the Devonian ancestor of pterygote insects, and that some lineages have since lost or added receptors as a result of different selection pressures. Electroretinogram assessments of Coleoptera have been limited, but within this order, *Leptinotarsa decemlineata* (Say) (Chrysomelidae) was found to have a visual system with λ_{max} = 360 and 510 nm (Mischke 1981); *Photuris lucicrescens* Barber (Lampyridae) has a visual system with λ_{max} = 350, 440 and 550 nm (Lall et al 1982); and *Carabus nemoralis* (Müller) and *C. auratus* L. (Carabidae) have λ_{max} = 348, 430, 500, and 620 nm (Hasselmann 1962).

Receptor pigments can have relatively broad sensitivities (Lall et al. 1989; Briscoe and Chittka 2001), so effects associated with 580 nm in *C. obstrictus* can most likely be attributed to reception by visual pigments with $\lambda_{max} \approx 550$ nm. Prokopy and Owens (1983) attribute 'supernormal' responses to yellow (560-590 nm) as an exaggerated foliage-type stimulus associated with stimulation of a 550 nm receptor with intensities greater than those associated with foliage.

Chittka (1996) found that a modeled system of spectral receptor types with $\lambda_{max} = 340$, 440 and 560 nm, approximating receptors typical of Apidae (Hymenoptera), was near optimal for distinguishing floral colours from background and flower colours among sympatric plant species. *Ceutorhynchus obstrictus* is a Brassicaceae oligophage; adults are capable of exploiting several members of this plant family (Dmoch 1965). Hosts for early season, newly emerged adults include wild mustard (*Sinapis arvensis* L.), hoary cress (*Lepidium draba* L. = *Cardaria draba* (L.)), field pennycress (*Thlaspi arvense* L.), flixweed (*Descurania sophia* (L.) Webb), shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.), radish (*Raphanus* spp.) and volunteer canola (*B. napus* L. and *B. rapa*) (Dmoch 1965; Dosdall and Moisey 2004). Most of these plants provide sustenance for newly emerged overwintered weevils, resources for ovarian development and opportunities to aggregate and mate (Fox and Dosdall 2003). Plant species suitable for larval development are much more limited. Oviposition occurs in developing siliques of *B. napus, B. rapa, Raphanus* spp. and to a lesser extent *S. arvensis*; only these species can sustain larvae (Dmoch 1965).

Significant differences in the proportions of 350 nm and 580 nm light reflected from *S. alba* and *B. napus* flowers and in proportions of 350 nm reflected from foliage have been detected; more 580 nm and less 350 nm light was reflected from *B. napus* than from *S. alba* flowers (Tansey et al. 2008). Differences in the UV colour proportions (the proportion of light below and above 365 nm reflected from flowers) among cultivars of *B. rapa* have also been detected and implicated in pollinator choices (Yoshioka et al. 2005). Although *C. obstrictus* will oviposit in developing *S. alba* if caged on plants in a no-choice environment, oviposition occurs with low frequency in the field and emergence of mature larvae is rare (Kalischuk and Dosdall 2004; Dosdall and Kott 2006). Reduced modeled responses of gravid female *C. obstrictus* to UV-reflectance beyond a proportion of 0.18 suggest that visual cues are important for *C. obstrictus* oviposition site selection and that the proportion of UV: yellow is particularly important. Greater sensitivity of female *C. obstrictus* to this interaction and differential larval development among hosts with different floral reflectance properties suggests the adaptive significance of this response.

Another possible explanation for decreased responses to UV proportion beyond a threshold value of 0.18 may be UV-green antagonism. Möller (2002) suggested that UV and green receptors of insect eyes could allow separation between foreground and sky under a multitude of illumination conditions and could be associated with a threshold of UV sensitivity. Menzel and Blakers (1976) reported that UV and green receptors occur within the same ommatidium in bee eyes. However, I did not detect an interaction of UV and green and the interaction

of yellow and UV was positive although the reflectance associated with these traps did not approach levels typical of the sky.

Chittka (1996) also suggested that spectral receptor types with λ_{max} = 340, 440 and 560 nm was near optimal for discrimination of green foliage. Both *S. alba* and *B. napus* flowers reflect much 580 and 530 nm but little 350 or 450 nm (proportions ca. 0.05-0.08); foliage of both *S. alba* and *B. napus* reflects moderate amounts of 530-580 nm (proportions ca. 0.12- 0.14). Greater responses of *C. obstrictus* to increased 530 nm reflectance and reduced responses to increased 450 nm reflectance suggest a possible mechanism for discrimination of flowers from foliage and among foliage (Tansey et al. 2008). The negative effects of the interaction of blue and green detected for females in these models support this conclusion.

Although linear trends associated with sampling interval and trap colour were not apparent for proportions of males, or total and gravid females, significant effects of sampling interval were detected in modeled responses of males and gravid females. Evaluation of each group as a proportion of total captures may have been confounded by differential proportions of each group present in captures at different times of year. Modeled responses were based on captures of each group expressed as proportions of each group rather than as proportions of total captures. Modeled responses indicated male responses to yellow decreased with time, gravid female responses increased. These results indicate ontogenic plasticity in the responses of adult *C. obstrictus* to visual cues. Greater gravid female responses with time suggest a physiological state change

associated with prolonged egg retention. Stimuli associated with an appropriate oviposition site can cause increased juvenile hormone titre, initiating oogenesis in *Nicrophorus orbicollis* Fab. (Coleoptera: Silphidae) (Trumbo et al. 1995). Pimentel et al. (1998) detected increases in *Rhagoletis juglandis* Cresson (Diptera: Tephritidae) egg load when they were held near yellow spheres. Henneman and Rapaj (1999) found that intensity of *R. juglandis* search for oviposition sites was dependent on egg load. Fewer canola flowers are present as plants mature so increased sensitivity of *C. obstrictus* to floral cues is likely adaptive.

Decreased male *C. obstrictus* responses to visual cues with time may be associated with an interaction of visual and olfactory cues. Male *Dendroctonus pseudotsugae* Hopkins (Coleoptera: Curculionidae) respond in an additive manner to a combination of olfactory cues (a mix of aggregation pheromones and ethanol) and visual cues associated with simulated host visual cues (Campbell and Borden 2006). Evans and Bergeron (1994) found that unmated female *C. obstrictus* from the spring generation produce a volatile chemical cue that is attractive to both males and females; this cue was not produced by mated females or females of the fall generation. Males of the fall generation cannot detect this substance (Evans and Bergeron 1994).

Population densities could influence responses. Populations were higher in 2007 than 2008 and Yellow: Ti (3:1), Yellow: Pb (3:1), Yellow, Green: Ti (3:1), Green, Ti Black, Pb Black, and Blue: Ti (1:3) traps caught greater proportions of gravid females in 2008 than 2007. Herzig (1995) found that *Trirhabda virgata* Le Conte (Coleoptera: Chrysomelidae) females emigrated more

frequently from host patches with severe defoliation or high densities of conspecifics. Greater population densities in 2007 than 2008 may have also resulted in greater *C. obstrictus* flight activity and influenced trap captures. This hypothesis requires testing.

Insects respond to objects based on specific predetermined templates and ignore stimuli that do not conform (Wehner 1981). Wallin and Raffa (2004) detected a broadening host range in eruptive versus endemic *Dendroctonus rufipennis* (Kirby) (Coleoptera: Curculionidae) populations, suggesting a relaxation of the template of suitable host-association stimuli. Although Wallin and Raffa (2004) attributed this trend in *D. rufipennis* to an increase in the range of acceptable phytochemical concentrations, results of this study suggest a potential broadening in the acceptability of visual cues in response to greater population densities. The effects of crowding on visual responses have not been tested on any insect to my knowledge.

These data indicate sensitivity of *C. obstrictus* to UV, blue and green wavelengths consistent with trichromatic systems. This knowledge, particularly the influence of UV and the interaction between yellow and UV, could have a number of potential applications, including improvements in trap design. Incorporation of yellow reflectance and moderate UV reflectance can potentially greatly increase the effectiveness of traps and so the accuracy of population assessments. Understanding the behavioural implications for the interaction between yellow and UV also offers insights into host association cues that are important to economically important anthophilous insects like *C. obstrictus*. This

understanding can be exploited to manipulate *B. napus*, *B. rapa* or other commercially important host plant flowers to reflect levels of yellow or UV that are suboptimal in their behavioural effects on *C. obstrictus* attraction. Manipulating cues associated with host attraction also has the potential to reinforce other modes of resistance such as the antixenosis demonstrated in several *S. alba* x *B. napus* genotypes (McCaffrey et al. 1999; Dosdall and Kott 2006).

Attractive effects of the interaction of UV and yellow can also offer insights into an important aspect of *C. obstrictus* behaviour: spring-time mass migration into crops (Bonnemaison 1957). Dosdall and Moisey (2004) reported that few *C. obstrictus* migrated to spring canola when crops were in the seedling and rosette stages; migration reached its maximum in the bud and flowering stages. Cárcamo et al. (2007) found that a perimeter of *B. rapa* around a *B. napus* main crop flowered one week earlier than the main crop and concentrated weevil populations. Trap catches reported at 4.8 times higher in flowering winter oilseed rape than in non-flowering spring rape (Šedivý and Vašák 2002) also suggest the importance of visual cues although they cannot discount the importance of olfactory cues in early season host association. However, strong attraction of *C. obstrictus* to flowering crops and early-season attraction of weevils to yellow objects (J. Tansey, unpublished data) suggest a great affinity of these weevils to the visual cues found to be attractive in this study. As attractive effects were detected in the absence of olfactory cues, attraction can be attributed to vision alone.

Table

Table 2.1. Models developed by stepwise multiple regression procedure for proportions of weevils sampled weekly from mid June to early August 2007 and 2008. G_m: log (x + 0.1) proportion male *C. obstrictus* captured per sampling interval. G_f: log (x + 0.1) corrected proportion of all female *C. obstrictus* captured per sampling interval. G_{gf}: log (x + 0.1) corrected proportion of gravid female *C. obstrictus* captured per sampling interval. *UV*: absolute reflectance of 350 nm light from painted traps. *B*: 450 nm. *G*: 530 nm. *Y*: 580 nm. *UVY*: variable created to represent interaction of *UV* and *Y*; interaction of *B* and *G*; *UVB*: interaction of *UV* and *B*; *YG*: interaction of *Y* and *G*. *SI*: sampling interval; *YSI*: variable created to represent the interaction of *Y* and *SI*.

Modeled system	Models developed by multiple regression stepwise procedure	*F*	df	R^2	*P*
Males	G_m = – 0.85229 + 0.41882 (*G*) – 0.93095 (*B*) + 8.80952 (*UVY*) – 2.06857 (*BG*) – 26.15332 (UV^2) – 0.00914 (*YSI*)	167.44	7, 1599	0.4273	< 0.0001
Total Females	G_{tf} = – 0.83681 + 0.91539 (*G*) – 1.36029 (*B*) + 12.188 (*UVY*) – 1.80406 (*BG*) – 36.18053 (UV^2) + 2.33536 (B^2) – 0.82901 (G^2)	276.95	7, 1599	0.5491	< 0.0001
Gravid Females	G_{gf}: = – 0.92863 + 0.82662 (*G*) - 1.12209 (*B*) + 12.47426 (*UVY*) - 1.39674 (*BG*) -35.45179 (UV^2) + 1.87464 (B^2) -0.94033 (G^2) + 0.00994 (*SI*)	87.87	8, 1599	0.3064	< 0.0001

Figures

Figure 2.1. Array of painted bowl traps near Lethbridge, Alberta.

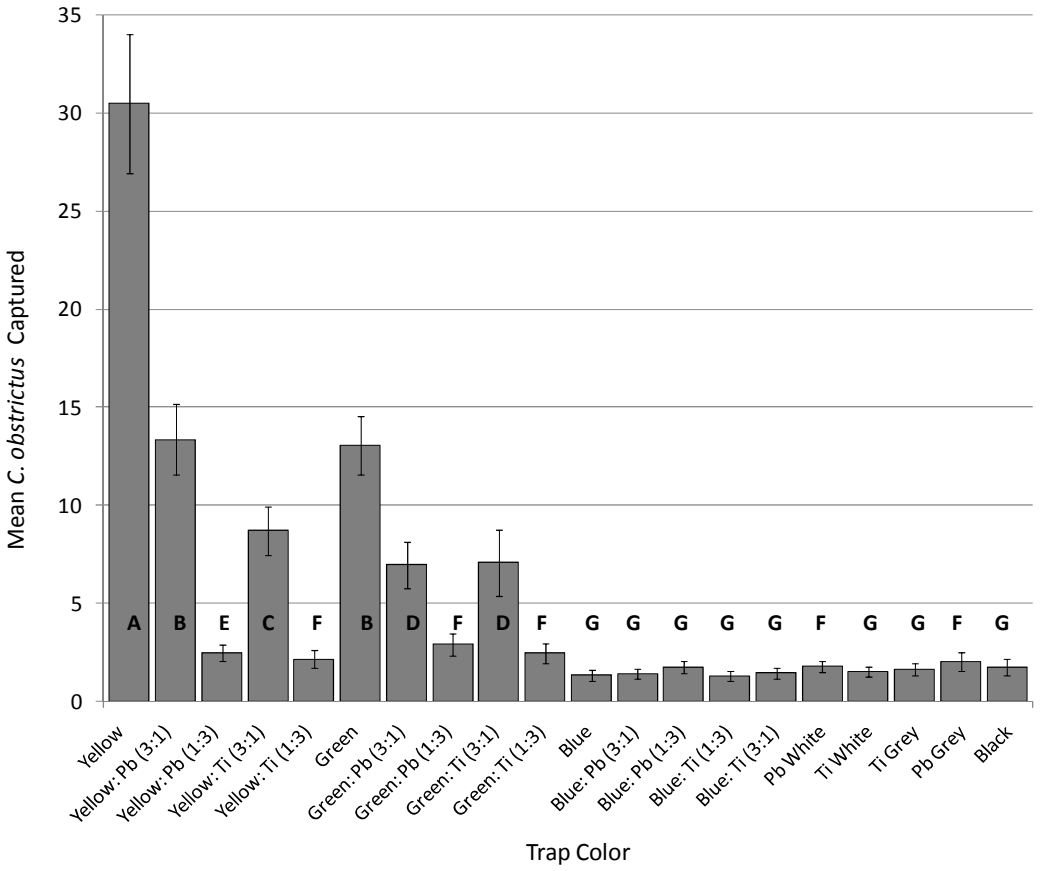

Figure 2.2. Mean *Ceutorhynchus obstrictus* captures by trap colour for weekly sampling intervals from late May to early August 2007 and 2008. Each trap was replicated four times. Bars with different letters indicate significant differences by pair-wise comparisons using Wald's Chi-square test ($\alpha = 0.0025$).

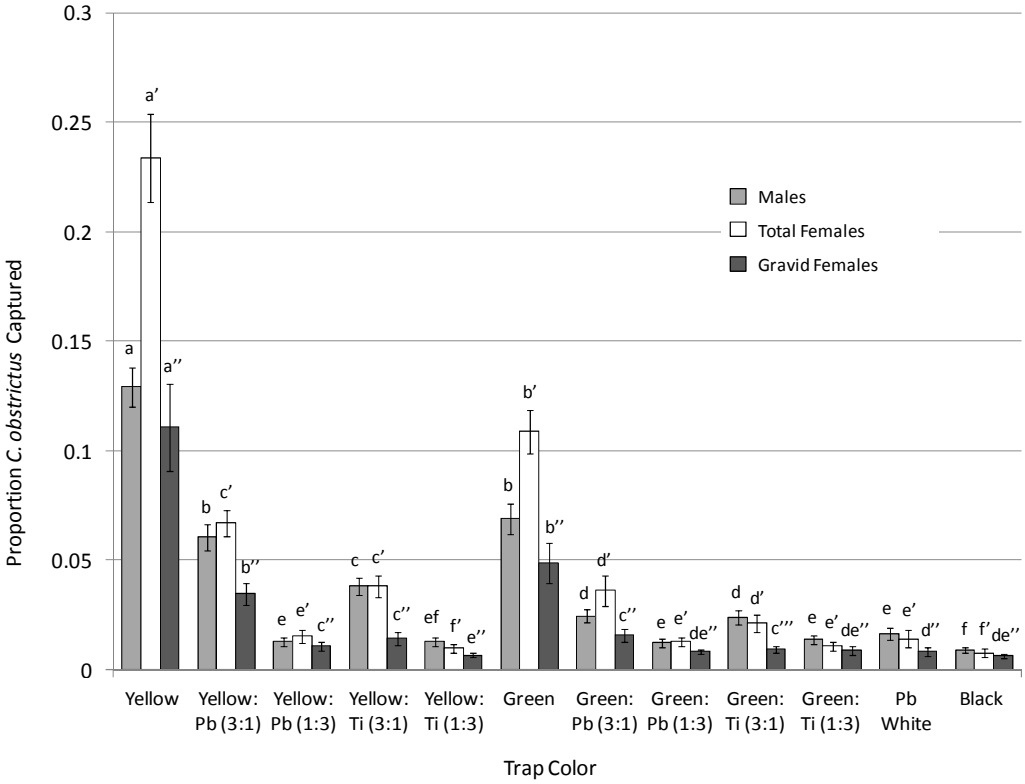

Figure 2.3. Mean proportions of *Ceutorhynchus obstrictus* males, total and gravid females of total weekly captures in all traps for weekly sampling intervals from late May to early August 2007 and 2008. Bars with different letters indicate significant differences by pair-wise comparisons using Wald's Chi-square test ($\alpha = 0.0025$). Data for Blue, Blue: Pb (3:1), Blue: Pb (1:3), Blue: Ti (3:1), Blue: Ti (1:3), Ti White, Pb Grey, and Ti Grey are not shown; these were not significantly different than Black.

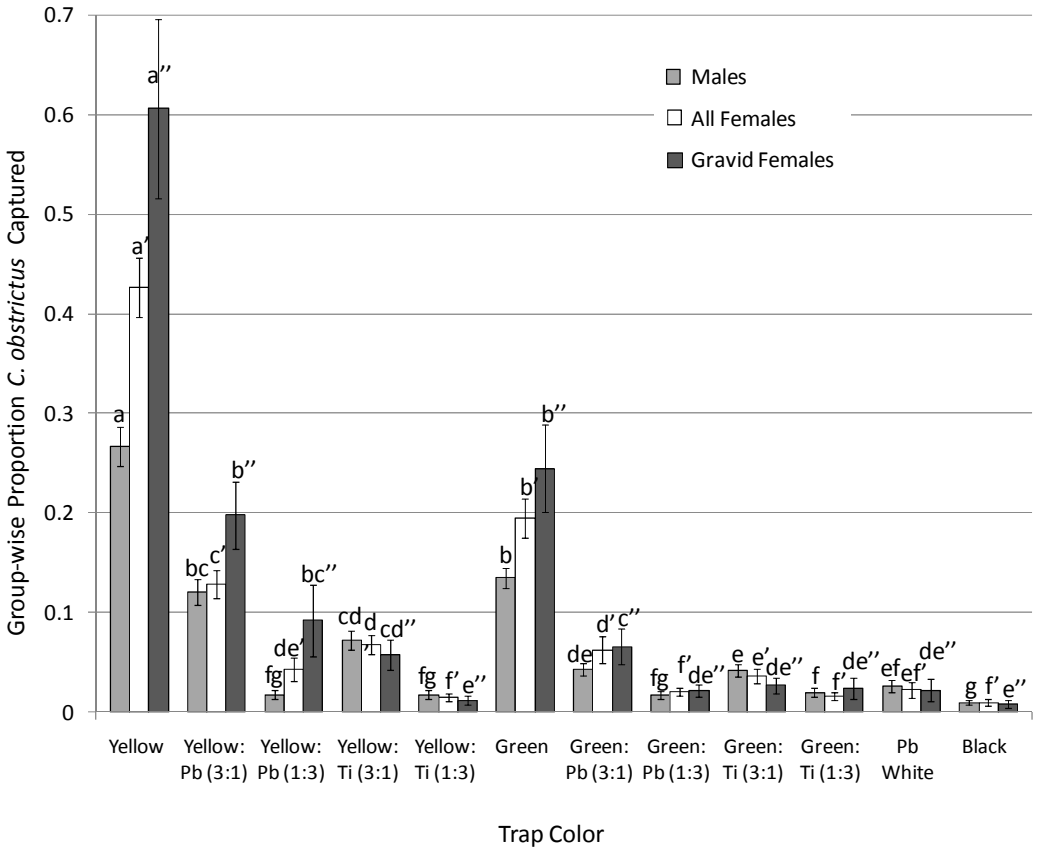

Figure 2.4. Mean numbers of males, total and gravid females per colour expressed as a proportion of total male, female and gravid female *C. obstrictus*, respectively, for weekly sampling intervals from late May to early August 2007 and 2008. Bars with different letters indicate significant differences by pair-wise comparisons using Wald's Chi-square test ($\alpha = 0.0025$). Data for Blue, Blue: Pb (3:1), Blue: Pb (1:3), Blue: Ti (3:1), Blue: Ti (1:3), Ti White, Pb Grey, and Ti Grey are not shown; these were not significantly different than Black.

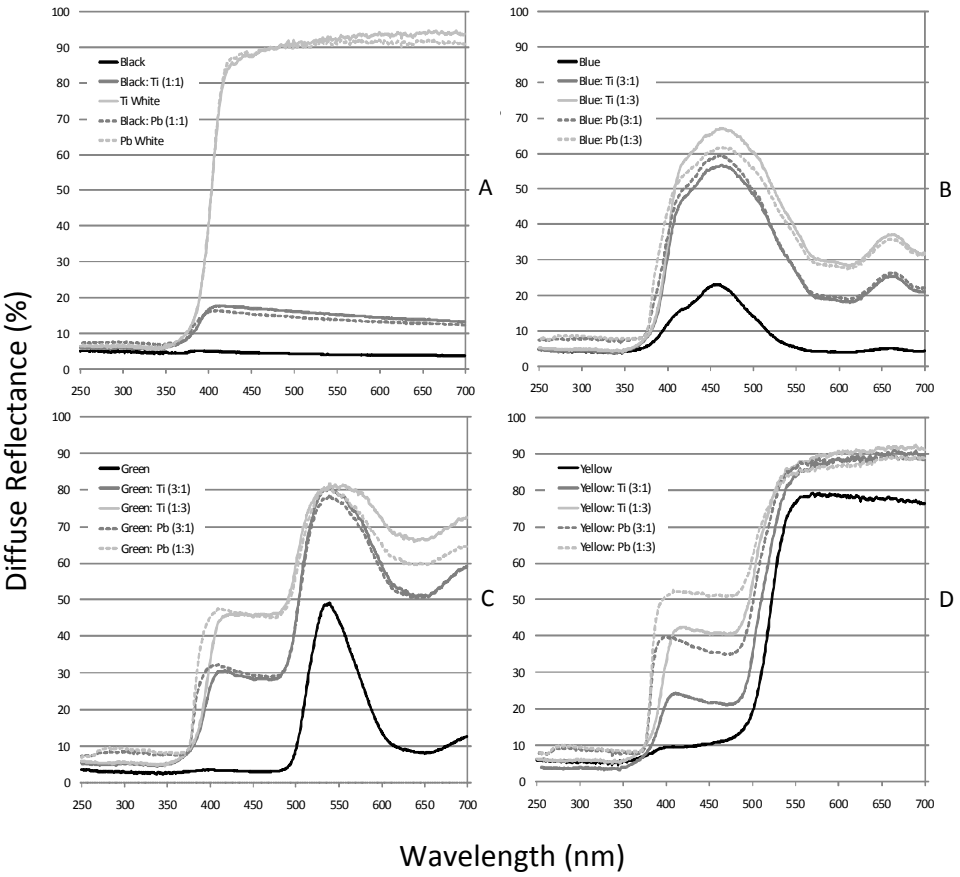

Figure 2.5. Reflectance properties associated with yellow bowl traps and paints and paint mixes applied to bowl traps and presented to a large naturalized *C. obstrictus* population near Lethbridge, AB in 2007 and 2008. Ratios indicate the proportion of each tint in the mix. A: Black, white and grey paints. B: Blue paints. C: Green paints. D: Yellow paints. Black: Ti (1:1) indicates a mix of one part black paint and TiO_2 white paint at (1:1). Black: Pb (1:1) indicates a mix of black paint and $PbCO_3$ white paint at (1:1). Colours and either TiO_2 or $PbCO_3$ white paints were mixed at (1:3) or (3:1).

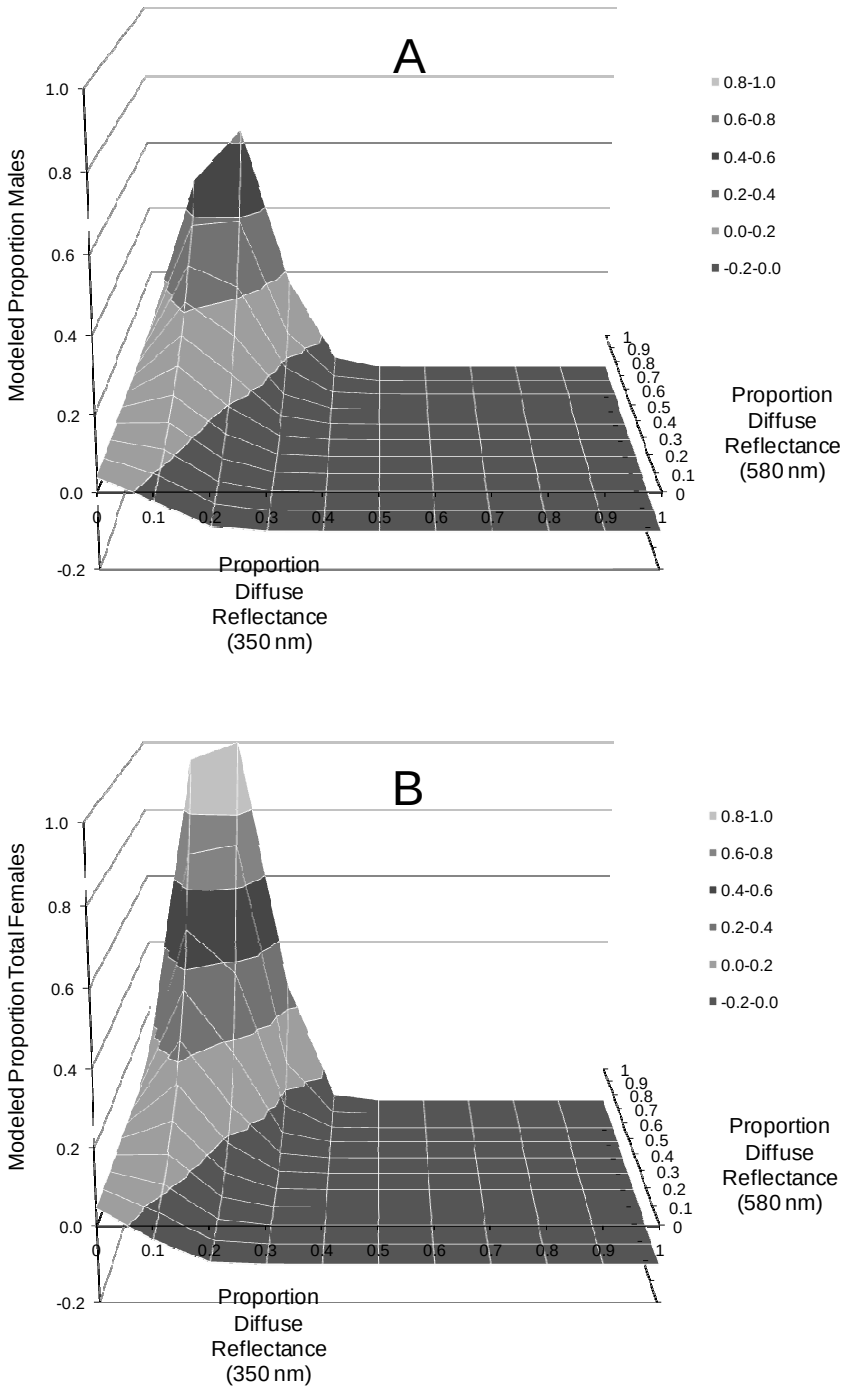

Figure 2.6.

Surface plots representing interaction of near ultraviolet (350 nm) and yellow (580 nm) reflection associated with painted bowl traps and predicted proportions of males (A) and total females (B) from weekly *C. obstrictus* captures late May to early August, 2007 and 2008.

References

Bonnemaison, L. 1957. Le charançon des siliques (*Ceutorhynchus assimilis* Payk.) biologie et méthode de lutte. Annales des Épiphyties 4: 387–543.

Briscoe, A.D. and L. Chittka. 2001. The evolution of colour vision in insects. Annual Review of Entomology 46: 471–510.

Brodeur J., L. Leclerc, M. Fournier, and M. Roy. 2001. Cabbage seedpod weevil (Coleoptera: Curculionidae) a new pest of canola in north-eastern North America. The Canadian Entomologist 133: 709–711.

Buechi, R. 1990. Investigations on the use of turnip rape as trap plant to control oilseed rape pests. IOBC/wprs Bulletin 13(4): 32–39.

Buntin, G.D., J.P. McCaffrey, P.L. Raymer, and J. Romero J. 1995. Quality and germination of rapeseed and canola seed damaged by adult cabbage seedpod weevil *Ceutorhynchus assimilis* (Paykull) [Coleoptera: Curculionidae]. The Canadian Journal of Plant Science 75: 539-541.

Campbell, S.A., and J.H. Borden. 2006. Integration of visual and olfactory cues of hosts and non-hosts by three bark beetles (Coleoptera: Scolytidae). Ecological Entomology 31: 437-449.

Cárcamo, H.A., L. Dosdall, M. Dolinski, O. Olfert, and J.R. Byers. 2001. The cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae) - a review. Journal of the Entomological Society of British Columbia 98: 201-210.

Cárcamo, H.A., R. Dunn, L.M. Dosdall, and O. Olfert. 2007. Managing cabbage seedpod weevil in canola using a trap crop – a commercial field-scale study in western Canada. Crop Protection 26: 1325-1334.

Chittka, L. 1996. Optimal sets of colour receptors and colour opponent systems for coding of natural objects in insect vision. Journal of Theoretical Biology 181: 179–196.

Dmoch, J. 1965. The dynamics of a population of the cabbage seedpod weevil (*Ceutorhynchus assimilis* Payk.) and the development of winter rape. Part I. Ekologiya Polska. Seria A 13: 249–287.

Dosdall L.M., and D.W.A. Moisey. 2004. Developmental biology of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae), in spring canola, *Brassica napus*, in western Canada. Annals of the Entomological Society of America 97: 458-465.

Dosdall, L. M., and L.S. Kott. 2006. Introgression of resistance to cabbage seedpod weevil to canola from yellow mustard. Crop Science 46: 2437-2445.

Dosdall, L. M., D. Moisey, H. Cárcamo, and R. Dunn. 2001. Cabbage seedpod weevil fact sheet. Alberta Agriculture, Food and Rural Development, Edmonton, AB, Canada.

Dosdall, L.M., R.M. Weiss, O. Olfert, and H.A. Cárcamo. 2002. Temporal and geographical distribution patterns of cabbage seedpod weevil (Coleoptera: Curculionidae) in canola. The Canadian Entomologist 134: 403-418.

Dosdall, L.M., G.A.P. Gibson, O.O. Olfert, and P.G. Mason. 2009. Responses of Chalcidoidea (Hymenoptera) parasitoids to invasions of the cabbage seedpod weevil (Coleoptera: Curculionidae) in western Canada. Biological Invasions.11:109–125.

Evans, K.A., and J. Bergeron. 1994. Behavioral and electrophysiological response of cabbage seed weevils (*Ceutorhynchus assimilis*) to conspecific odor. Journal of Chemical Ecology 20: 979-989.

Fox, A.S., and L.M. Dosdall. 2003. Reproductive biology of *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae) on wild and cultivated Brassicaceae in southern Alberta. Journal of Entomological Science 38: 365-376.

Hasselmann, E.M. 1962. Über die relative spektrale Empfindlichkeit von Käfer- und Schmetterlingsaugen bei verschiedenen Helligkeiten. Zoologische Jahrbuecher Abteilung fuer Allgemeine Zoologie und Physiologie der Tiere 69: 537– 576.

Hausmann, C., J. Samietz, and S. Dorn. 2004. Visual orientation of overwintered *Anthonomus pomorum* (Coleoptera: Curculionidae). Environmental Entomology 33: 1410–1415.

Henneman, M.L., and D.R. Papaj. 1999. Role of host fruit colour in the behaviour of the walnut fly *Rhagoletis juglandis.* Entomologia Experimentalis et Applicata 93: 249-258.

Herzig, A.L. 1995. Effects of population density on long-distance dispersal in the goldenrod beetle *Trirhabda virgata*. Ecology 76: 2044–2054.

Judd, G.J.R., J.H. Borden, and A.D. Wynne. 1998. Visual behaviour of the onion fly *Delia antiqua*: antagonistic interaction of ultraviolet and visible wavelength reflectance. Entomologia Experimentalis et Applicata 49: 221-234.

Kalischuk, A.R., and L.M. Dosdall. 2004. Susceptibilities of seven Brassicaceae species to infestation by the cabbage seedpod weevil (Coleoptera: Curculionidae). The Canadian Entomologist 136: 265-276.

Kennedy, J.S. 1965. Mechanisms of host plant selection. Annals of Applied Biology 56: 317 – 322.

Laffin, R.D., L.M. Dosdall, and F.A.H. Sperling. 2005. Population structure of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae). Origins of North America introductions. Environmental Entomology 34: 504-510.

Lall, A.B., and J.E. Lloyd. 1989. Spectral sensitivity of the compound eyes in two day-active fireflies (Coleoptera: Lampyridae: *Lucidota).* Journal of Comparative Physiology A 166: 257-260.

Lall, A.B., E.T. Lord, and C.O. Trouth. 1982. Vision in the firefly *Photuris lucicrescens* (Coleoptera: Lampyridae): spectral sensitivity and selective adaptation in the compound eye. Journal of Comparative Physiology A 147: 192-200.

Mason, P.G., T. Baute, O. Olfert, and M. Roy. 2004. Cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae) in Ontario and Quebec. Journal of Entomological Science Ont. 134: 107–113.

McCaffrey, J. P. 1992. Review of the U.S. canola pest complex: cabbage seedpod weevil, pp. 140-143. *In* Proceedings, 1992 U.S. Canola Conference, 5-6 March 1992. Ameri-Can Pedigreed Seed Company, Memphis, TN.

McCaffrey, J.P., B.L. Harmon, J. Brown, A.P. Brown, and J.B. Davis. 1999. Assessment of *Sinapis alba*, *Brassica napus* and *S. alba* x *B. napus* hybrids for resistance to cabbage seedpod weevil, *Ceutorhynchus assimilis* (Coleoptera: Curculionidae). Journal of Agricultural Science 132: 289–295.

McLeod, J. H. 1962. Cabbage seedpod weevil - *Ceutorhynchus assimilis* (Payk.) Curculionidae, pp. 5-6. *In* J. H. McLeod, B. M. McGugan, and H. C. Coppel (eds.). A review of the biological control attempts against insects and weeds in Canada. Commonwealth Institute of Biological Control, Trinidad

Menzel, R. and M. Blakers. 1976. Colour receptors in the bee eye morphology and spectral sensitivity. Journal of Comparative Physiology A 108: 11-33.

Mischke, U. 1981. Spektrale Eigenschaften des visuellen Systems von *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Zoologica Beiträge 27: 319-334.

Moericke, V. 1952. Farben als Landereize für geflügelten Blattläusen (Aphidoidea). Zeitschrift für Naturforschung 7: 304–324.

Moericke, V. 1969. Host plant specific colour behaviour by *Hyalopterus pruni* (Aphididae). Entomologia Experimentalis et Applicata 13: 524- 534.

Möller, R. 2002. Insects could exploit UV-green contrast for landmark navigation. Journal of Theoretical Biology 214: 619-631.

Pimentel, H.A., J. Korer, C. Nufio**,** and D. Papaj. 1998. Role of colour and shape stimuli in host-enhanced oogenesis in the walnut fly *Rhagoletis juglandis*. Physiological Entomology 23: 97–104.

Prokopy, R. J., B.D. Roitberg, and A.L. Averill. 1984. Resource partitioning. pp. 301-330. *In* Bell, W.J., and R.T. Cardé (eds.). Chemical ecology of insects. Chapman and Hall, London.

SAS Institute (2005) SAS Version 9.1. SAS Institute Inc. Cary, North Carolina.

Šedivý, J., and J. Vašák. 2002. Differences in flight activity of pests on winter and spring oilseed rape. Plant Protection Science 38: 138-144.

Sivinski, J. 1990. Coloured spherical traps for capture of Caribbean fruit fly *Anastrepha suspensa*. Florida Entomologist 73: 123-128.

Smart, L. E., M.M. Blight, and A.J. Hick. 1997. The effect of visual cues and a mixture of isothiocyanates on trap capture of cabbage seed weevil, *Ceutorhynchus assimilis.* Journal of Chemical Ecology 23: 889-902.

Tansey, J.A., S.D. Noble, L.M. Dosdall, and B.A. Keddie. 2008. Visual cues influence host choices of the cabbage seedpod weevil. Proceedings of the Entomological Society of Alberta, 56th annual meeting. Edmonton, Alberta. 6-8 November, 2008.

Trumbo, S.T., D.W. Borst, and G.E. Robinson. 1995. Rapid elevation of juvenile hormone titre during behavioural assessment of the breeding resources by the burying beetle *Nicrophorus orbicollis.* Journal of Insect Physiology 41: 535–543.

Ulmer, B.J., and L.M. Dosdall. 2006. Spring emergence biology of the cabbage seedpod weevil (Coleoptera: Curculionidae). Annals of the Entomological Society of America 99: 64-69.

Wallin, K.E., and K.E. Raffa. 2004. Feedback between individual host selection behaviour and population dynamics in an eruptive herbivore. Ecological Monographs 74: 101-116.

Wehner, R. 1981. Spatial vision in arthropods, pp. 287-616. *In*: Autrum, H., (ed.). 1981. Handbook of Sensory Physiology, Vol. 7, Sect. 6C. Springer, Berlin/New York.

Yoshioka, H., A. Horisaki, K. Kobayashi, Syafaruddin, S. Niikura, S. Ninomiya, and R. Ohsawa. 2005. Intraspecific variation in the ultraviolet colour proportion of flowers in *Brassica rapa* L. Plant Breeding 124: 551-556.

Chapter 3

Contributions of visual cues to cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae), resistance in novel host genotypes

Introduction

The cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (syn. *C. assimilis* (Paykull)) (Coleoptera: Curculionidae), is damaging to brassicaceous oilseed crops both in Europe and North America (McCaffrey 1992; Buntin et al. 1995; Dosdall et al. 2001). It is native to Europe and was reported in south-western British Columbia in 1931 (McLeod 1962), the Mixed Grassland Ecoregion near Lethbridge, Alberta in 1995 (Cárcamo et al. 2001), western Saskatchewan in 2000 (Dosdall et al. 2002) and in Québec and Ontario 2000-2001 (Brodeur et al. 2001; Mason et al. 2003). Western North American populations were likely introduced from source locations in western or northern Europe, whereas north-eastern North American populations were introduced separately from Scandinavia or Russia (Laffin et al. 2005). *Ceutorhynchus obstrictus* is dispersing north and east from western Canadian populations at approximately 55 km per year and will eventually occupy all regions in western Canadian associated with canola production (Dosdall et al. 2002).

Adult weevils overwinter in the ground below leaf litter (Dmoch 1965; Ulmer and Dosdall 2006), emerge late spring to feed on brassicaceous plants near their overwintering sites before undergoing host-seeking migrations (Bonnemaison 1957). Early season hosts provide resources for ovarian development and aggregation and mating sites (Fox and Dosdall 2003). Oviposition occurs in developing siliques of *Brassica napus* L., *Brassica rapa* L., *Raphanus* spp. and to a lesser extent *Sinapis arvensis* L. (Dmoch 1965). Larvae consume five to six seeds during three instars (Dmoch 1965), chew through pod

walls when mature and drop to the soil where they pupate in earthen cells. In western Canadian spring canola, development times from egg to emergence of adults are typically 31-58 d (Dosdall and Moisey 2004).

Interspecific crosses of *Sinapis alba* L. x *B. napus* has produced several lines demonstrated to be *C. obstrictus*-resistant in field trials and laboratory experiments (Dosdall and Kott 2006). Mechanisms of weevil resistance associated with these lines likely include antixenosis and antibiosis (Kogan and Ortman 1978; Painter 1951); oviposition and larval weights are reduced and larval development times are prolonged when reared on resistant lines (Dosdall and Kott 2006; Chapter 5). Resistance mechanisms may also include variable visual factors that influence attractiveness and host selection. Visual factors may influence choices of 'food' hosts shortly after emergence and 'true' hosts for oviposition.

Yellow is a relatively ubiquitous flower colour, is attractive to many herbivorous insects and pollinators and is considered a means for herbivores and pollinators to discriminate foliage from non-foliage (Prokopy and Owens 1983). Smart et al. (1997) found that conspicuous yellow traps were attractive to *C. obstrictus* but black, white and green traps were not. Host plant flowers are attractive to *C. obstrictus* adults. Removing this colour by removing flowers from otherwise suitable host plants influences their attractiveness. Frearsen et al. (2006) demonstrated a marked reduction in levels of weevil attack on apetalous *B. napus*. However, petals also produce odours (Cook et al. 2005), so the influence of colour on host choices could not be explicitly determined from this study.

Herbivorous insects use both visual and olfactory cues for host location and can demonstrate great selectivity among visual cues (Prokopy and Owens 1983). Host location has been suggested to follow a stepwise process that incorporates directed flight in response to olfactory cues, visual responses to reflected spectral properties of potential host plants at relatively close range and chemical and tactile cues at intimate ranges (Kennedy 1965). Directed flight in response to visual cues has also been proposed (Moericke 1952). At relatively close ranges, spectral quality of potential host plants is the predominant cue associated with detection and alightment in many herbivorous insects (Prokopy and Owens 1983). Hausmann et al. (2004) found an affinity of female *Anthonomus pomorum* L. (Coleoptera: Curculionidae) for ultraviolet (UV), green, and blue transmitted light in laboratory choice tests and concluded that these weevils have a trichromatic visual system and incorporate visual cues for host plant location. *Ceutorhynchus obstrictus* responses to painted traps are strongly influenced by the amounts of light reflected at 580 nm (yellow) and 350 nm (UV) wavelengths and females are more sensitive to these frequencies than males (Chapter 2).

Here I present results of assessments of *C. obstrictus* responses to visual cues associated with Brassicaceae species that are susceptible (*B. napus*) and resistant (*S. alba*) to weevil infestations, and recombinant inbred lines from *S. alba* x *B. napus*. Differences in floral and foliar reflectance properties among genotypes and the relationship of reflectance properties and weevil responses are assessed. The relationship between reflectance properties, weevil responses and

results of field resistance trials are also determined. These results suggest courses for breeding strategies and deployment of resistant germplasm.

Methods and Materials

Plants and Insects

Seed for test plants was obtained from Dr. Laima S. Kott (University of Guelph, Guelph, ON); genotypes included *B. napus* var. Q2 (hereafter referred to as Q2), *Sinapis alba* var. AC Pennant (hereafter referred to as *S. alba*), and three *C. obstrictus*-resistant and two susceptible lines from *S. alba* x *B. napus* (Accessions 171S, 154S and 276R, 173R and 121R, respectively; 'S' denotes susceptible genotypes, 'R' denotes resistant genotypes). Plants were propagated in a soilless growth medium consisting of a modified Cornell mix based on the recipe of Boodly and Sheldrake (1982) (Agriculture and Agri-Food Canada (2002)) in a greenhouse chamber at the Agriculture and Agri-Food Canada centre, Lethbridge, Alberta and maintained at 16:8 (L:D) and 60% relative humidity. *Ceutorhynchus obstrictus* adults were captured using sweep nets from a commercial *B. napus* field near Lethbridge (49° 41′ 39″ N, 112° 49′ 58.3″ W). Plants were at growth stage 4.3 (many flowers open, lower pods elongating) (Harper and Berkenkamp 1975) when tested; this stage is most sensitive to *C. obstrictus* oviposition (Dosdall and Moisey 2004). *Ceutorhynchus obstrictus* adults were captured using sweep nets from a commercial *B. napus* field near Lethbridge, Alberta (49° 41′ 39″ N, 112° 49′ 58.3″ W) in late May, late June and mid-August 2007; weevils were maintained on potted, flowering *B. napus* var. Q2 in mesh cages in

the laboratory at 12:12 (L:D) and introduced to experiments within two weeks of capture.

Floral and foliar reflectance properties

Reflectance properties of genotypes (flower petals and foliage) were assessed using a dual-beam spectrophotometer operating between 250 and 700 nm (Cary 5G UV-Vis-NIR, Varian, Inc., Mississauga, ON). Petals (collected approximately 4 days after anthesis) were masked with black plastic film with a 4 mm diameter aperture to present a constant sample area to the instrument. This technique was found by Noble and Crowe (2007) to be effective for negating problems associated with samples smaller than the area of the illuminating beam. Reflectance was assessed at 1 nm increments and corrected to absolute diffuse reflectance by a 99% Spectralon reflectance standard (Labsphere, North Sutton, NH). Flower petals and foliage were randomly selected from at least four plants; 4 to 11 replicate petals and four replicate lower leaves were tested from each genotype.

Floral and foliar reflectance was compared using analysis of variance (proc MIXED). All factors were considered fixed. Differences between genotypes were assessed with the LSMEANS statement using the Tukey-Kramer adjustment in proc MIXED.

Bioassay: assessment of *C. obstrictus* visual responses to genotypes

Attractiveness of test plants to weevils was assessed using a plastic Y-maze (Figure 3.1). The body of the apparatus consisted of a 28 x 24 cm black acrylonitrile butadiene styrene T-joint. Colourless, semitransparent plastic tubes (23 cm-long; 10 cm-diameter) were mounted to the lateral openings of the apparatus; these were capped with a clear cellophane film (thickness < 0.2 mm) so as to minimize potential influences of olfactory cues associated with test plants. A 0.2 mm thickness of cellophane allows 89% transmission of 275 nm (Johnson 1934). A flowering plant was set directly next to one capped end of the Y-maze. A comparison of responses of individual weevils and groups to flowering, whole Q2 in olfactometer tests indicated no significant differences (Chapter 4). Groups of 20 randomly selected, mixed sex *C. obstrictus* were introduced to the apparatus and allowed 20 min to acclimate and move to the blank or test plant side. Similar numbers of males and females were tested: 395 of 827 (48%) *C. obstrictus* tested were male, 432 of 827 (52%) were female. Each genotype was replicated four times with different plants of comparable size. Replicates of genotypes were tested in random order over the course of the experiment and sides of the Y-maze were alternated for successive runs. This experiment was conducted outdoors under natural light. The experiment was conducted in late June 2008 with specimens of the overwintered spring generation of *C. obstrictus* and in mid-August 2008 with specimens of the newly emerged fall-generation. Runs were restricted to 10:00 – 14:00 h on bright, sunny days and with temperatures ranging

from 20 to 24^{o}C. Frequency-specific photon flux density associated with each test was not assessed.

Proportions of *C. obstrictus* associated with the blank or test sides of the Y-maze and proportions males or females associated with the blank or test sides of the Y-maze were compared by analysis with binomial generalized estimating equations (proc GENMOD). Pair-wise comparisons for genotype and generation of the proportion of total *C. obstrictus*, males and females were made using a Wald chi-square test (LS MEANS statement with 'diff' option in proc GENMOD).

Relating *C. obstrictus* responses to floral and foliar reflectance

Assessments of relationships between floral and foliar reflective properties and proportions of male or female *C. obstrictus* associated with the blank or test sides of the Y-maze were conducted using multiple regression stepwise procedure analysis (proc REG with the Selection = Stepwise option). Models were developed to represent the responses of male and female *C. obstrictus* to amounts of ultraviolet (350 nm), blue (450 nm), green (550 nm) and yellow (580 nm) light reflected from flowers and foliage. These frequencies will hereafter be referred to as *UV*, *B*, *G*, and *Y*, respectively. Tested factors also included variables created to represent interactions between main effects and quadratic functions associated with main effects. Interactions between main effects will be referred to as *UVG*, the interaction between *UV* and *G*; *UVY*, the interaction between *UV* and *Y*; and *BG*, the interaction between *B* and *G*. All possible combinations of main effects

were tested. Quadratic functions associated with *UV*, *B*, *G* and *Y* will hereafter be referred to as UV^2, B^2, G^2 and Y^2.

Relating *C. obstrictus* resistance to floral and foliar reflectance

Assessments of relationships between weevil infestation scores associated with each genotype and reflectance properties were also conducted using a similar multiple regression stepwise procedure analysis. Weevil infestation scores were calculated as the mean numbers of weevil larvae emergence holes per pod associated with each genotype from replicated field resistance trials near Lethbridge, AB in 2004 and 2005 (Shaw 2008).

Relating *C. obstrictus* resistance to responses

Assessments of relationships between weevil infestation scores associated with each genotype and Y-maze responses were conducted using a linear regression analysis (SAS proc REG).

All analyses were conducted with SAS version 9.1 (SAS Institute 2005).

Results

Floral reflectance

Floral reflectance properties of genotypes are presented in Figure 3.2. Significant differences in 580 nm reflectance were detected among genotypes ($F = 3.84$; df = 6, 29; $P = 0.0062$). Pair-wise comparisons indicated that Q2, 171S, 154S, 173 R,

276R and *S. alba* reflected similar amounts of 580 nm ($P > 0.05$ for all comparisons); Q2 and 154S reflected more 580 nm than 121R ($P < 0.05$ for both comparisons). Significant differences in 550 nm reflectance were also detected ($F = 4.13$; df = 6, 29; $P = 0.0011$). Pair-wise comparisons indicated that Q2, 171S, 154S, 173 R, 276R and *S. alba* reflected similar amounts of 550 nm ($P > 0.05$ for all comparisons); Q2, 171S, 154S, 173R and 276R reflected more 550 nm than 121R ($P < 0.05$ for all comparisons). Genotypes also reflected significantly different amounts of 450 nm light ($F = 5.10$; df = 6, 29; $P = 0.0041$). Pair-wise comparisons indicated that Q2, 171S, 173R, *S. alba* and 276R flowers reflected similar amounts of 450 nm ($P > 0.05$ for all comparisons). Q2, 171S and 276R reflected significantly more 450 nm than 121R ($P < 0.05$ for all comparisons). Comparisons of reflectance of 350 nm from genotypes also indicated differences ($F = 16.05$; df = 6, 29; $P < 0.0001$). *Sinapis alba* reflected significantly more 350 nm than any other genotype ($P < 0.05$ for all comparisons). Q2 and 171S reflected similar amounts of 350 nm ($t = 0.37$; df = 29; $P = 0.9998$) and significantly more than 121R, 154S, 173R, or 276R ($P < 0.05$ for all comparisons).

Foliar reflectance

Foliar reflectance properties of genotypes are presented in Figure 3.3. Significant differences among genotypes were detected in the proportion of 580 nm ($F = 4.16$; df = 6, 22; $P = 0.0060$). Pair-wise comparison indicated that 276R reflected a significantly more 580 nm than 154S, 173R, or *S. alba* ($P < 0.05$ for all comparisons). There were also significant differences in 550 nm reflectance

among genotypes (F = 3.13; df = 6, 22; P = 0.0225). Foliage of 276R reflected significantly more 550 nm than 173R or 154S (P <0.05 for both comparisons). Comparisons of amounts of 450 nm reflected among genotypes also indicated differences (F = 8.93; df = 6, 22; P < 0.0001). *Sinapis alba* reflected significantly less 450 nm that all other genotypes (P < 0.01 for all comparisons). Reflectance at 350 nm reflectance also differed among genotypes (F = 9.75; df = 6, 22; P < 0.0001). *Sinapis alba* reflected significantly less 350 nm that all other genotypes (P < 0.001 for all comparisons).

***Ceutorhynchus obstrictus* responses to genotypes**

Significant differences in responses of weevils to genotypes were detected (χ^2 = 68.21; df = 6; P < 0.0001). Similar proportions of weevils were attracted to Q2 and 171S (χ^2= 0.46; df = 1; P = 0.4993). These were more attractive than 154 S, 173R, 276R, 121R or *S. alba* (P < 0.05 for all comparisons). No significant differences were detected among 154S, 173R, 121R or 276R (P > 0.05 for all comparisons). Of these, only 154S attracted greater proportions than *S. alba* (χ^2 = 7.98; df = 1; P = 0.0047). Although there were no significant differences between responses of spring and fall generations of *C. obstrictus* to these genotypes (χ^2 = 1.06; df = 1; P = 0.3303), a significant interaction of generation and genotype was detected (χ^2= 19.21; df = 6; P = 0.0038). Significantly higher proportions of spring than fall generation weevils were attracted to 154S (χ^2 = 18.15; df = 1; P < 0.0001).

Significant differences in the proportions of males responding to these genotypes were apparent (χ^2 = 22.28; *df* = 6; *P* = 0.0011). Similar proportions were attracted to 171S and Q2; Q2, 154S and 276R; and 154S, 276R, 173R, 121R and *S. alba* (*P* > 0.05 for all comparisons) (Figure 3.4). Although a significant generation effect was not apparent (χ^2= 2.78; *df* = 1; *P* = 0.0957), a significant interaction of generation and genotype (χ^2 = 15.76; *df* = 6; *P* = 0.0151) indicated differences in the responses of spring and fall males to visual cues associated with these genotypes. A significantly higher proportion of spring generation than fall generation males was attracted to 154S (χ^2 = 14.33; *df* = 1; *P* = 0.0002). No other differences in responses to genotypes by spring and fall generation *C. obstrictus* were apparent (*P* > 0.05 for all comparisons). Blank sides and 121R attracted similar proportions of males (χ^2 = 0.93; *df* = 1; *P* = 0.3338).

Significant differences in the proportions of female *C. obstrictus* attracted to genotypes were apparent (χ^2 = 52.74; *df* = 6; *P* < 0.0001). Greatest (albeit not significantly) proportions were attracted to Q2. Similar proportions were attracted to Q2 and 171S (χ^2 = 0.57; *df* = 1; *P* = 0.4492); similar proportions were also attracted to 171S and 154S (χ^2 = 2.90; *df* = 1; *P* = 0.0887). Similar proportions of female weevils were attracted to 154S, 121R, and 173R (*P* > 0.05 for all comparisons), and similar proportions were attracted to 121R, 173R, 276R, and *S. alba* (*P* > 0.05 for all comparisons) (Figure 3.4). There were no significant differences between responses of spring and fall generation female *C. obstrictus* to these genotypes (χ^2= 0.08; *df* = 1; *P* = 0.7786), and no significant interaction of

generation and genotype (χ^2 = 8.58; *df* = 6; *P* = 0.1986). Blank sides, *S. alba* and 276R attracted similar proportions of females (*P* > 0.05 for all comparisons).

Relating *Ceutorhynchus obstrictus* responses to floral and foliar reflectance

The multiple regression stepwise procedure indicated that female responses (the proportions of females responding to test plants) to flowers could be attributed to the interaction of yellow and UV, as well as the quadratic function associated with UV reflected from test plants. The model developed to describe this relationship is: $x = -0.0632 - 0.0003\ UV^2 + 0.0003\ UVY$ ($F_{3, 53} = 18.86$; $R^2 = 0.4158$; $P < 0.0001$) (Figure 3.5). This model suggests increased female responses with increased floral 580 nm in the presence of 350 nm reflection. Greatest modeled weevil scores are associated with proportions of floral 580 nm reflectance = 100% and proportions of floral 350 nm reflection of approximately 50%. Decreased weevil scores are associated with levels of 350 nm reflectance above and below these values. No linear relationships of male responses to floral yellow, green, blue or UV reflectance were detected (*P* > 0.05 for all assessments). No linear or quadratic relationships between female or male responses and proportions of foliar yellow, green, blue or UV or interactions among these frequencies was detected (*P* > 0.05 for all assessments). Female responses increased linearly with greater amounts of 550 nm reflected by flowers relative to foliage ($F_{1, 54} = 24.69$; $R^2 = 0.3137$; $P < 0.0001$). No relationships between proportions of yellow, green, blue or UV reflected by flowers relative to foliage were detected for males (*P* > 0.05 for all assessments).

Relating *Ceutorhynchus obstrictus* resistance to floral and foliar reflectance

The multiple regression stepwise procedure indicated that weevil infestation scores as measured by larval exit-holes in mature siliques were related to the interaction of yellow and UV and the quadratic function associated with UV reflected from test plant flowers ($F_{3,53} = 95.46$; $R^2 = 0.7827$; $P < 0.0001$). The model developed to describe this relationship is: $x = -0.3906 - 0.0007\ UV^2 + 0.0006\ UVY$ (Figure 3.6). This model suggests increased weevil infestation with increased floral 580 nm in the presence of 350 nm reflection. Greatest modeled weevil scores are associated with proportions of floral 580 nm reflectance = 100% and proportions of floral 350 nm reflection of ca. 45%. Decreased weevil scores are associated with levels of 350 nm reflectance above and below these values. No relationship of foliar reflectance and weevil scores was detected ($P > 0.05$ for all assessments). However, a significant linear relationship between increasing weevil scores and increasing ratios of green reflected by flowers relative to foliage was apparent ($F_{1, 54} = 48.52$; $R^2 = 0.4733$; $P < 0.0001$).

Relating *Ceutorhynchus obstrictus* visual responses to resistance

Significant linear relationships in Y-maze assessments between weevil scores associated with each of the tested genotypes and responses of combined male and female *C. obstrictus* were apparent ($F_{1, 54} = 39.50$; $R^2 = 0.4224$; $P < 0.0001$). The model representing the relationship of weevil scores (*WS*) (the mean numbers of larval exit-holes per genotype from Shaw (2008)) and *C. obstrictus* responses is: x

= 0.40702 + 0.69349 (*WS*). A significant linear relationship between female responses to genotypes in Y-maze assessments and weevil scores was also apparent ($F_{1, 54} = 41.40$; $R^2 = 0.4340$; $P < 0.0001$). The model representing the relationship of weevil scores (*WS*) and female *C. obstrictus* response is: x = 0.18520 + 0.52976 (*WS*). The relationship of male responses and weevil scores approached significance ($F_{1, 54} = 3.91$; $R^2 = 0.0675$; $P = 0.0532$).

Discussion

I found differences in reflectance properties among genotypes associated with frequencies of light known to be important to many anthophilous insects. Most insect visual systems examined to date have near-ultraviolet receptors with response maxima near 350 nm ($\lambda_{max} \approx 350$ nm), blue receptors ($\lambda_{max} \approx 450$ nm), and green receptors ($\lambda_{max} \approx 550$ nm) (Briscoe and Chittka 2001). Chittka (1996) suggested that this set of UV, blue, and green photoreceptors is ancestral to Insecta and that some lineages have since lost or added receptors as a result of different selection pressures. Chittka (1996) found that a modeled system of spectral receptor types with λ_{max} = 340, 440 and 560 nm, approximating those typical of Apidae (Hymenoptera), was near optimal for distinguishing floral colours from background, flower colours among sympatric plant species and discrimination of green foliage. Many herbivorous insects also respond to yellow (560-590 nm) as a 'supernormal' foliage-type stimulus (Prokopy and Owens 1983), causing exaggerated responses. Prokopy (1972) suggested that the great attraction of many herbivorous insects to yellow may be a function of affecting

insect visual receptors with response maxima near 550 nm with greater intensity than that typically emitted from foliage. Trichromatic vision in *C. obstrictus* with λ_{max} values near those described for bees (Hymenoptera: Apidae) has been detected (Chapter 2).

I found that differences in floral yellow, green, blue and UV reflectance properties were significant. The susceptible genotypes Q2 and 154S reflected more yellow than the resistant genotype 121R, suggesting a relationship of greater attractiveness with increased yellow reflectance. Genotypic differences in floral UV reflectance were somewhat surprising; the susceptible 171S and Q2 reflected significantly more UV than any of the resistant introgressed genotypes, suggesting a relationship of greater UV reflectance with attractiveness. However, the highly resistant *S. alba* reflected significantly more UV than any other genotype. These results indicate that *C. obstrictus* is attracted to moderate amounts of UV reflection and that response decreases beyond a threshold UV level. These results are consistent with those of Chapter 2.

Visual responses of *C. obstrictus* to these genotypes differed. The susceptible genotypes, 171S, 154S and Q2 were significantly more attractive to female *C. obstrictus* than the resistant 121R, 173R, 276R or *S. alba.* The genotypes 276R and *S. alba* were not significantly more attractive to females than blank sides. Males were less discriminating. Susceptible genotypes Q2 and 154S were the most attractive to males although not significantly more than the resistant genotypes 276R and 173R. There were no significant differences in attractiveness to males of the susceptible genotype 171S and highly resistant *S.*

alba. Sivinski (1990) determined that female *Anastrepha suspense* (Loew) (Diptera: Tephritidae) showed strong preferences among five colours of spherical fruit models but that males were less discriminating. Female *Anthonomus pomorum* L. (Coleoptera: Curculionidae), were more responsive to UV, green, and blue light than males (Hausmann et al. 2004), a response attributed to greater affinity of females to host plant-associated visual cues.

In addition to differences in the responses of both male and female *C. obstrictus* to visual cues associated with these genotypes, spring (overwintered) males were more attracted to 154S than fall generation males. Newly emerged spring generation adults feed on several brassicaceous plant species near their overwintering sites ('food' hosts) for a few days before undergoing oviposition host-seeking migrations (Bonnemaison 1957). Reduced responses of males from *C. obstrictus* field populations to visual cues with time have been detected (Chapter 2). Greater responses of spring generation males to 154S are consistent with decreasing male responses with time. I have observed weevils hovering over and briefly visiting yellow objects in the spring (J. Tansey, unpublished data); although the sexes of these insects were not determined, visual cues are likely very important to early season host location for both sexes and discrimination among attractive 'food' host visual cues is likely low.

Importantly, weevils of both the spring and fall generations were held at 12:12 (L;D) in the laboratory before being introduced to experiments. Plasticity in pheromone responses of male *Pseudaletia unipuncta* (Haworth), (Lepidoptera: Noctuidae) is influenced by photoperiod (Dumont and McNeil 1992). Behavioural

response to pheromone by reproductively inactive male *Caloptilia fraxinella* (Ely) (Lepidoptera: Gracillariidae) can be increased with methoprene (JHA) treatment (Lemmen and Evenden 2009). Juvenile hormone levels and responses to food odours are influenced by circadian rhythms in *Drosophila melanogaster* L. (Diptera: Drosophilidae) (Krishnan et al. 1999). In the cotton boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae), juvenile hormone levels are greater in reproductive females (Taub-Montemayor et al. 1997). Acclimation of *C. obstrictus* to a 12:121 (L:D) schedule from the 16:8 (L:D) at time of capture may have caused juvenile hormone levels to rise and increased responses of fall generation weevils to host cues.

Chittka (1996) proposed that hymenopterans with trichromatic vision are capable of differentiating flowers with similar reflectance properties. Although significant differences in the amounts of floral yellow, green, blue and UV were detected among genotypes, the greatest contributions to relationships of female responses and reflectance properties were associated with interactions of yellow and UV and a quadratic response to UV. The attractiveness of yellow was greatly influenced by the amounts of UV reflected; increased attractiveness was associated with increased yellow and increased UV to a threshold UV proportion. Proportions greater and less than this amount resulted in reduced attractiveness. This peak in attractiveness associated with amounts of yellow and UV reflection suggest the potential for great specificity of female responses to potential host plants with variable reflectance properties. Males were less discriminating and,

although an effect of yellow and UV interaction on males has been detected (Chapter 2), this interaction was not found to be influential in this study.

Responses to visual cues were related to weevil infestation scores in mature pods. This relationship was limited to females, again suggesting greater discrimination of visual cues associated with host selection by females than males. Weevil scores are a partial measure of oviposition preference. Early-season oviposition is generally associated with lower, early-maturing pods (Cárcamo et al. 2001; Dosdall and Moisey 2004). Large numbers of flowers are also present on upper raceme branches at this stage of plant development and likely influence attractiveness of potential host plants to females. Oviposition occurs in developing siliques of relatively few Brassicaceous species: *B. napus, B. rapa, Raphanus* spp. and to a lesser extent *S. arvensis* (Dmoch 1965). Although exhaustive spectrographic analysis of these species has not been conducted, results of this study suggest that they may share some visual features. Dosdall and Moisey (2004) also found that in a mixed stand of *S. arvensis* L., *Thlaspi arvense* L., and *Descurania sophia* (L.) Webb, weevil numbers were significantly highest on *S. arvensis*, the only species in this list with yellow flowers.

Weevil infestation scores were also related to the amounts of floral yellow and UV and the contrast between floral and foliar green. The model developed to explain this relationship was similar to the model developed to explain female *C. obstrictus* responses to floral reflectance properties. A significant relationship between weevil scores and visual cues incorporating an interaction of yellow and UV and a quadratic relationship with UV was again detected. Effects of yellow

reflectance in the presence of UV, a peak in weevil scores associated with moderate UV reflectance were apparent. Models associated with female responses to reflectance patterns and weevil infestation scores to reflectance patterns were similar. The relationship of weevil scores and female *C. obstrictus* responses to visual cues associated with test genotypes was significant. These results indicate the great importance of floral visual cues to weevil oviposition choices. These results also suggest that *C. obstrictus* females are capable of discrimination among flowers that are highly similar.

Visually, flowers can differ in size, shape, symmetry, depth, spatial frequency, spatial orientation and height; these traits can influence anthophilous insects (Dafni et al. 1997). These characters were similar among the genotypes tested, both in field resistance trials and assessments of *C. obstrictus* responses to visual cues. Consequently, differences in response and at least some of the difference demonstrated in resistance trials can be attributed to differences in floral spectral reflectance properties of these genotypes. Yoshioka et al. (2005) found that the proportion of UV reflected and absorbed from *B. rapa* flowers was genotypically stable and attributed differences in visitation by anthophilous insects to these differences.

Despite differences in reflectance properties among the genotypes evaluated in the current study, weevil infestation scores cannot be attributed exclusively to oviposition choices based on differences in reflectance. Dosdall and Moisey (2004) found that *C. obstrictus* adults were found only on members of the Brassicaceae, even though some sympatric non-brassicaceous species that

flowered simultaneously also produce yellow flowers. The amounts of yellow and UV reflected from these flowers have not yet been examined. Differences in the responses to *B. napus*, *S. alba* and several *S. alba* x *B. napus* genotypes have been demonstrated in an olfactometer and in feeding and oviposition preference assessments in the absence of floral visual cues (Chapters 4, 5). The link between visual and chemical factors is as yet unknown for these *S. alba* x *B. napus* genotypes. Strauss et al. (2004) detected differences in the levels of induced glucosinolates among *Raphanus sativus* L. (Brassicaceae) petal colour variants (yellow, white; anthocyanin-containing bronze and pink). Strauss et al. (2004) suggested that petal colour and glucosinolate induction were likely associated with pleiotropic effects between petal colour and defence loci.

An altered glucosinolate profile with reduced foliar aliphatic glucosinolates and increased indolyl glucosinolates was detected in an *Arabidopsis* mutant with a defective gene encoding *CYP83A1* (a cytochrome P450 highly similar to *CYP83B1*, an enzyme associated with glucosinolate biosynthesis) (Hemm et al. 2003). This mutant also demonstrated altered phenylpropanoid pathway products (Hemm et al. 2003). Because anthocyanin (yellow) and colourless flavonoid (UV absorbing) pigments are derived from phenylpropanoid metabolism (Schwinn and Davies 2004; Kitamura 2006), this gene can influence expression of aliphatic and indolyl glucosinolates and alter floral reflective properties.

Many insects respond to objects based on a specific predetermined template of stimuli and ignore stimuli that do not conform (Laughlin 1981;

Wehner 1981). Results of this study suggest that the *C. obstrictus* template incorporates a well-defined visual component. These results also suggest that plants that conform more closely to the visual component of this template may be more 'apparent'. Apparency theory (Feeny 1976; Rhoades and Cates 1976) predicts that less apparent plants should be more heavily defended by qualitative toxins; more apparent plants are protected by quantitative defences. Antibiosis resistance has been demonstrated for *S. alba* and several resistant *S. alba* x *B. napus* genotypes and is associated with reduced larval weights and prolonged development times (Dosdall and Kott 2006, Chapter 5). Chemical analysis of these genotypes indicates differences in glucosinolate profiles among resistant and susceptible lines. Shaw (2008) examined upper cauline leaves; HPLC analysis detected consistent differences in the heights of one peak among cauline leaves of resistant and susceptible *S. alba* x *B. napus* lines. The peak was associated with an as yet uncharacterised glucosinolate (retention time 21.4 ± 0.03 min); peak height was inversely correlated with weevil infestation scores and on average, 3.5 times larger in resistant than susceptible lines (Shaw 2008). The role of this glucosinolate as a *C. obstrictus* antifeedant or mechanism of antibiosis resistance is unknown. Shaw (2008) also detected differences among resistant and susceptible *S. alba* x *B. napus* lines in the amounts of another as yet uncharacterised glucosinolate (retention time 20.5 ± 0.01 min) in seeds of immature pods; peak heights were correlated with weevil infestation scores and were, on average, 3.5 times greater in susceptible than resistant lines (Shaw 2008).

Given the antibiotic qualities of resistant genotypes and *S. alba* (Chapter 5), floral visual cues are apparently indicative of host plant quality and responses of female *C. obstrictus* are likely adaptive. Also, greater numbers of progeny would be associated with genotypes that are less inhibitory to larval growth and development. Given the prevalence of susceptible *B. napus* in the Lethbridge region, the vast majority of *C. obstrictus* sampled for this study were certainly associated with this host plant and would likely express an affinity for it. This suggests that differences in responses may also be attributed to behaviour analogous to flower constancy in bees, where individuals ignore flowers of non-preferred species, even if these are more rewarding (Hill et al. 1997).

Importantly, apparency theory also predicts reduced colonization of less apparent (resistant) lines (Bernal et al. 2004). Although initial resistance of novel genotypes in the field may be enhanced by being less apparent, herbivore search efficiency evolves after colonization of novel hosts regardless of apparency (Parmesan 1991). All of the genotypes tested can support larval development and so facilitate modification of search efficiency. Also, toxic cultivars are not evolutionarily sustainable in monoculture (Bernal et al. 2004). Therefore I suggest introduction of resistant genotypes in a manner that incorporates susceptible refugia as a means of reducing the potential evolution of search efficiency and selective pressures associated with antibiosis.

Figures

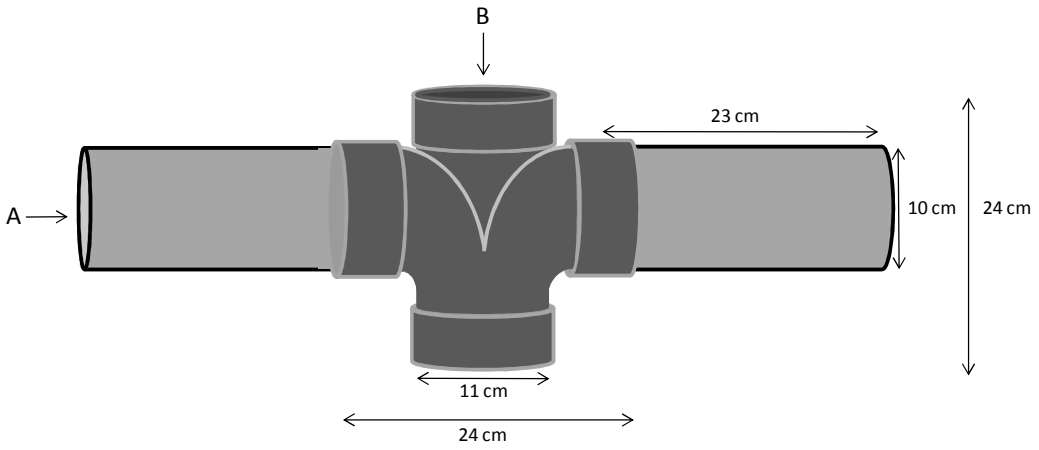

Figure 3.1. Y-maze used to assess visual responses of *C. obstrictus* to test plants. Ends of translucent arms of the apparatus were capped with cellophane (A). Flowers of test plants were placed next to the capped opening of one arm. Weevils were introduced through the opening in the top (B); this end was then capped. Attraction to a test plant or a blank side was recorded after 20 min.

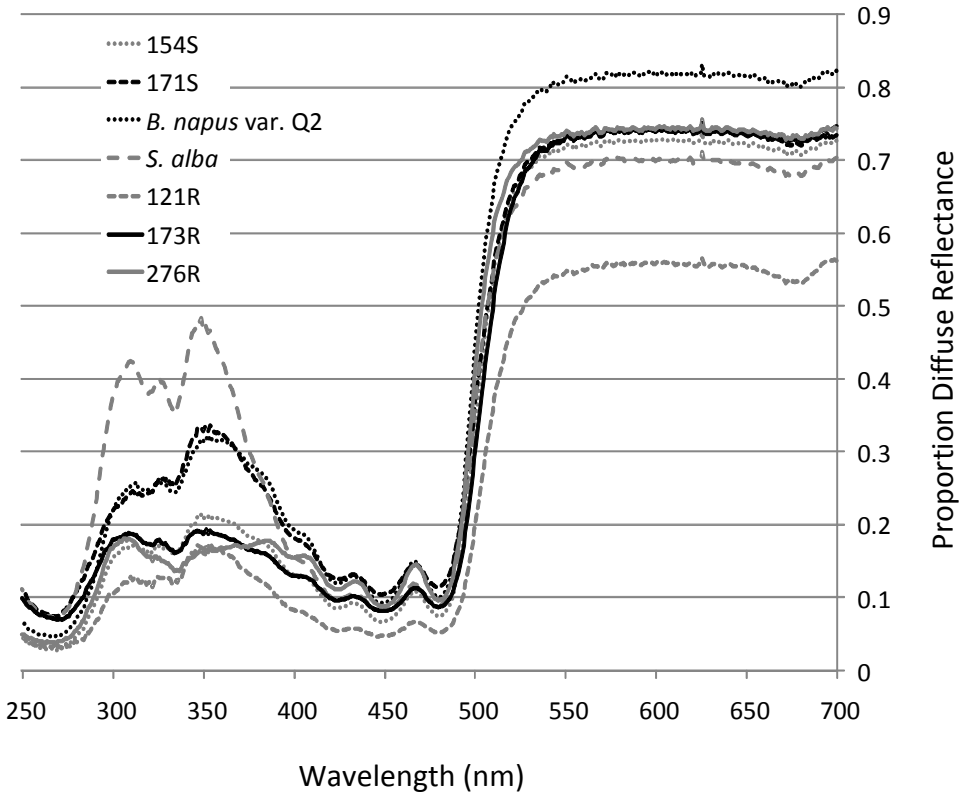

Figure 3.2. Mean floral reflectance properties of genotypes evaluated including *Brassica napus* var. Q2, *Sinapis alba,* and *S. alba* x *B. napus* lines.

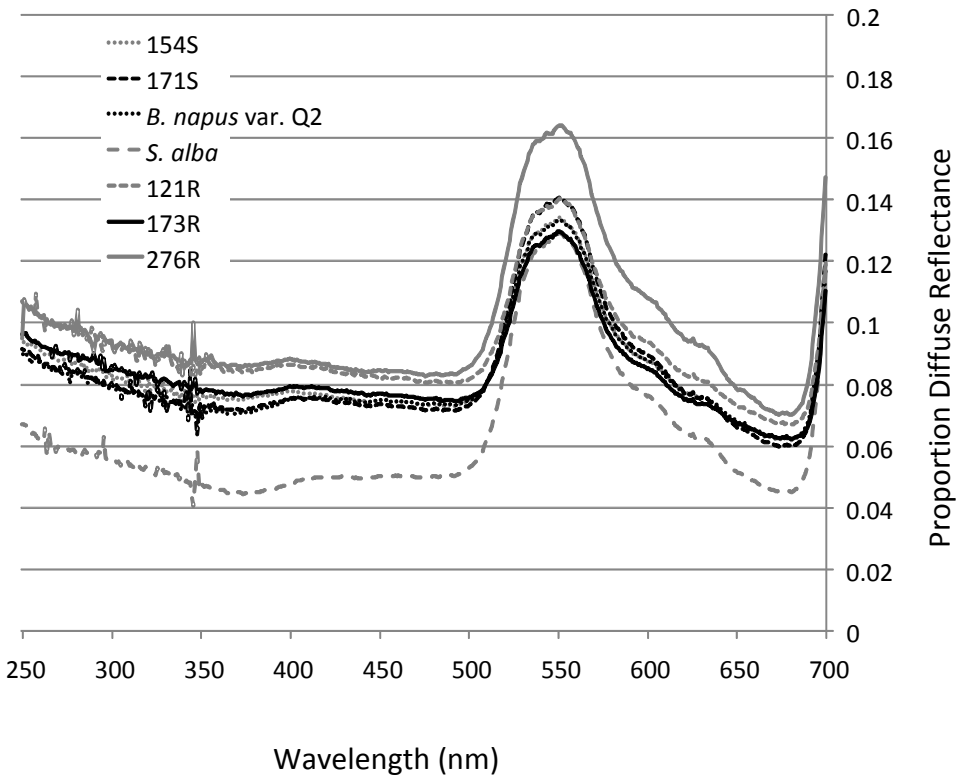

Figure 3.3. Mean foliar reflectance properties of test genotypes evaluated including *Brassica napus* var. Q2, *Sinapis alba*, and *S. alba* x *B. napus* lines.

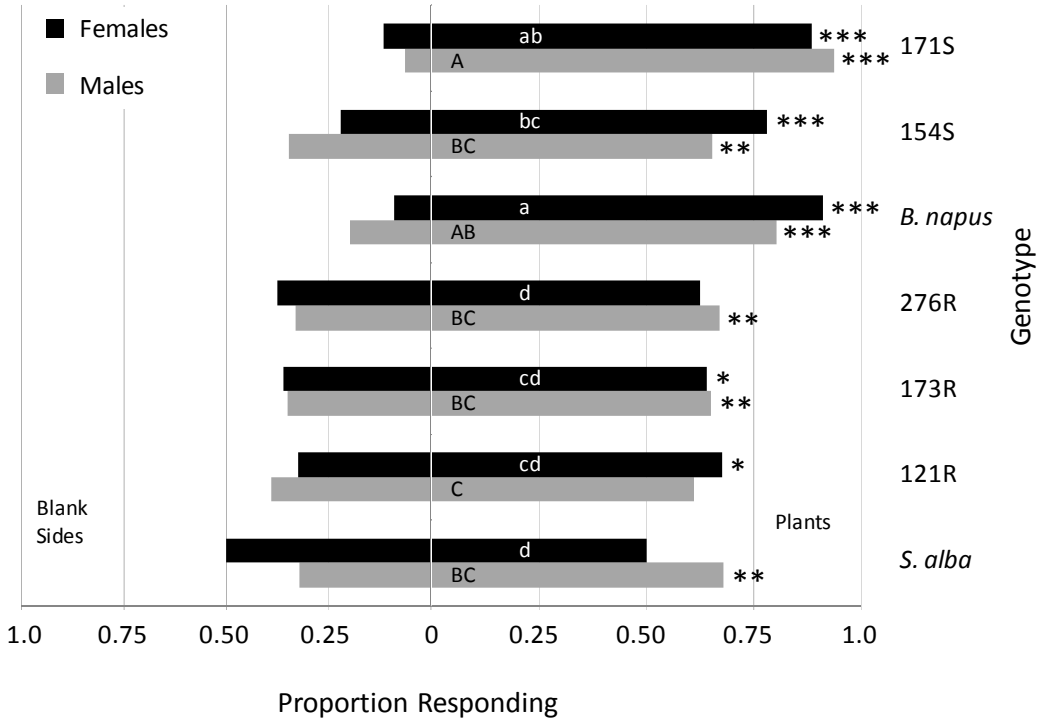

Figure 3.4. Responses of *C. obstrictus* to flowering plants of several *Sinapis alba* x *Brassica napus* genotypes, *S. alba* var. AC Pennant and *B. napus* var. Q2 versus blank sides in a visual Y-maze. 'S' indicates susceptible genotypes; 'R' resistant. Different letters on bars indicate significant differences in *C. obstrictus* responses by a Wald's χ^2test: $\alpha = 0.05$. * Significantly higher proportion than blank sides by a Wald's χ^2 test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. There are no significant differences ($P < 0.05$) by Wald chi-square tests among like-lettered groups.

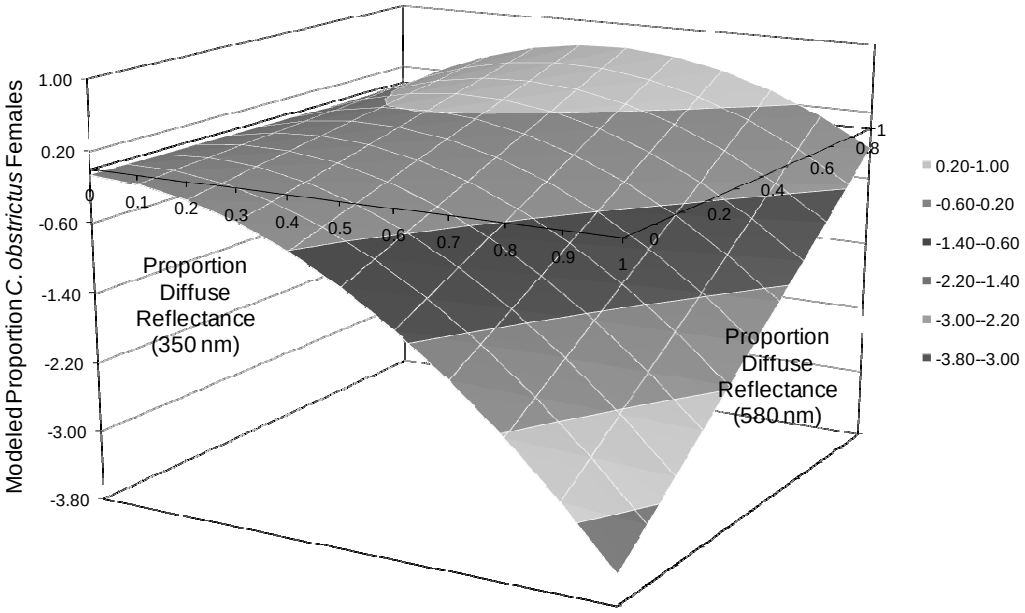

Figure 3.5. Surface plot representing modeled relationship of proportions of female *C. obstrictus* responding to 580 and 350 nm reflectance associated with flowers of *S. alba*, *B. napus* and resistant and susceptible *S. alba* x *B. napus* genotypes. Negative modeled responses are assumed to represent non-response.

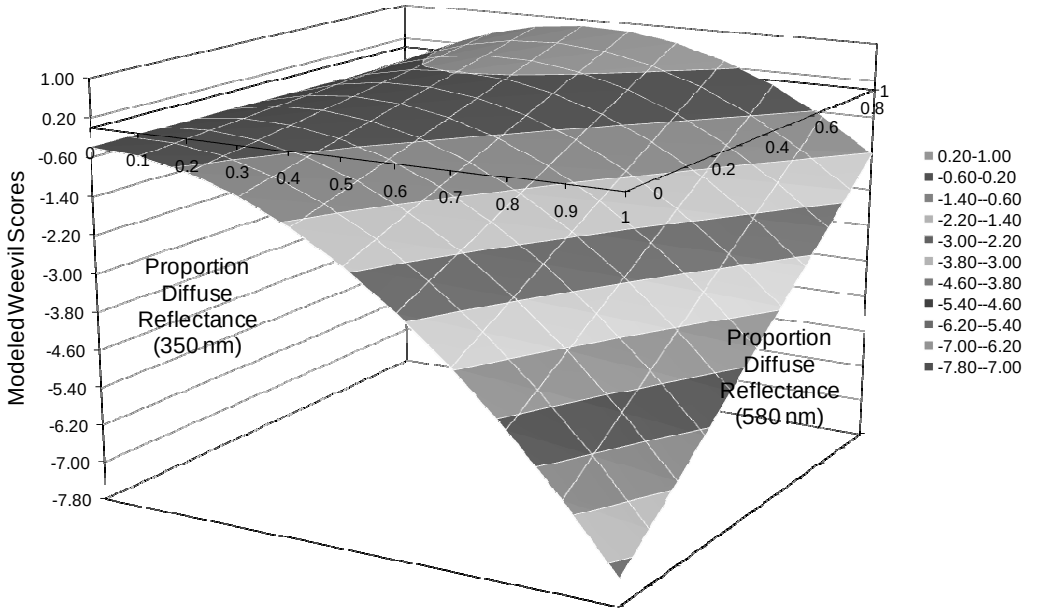

Figure 3.6. Surface plot representing the modeled relationship between 580 and 350 nm reflectance associated with flowers of *S. alba*, *B. napus* and resistant and susceptible *S. alba* x *B. napus* genotypes and weevil resistance scores. Weevil scores indicate the mean numbers of *C. obstrictus* per pod that emerged from resistant and susceptible *S. alba* x *B. napus* genotypes in field trials near Lethbridge, AB in 2004 and 2005. Negative weevil score values are assumed to represent no oviposition associated with non-response.

References

Agriculture and Agri-Food Canada. 2002. Soilless growth medium for plants: preparation. SOP code: 120.0000.00. 6 pp.

Bernal, J.S., J. Prsifka, M. Sétamou, and K.M. Heinz. 2004. Transgenic insecticidal cultivars in integrated pest management: challenges and opportunities. *In*: Koul, O., G.S. Dhaliwal, G.W. Cuperus (eds.). pp. 123-145. Integrated pest management: potential, constraints and challenges. CABI Publishing, Oxon, United Kingdom.

Bonnemaison, L. 1957. Le charançon des siliques (Ceutorhynchus assimilis Payk.) biologie et méthode de lutte. Annales des Épiphyties 4: 387–543.

Boodly, J.W., and R. Sheldrake. 1982. Cornell Peat-Lite Mixes for Commercial Plant Growing. An extension publication of the New York State College of Agriculture and Life Sciences, a statutory college of the state university, at Cornell University, Ithaca, New York.

Briscoe, A.D., and L. Chittka. 2001. The evolution of colour vision in insects. Annual Review of Entomology 46: 471-510.

Brodeur J., L. Leclerc, M. Fournier, and M. Roy. 2001. Cabbage seedpod weevil (Coleoptera: Curculionidae) a new pest of canola in northeastern North America. The Canadian Entomologist 133: 709-711.

Buntin, G. D., J.P. McCaffrey, P.L. Raymer, and J. Romero. 1995. Quality and germination of rapeseed and canola seed damaged by adult cabbage seedpod weevil, *Ceutorhynchus assimilis* (Paykull) [Coleoptera: Curculionidae]. The Canadian Journal of Plant Science 75: 539-541.

Cárcamo, H.A., L. Dosdall, M. Dolinski, O. Olfert, and J.R. Byers. 2001. The cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae) - a review. Journal of the Entomological Society of British Columbia 98: 201-210.

Chittka, L. 1996. Optimal sets of colour receptors and colour opponent systems for coding of natural objects in insect vision. Journal of Theoretical Biology 181: 179-196.

Cook, S.M., J.S. Sandoz, A.P. Martin., D.A. Murray, G.M Poppy, and I.H. Williams. 2005. Could learning of pollen odours by honey bees (*Apis mellifera*) play a role in their foraging behaviour? Physiological Entomology 30: 164-174.

Dafni, A., M. Lehrer, and P. G. Kevan. 1997. Spatial flower parameters and insect spatial vision. Biological Reviews 72: 239-282.

Dmoch, J. 1965. The dynamics of a population of the cabbage seedpod weevil (Ceutorhynchus assimilis Payk.) and the development of winter rape. Part I. Ekologiya Polska. Seria A 13: 249-287.

Dosdall, L. M., D. Moisey, H. Cárcamo, and R. Dunn. 2001. Cabbage seedpod weevil fact sheet. Alberta Agriculture, Food and Rural Development, Edmonton, AB, Canada.

Dosdall, L.M., R.M. Weiss, O. Olfert, and H.A. Cárcamo. 2002. Temporal and geographical distribution patterns of cabbage seedpod weevil (Coleoptera: Curculionidae) in canola. The Canadian Entomologist. 134: 403-418.

Dosdall, L.M., and D.W.A. Moisey. 2004. Developmental biology of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae), in spring canola, *Brassica napus*, in western Canada. Annals of the Entomological Society of America 97: 458-465.

Dosdall, L. M., and L.S. Kott. 2006. Introgression of resistance to cabbage seedpod weevil to canola from yellow mustard. Crop Science 46: 2437-2445.

Dumont, S., and J.N. McNeil. 1992. Responsiveness of *Pseudaletia unipuncta* (Lepidoptera: Noctuidae) males, maintained as adults under different temperature and photoperiodic conditions, to female sex pheromone. Journal of Chemical Ecology 18: 1797- 1807.

Feeny, P. 1976. Plant apparency and chemical defence. Recent Advance in Phytochemistry 10: 1–40.

Fox, A.S., and L.M. Dosdall. 2003. Reproductive biology of *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae) on wild and cultivated Brassicaceae in southern Alberta. Journal of Entomological Science 38: 533-544.

Frearson, D.J.T., A.W. Ferguson, N. Laugier, and I.H. Williams. 2006. The influence of petals on numbers of pests and their parasitoids in oilseed rape. *In* Proceedings of the International Symposium on Integrated Pest Management in Oilseed Rape. April 3-5, 2006. Paulinerkirche, Göttingen, Germany.

Harper, F.R., and B. Berkenkamp.1975. Revised growth-stage key for *Brassica campestris* and *B. napus*. The Canadian Journal of Plant Science 55: 657–658.

Hausmann, C., J. Samietz, and S. Dorn. 2004. Visual orientation of overwintered *Anthonomus pomorum* (Coleoptera: Curculionidae). Environmental Entomology 33: 1410–1415.

Hemm, M.R., M.O. Ruegger, and C. Chapple. 2003. The *Arabidopsis* ref2 mutant is defective in the gene encoding CYP83A1 and shows both phenylpropanoid and glucosinolate phenotypes. Plant Cell 15: 179–194.

Hill, P.S.M., P.H. Wells, and H. Wells. 1997. Spontaneous flower constancy and learning in honey bees as a function of colour. Animal Behavior 54: 615–627

Johnson, B.K. 1934. An ultra-violet microscope for the examination of opaque objects. Journal of Scientific Instruments 11: 384-394.

Kennedy, J. S. 1965. Mechanisms of host plant selection. Annals of Applied Biology 56: 317 – 322.

Krishnan, B., S.E. Dryer, and P.E. Hardin. 1999. Circadian rhythms in olfactory responses of *Drosophila melanogaster*. Nature 400: 375-378.

Kogan, M., and E.F. Ortman. 1978. Antixenosis—A new term proposed to define Painter's 'non preference' modality of resistance. Bulletin of the Entomological Society of America 24: 175–176.

Laffin, R.D., L.M. Dosdall, and F.A.H. Sperling. 2005. Population structure of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera:

Curculionidae). Origins of North America introductions. Environmental Entomology 34: 504-510.

Laughlin, S. 1981. Neural principles in the peripheral visual systems of invertebrates. pp. 133-280. *In* Autrum, H. (ed.). Handbook of Sensory Physiology, Vol. 7, Sect. 6B. Springer, Berlin/New York.

Lemmen, J., and M. Evenden. 2009. Peripheral and behavioral plasticity of pheromone response and its hormonal control in a long-lived moth. The Journal of Experimental Biology 212: 2000-2006.

Mason, P.G., T. Baute, O. Olfert, and M. Roy. 2003. Cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae) in Ontario and Quebec. Journal of the Entomological Society of Ontario 134: 107-113.

McCaffrey, J.P., B.L. Harmon, J. Brown, A.P. Brown, and J.B. Davis. 1999. Assessment of *Sinapis alba*, *Brassica napus* and *S. alba* x *B. napus* hybrids for resistance to cabbage seedpod weevil, *Ceutorhynchus assimilis* (Coleoptera: Curculionidae). Journal of Agricultural Science 132: 289-295.

McCaffrey, J. P. 1992. Review of the U.S. canola pest complex: cabbage seedpod weevil, pp. 140-143. *In* Proceedings, 1992 U.S. Canola Conference, 5-6 March 1992. Ameri-Can Pedigreed Seed Company, Memphis, TN.

McCaffrey, J.P., B.L. Harmon, J. Brown, and J.B. Davis. 2004. Resistance of canola-quality cultivars of yellow mustard, *Sinapis alba* L., to the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham). The Canadian Journal of Plant Sciences 84: 397-399.

McLeod, J. H. 1962. Cabbage seedpod weevil – *Ceutorhynchus assimilis* (Payk.) Curculionidae. *In* J. H. McLeod, B. M. McGugan, and H. C. Coppel (eds.). A review of the biological control attempts against insects and weeds in Canada. 5-6. Commonwealth Institute of Biological Control Trinidad.

Moericke, V. 1952. Farben als Landereize für geflügelten Blattläusen (Aphidoidea). Zeitschrift für Naturforschung 7: 304–324.

Noble, S.D., and T.G. Crowe. 2007. Sample holder and methodology for measuring the reflectance and transmittance of narrow-leaf samples. Applied Optics 46: 4968-4976.

Painter, R.H. 1951. Insect resistance in crop plants. University of Kansas, Lawrence, KS.

Parmesan, C. 1991. Evidence against plant "apparency" as a constraint on evolution of insect search efficiency (Lepidoptera: Nymphalidae). Journal of Insect Behaviour 4: 417-430.

Prokopy, R . J. 1972. Response of apple maggot flies to rectangles of different colours and shades. Environmental Entomology 1: 720-726.

Prokopy, R.J., and E.D. Owens. 1983. Visual detection of plants by herbivorous insects. Annual Review of Entomology 28: 337-364.

Rhoades, D.F., and R.G. Cates. 1976. Toward a general theory of plant antiherbivore chemistry. Recent Advances in Phytochemistry 10: 168–213.

SAS Institute. 2005. SAS, Version 9.1. SAS Institute, Inc., Cary, NC.

Sivinski J. 1990. Coloured spherical traps for capture of Caribbean fruit fly *Anastrepha suspensa*. Florida Entomologist 73: 123-128.

Shaw, E. 2008. The detection of biochemical markers for cabbage seedpod weevil (*Ceutorhynchus obstrictus*) resistance in *Brassica napus* L. x *Sinapis alba* L. germplasm. M.Sc. Thesis, University of Guelph, Guelph, ON. 155 pp.

Smart, L. E., M.M. Blight, and A.J. Hick. 1997. The effect of visual cues and a mixture of isothiocyanates on trap capture of cabbage seed weevil, *Ceutorhynchus assimilis.* Journal of Chemical Ecology 23: 889-902.

Strauss, S.Y., R.E. Irwin, and V.M. Lambrix. 2004. Optimal defence theory and flower petal colour predict variation in the secondary chemistry of wild radish. Ecology 92: 132–141.

Taub-Montemayor, T.E., K.H. Dahm, G. Bhaskaran, and M.A. Rankin. 1997. Rates of Juvenile Hormone biosynthesis and degradation during reproductive development and diapause in the boll weevil, *Anthonomus grandis*. Physiological Entomology 22: 269 – 276.

Ulmer, B.J., and L.M. Dosdall. 2006. Spring emergence biology of the cabbage seedpod weevil (Coleoptera: Curculionidae). Annals of the Entomological Society of America 99: 64-69.

Wehner, R. 1981. Spatial vision in arthropods. pp. 287-616 *In* Autrum, H. (ed.).Handbook of Sensory Physiology, Vol. 7, Sect. 6C. Springer, Berlin/New York.

Yoshioka, H., A. Horisaki, K. Kobayashi, Syafaruddin, S. Niikura, S. Ninomiya, and R. Ohsawa. 2005. Intraspecific variation in the ultraviolet colour proportion of flowers in *Brassica rapa* L. Plant Breeding 124: 551-556.

Chapter 4

Responses of *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae) to olfactory cues associated with novel *Sinapis alba* L. x *Brassica napus* L. genotypes

Introduction

The cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (syn. *C. assimilis* (Paykull)) (Coleoptera: Curculionidae) is a pest of brassicaceous oilseed crops in Europe and North America (McCaffrey 1992; Buntin et al. 1995; Dosdall et al. 2001). This European insect was first reported in south-western British Columbia in 1931 (McLeod 1962) and has spread throughout most of continental U.S.A. (McCaffrey 1992). The weevil was reported near Lethbridge, Alberta in 1995 (Cárcamo et al. 2001), western Saskatchewan in 2000 and is predicted to eventually occupy all western Canadian canola production regions (Dosdall et al. 2002. The weevil has also been found in Québec and Ontario (Brodeur et al. 2001; Mason et al. 2004). Dispersal from western Canadian populations is at a rate of approximately 55 km per year; eventual invasion of all western Canadian canola production regions is predicted (Dosdall et al. 2002).

Adult *Ceutorhynchus obstrictus* overwinter in the ground below leaf litter (Dmoch 1965; Ulmer and Dosdall 2006). Adults emerge in late spring to feed on brassicaceous plants near their overwintering sites; mass migration into canola crops occurs shortly after flowering (Bonnemaison 1957). Oviposition occurs and larvae feed on seeds in developing siliques of *Brassica* spp.; five to six seeds can be consumed during three instars (Dmoch 1965). Mature larvae chew holes in pod walls, emerge and drop to the soil where they pupate. Emergence of the next generation of adults occurs in mid August in western Canada (Dosdall and Moisey 2004).

Interspecific crosses of *Sinapis alba* L. x *B. napus* have produced several accessions that are resistant to *C. obstrictus* (Dosdall and Kott 2006; Ross et al. 2006; Shaw 2008). Fewer eggs are deposited and larvae show increased development times on resistant lines (McCaffrey et al. 1999, Dosdall and Kott 2006, Chapter 5). Reduced apparency (as per Feeny 1976) of resistant lines has also been demonstrated; *C. obstrictus* adults are less responsive to visual cues associated with resistant genotypes (Tansey et al. 2008; Chapter 3). Although *C. obstrictus* will oviposit and can complete development on marginally suitable host plants like *S. alba* (Kalischuk and Dosdall 2004; Dosdall and Kott 2006), given choices, these weevils discriminate among populations of potential hosts (for example, Moyes and Raybould 2001). In addition to differences in visual cues associated with potential hosts, mechanisms for this discrimination and differences in susceptibilities of introgressed lines are likely related to variations in volatile compounds emitted by resistant and susceptible hosts.

Visser (1986) noted that volatile chemical cues are used by many herbivorous insects for host association and location, and suggested that glucosinolates and their hydrolysis products, which are compounds relatively specific to Brassicaceae, should act as host association cues to Brassicaceae specialists. Evans and Allen-Williams (1993) found that field traps baited with *B. napus* extracts were located by *C. obstrictus* from distances of 20 m, and adults oriented toward odours of *B. napus* foliage and flowers and their extracts in olfactometer studies (Evans and Allen-Williams 1993, Bartlet et al. 1997). Smart et al. (1997) found that mixes of 3-butenyl, 4-pentenyl and 2-phenylethyl

isothiocyanates are attractive to *C. obstrictus* during its crop-colonizing migration. Smart and Blight (1997) determined that each of these compounds was also individually attractive to *C. obstrictus* in a field trapping study. Moyes and Raybould (2001) determined that a relationship exists between amounts of 3-butenyl glucosinolate associated with potential host plant populations and *C. obstrictus* oviposition, and suggested that hydrolysis products associated with this compound facilitated location and discrimination of host populations by the weevil.

Shaw (2008) examined upper cauline leaves using high performance liquid chromatography (HPLC) analysis and detected consistent differences in the heights of one peak among resistant and susceptible *S. alba* x *B. napus* lines. The peak was associated with an as yet unidentified compound (retention time 21.4 ± 0.03 min) that was determined, though myrosinase degradation, to be a glucosinolate. Peak height was inversely correlated with susceptibility to weevil attack as indicated by exit-hole frequencies in pods of susceptible and resistant genotypes. Peak height of the uncharacterised glucosinolate was, on average, 3.5 times larger in resistant than susceptible lines (Shaw 2008). Shaw (2008) also detected differences among resistant and susceptible *S. alba* x *B. napus* lines in the amounts of another as yet uncharacterised glucosinolate (retention time 20.5 ± 0.01 min) in seeds of immature pods; peak heights were correlated with weevil infestation scores and, on average, the peak height was 3.5 times greater in susceptible than resistant lines (Shaw 2008). Identities of these compounds were not assessed by liquid chromatography-mass spectrometry.

Here I present results of laboratory olfactometer assessments of the volatile cues associated with whole plants, racemes and cauline leaves of *C. obstrictus*-resistant and susceptible lines derived from *S. alba* x *B. napus* and the parental genotypes, *B. napus* and *S. alba.* Responses were assessed for both overwintered- and fall-generation weevils. I also investigated potential identities of the uncharacterised glucosinolate investigated by Shaw (2008). Potential effects of hydrolysis products of these glucosinolates were used to draw inferences regarding the influence of detected polymorphisms on attractiveness of volatile cues associated with specific host genotypes. Potential courses for resistance breeding and deployment strategies for novel germplasm are also addressed.

Methods and Materials

Plants and insects

Seed for test plants was obtained from Dr. Laima S. Kott (University of Guelph, Guelph, ON); genotypes evaluated included *B. napus* var. Q2 (hereafter referred to as Q2), *S. alba* var. AC Pennant (hereafter referred to as *S. alba*), and three *C. obstrictus*-resistant and two susceptible lines derived from *S. alba* x *B. napus* (Accessions 171S, 154S and 276R, 173R and 121R, respectively; 'S' denotes susceptible genotypes, 'R' denotes resistant genotypes). Plants were propagated in a soilless growth medium consisting of a modified Cornell mix based on the recipe of Boodly and Sheldrake (1982) (Agriculture and Agri-Food Canada 2002). Plants were grown in a greenhouse chamber at the Agriculture and Agri-Food

Canada Research Centre, Lethbridge, Alberta and maintained at 16:8 (L:D) and 60% relative humidity. Plants were at growth stage 4.3 (many flowers open, lower pods elongating) (Harper and Berkenkamp 1975) when tested because this is when most *C. obstrictus* oviposition occurs (Dosdall and Moisey 2004). *Ceutorhynchus obstrictus* adults were captured using sweep nets from a commercial *B. napus* field near Lethbridge, Alberta (49° 41′ 39″ N, 112° 49′ 58.3″ W) in late May, late June and mid-August 2007; weevils were maintained on potted, flowering *B. napus* var. Q2 in mesh cages in the laboratory and introduced to experiments within two weeks of capture.

Bioassay

A Y-tube olfactometer (Figure 4.1) was used to assess behavioural responses of *C. obstrictus* to olfactory cues associated with these genotypes. All tests were conducted in the laboratory at 21°C and between 10:00 and 15:00 hrs. The olfactometer was place squarely under ceiling fluorescent lighting (GE T8 F32T8/SPX41, General Electric, Fairfield, CT). The apparatus consisted of a modified 145-mm-diameter by 250-mm-high glass bell jar with a 29 mm circular opening in its side and a 42.5 mm circular opening in its top. A curved (45^{o}), 115 mm-long section of 38-mm-diameter glass tubing was attached to the top of the bell jar; another similarly curved piece was attached to this. A 160 mm glass Y-intersection (45^{o}) (42.5 mm internal diameter) was next in line; each arm was attached to a 50-mm-diameter by 200-mm-long glass bell jar that tapered to a 6.9 mm opening at its distal end. All glass sections of the apparatus were connected

using 33.5-mm-diameter Tygon™ tubing. A machined plastic funnel with a 12.8-mm-diameter distal opening was inserted into the last bell jar to allow weevils to move into the jar but restrict movement back into the Y-tube. The ends of each small bell jar attached to the Y-tube were connected to 950-mm-long by 190-mm-diameter Plexiglas™ cylinders using 15-mm-diameter Tygon™ tubing. Compressed air was filtered using activated charcoal and bubbled through distilled water to maintain consistent humidity before being pumped into the apparatus. Relative humidity in the device was measured at one second intervals over 2 hr and determined to be 48.98 ± 0.009 %. Airflow was maintained at 0.24 L min^{-1} using an air-flow regulator, through a splitter and to odour sources and controls. All parts of the apparatus were washed in dish soap and water, rinsed with 70% ethanol and dried between runs.

Replicates of genotypes were tested in random order over the course of the experiments. Preliminary analysis indicated no significant differences between the responses of 40 individual weevils (65%) and four groups of 20 randomly selected weevils (70%) to whole, flowering Q2 ($\chi^2 = 0.31$; df = 1; $P = 0.5805$), so randomly selected, mixed-sex groups of 20 *C. obstrictus* were tested in all evaluations of responses, as per Bartlet et al. (1997). Weevils were introduced through the opening in the side of the large glass bell jar; the opening was plugged with a rubber stopper after their introduction. Weevil positions in one arm or the other of the Y-tube were recorded after 20 minutes. Weevils were removed, preserved in 70% ethanol and dissected to determine sex. Proportions of *C. obstrictus* males or females associated with the blank or test sides of the Y-tube

olfactometer were compared by analysis with binomial generalized estimating equations (proc GENMOD) (SAS Institute 2005). Pair-wise comparisons of the proportions of males and females among genotypes, between whole plants and flowers and among sampling dates were made using Wald chi-square tests (LS MEANS statement with 'diff' option in proc GENMOD) (SAS Institute 2005).

Comparing responses to whole plants and flowering racemes

Whole plants were placed in Plexiglas™ cylinders and air was pumped past them and through the apparatus. Pots of soilless growth medium were used as a control for these tests. To test flowers, the top 20 cm of intact flowering plants were inserted into a 30 cm plastic cylinder through a 4 cm opening in its side. Parafilm™ was placed over the opening to prevent escape of pumped air. An empty chamber was used as a control for these tests. Responses were compared in mid June and mid August 2007.

Responses to flowering racemes at different points in the growing season

Responses to flowering racemes were assessed in a manner consistent with the previous section. An empty chamber was used as a control for these tests. Responses were compared in mid June, mid July and mid August 2007.

Responses to cauline leaves by weevil generation

Cauline leaves are the bracts just below an inflorescence. Fresh cauline leaves, excised and macerated (with scissors), freeze-dried (0.1 g reconstituted with 0.1 mL distilled water) and freeze-dried with myrosinases denatured (0.1 g heated to

110°C for 45 min then reconstituted with 0.1 mL distilled water) were introduced into a sealed 250 mL flask. Air was forced through a hole in the rubber stopper sealing the flask, out another hole in the stopper and through the apparatus. The control for these tests was an empty Plexiglas™ cylinder. Responses to macerated and excised cauline leaves were assessed in mid June and mid August 2008, and responses to freeze-dried cauline leaves were assessed in mid August 2008.

Relationships of glucosinolate content and weevil responses

Relationships of the mean peak heights associated with uncharacterised glucosinolates evaluated from immature seeds and cauline leaves Shaw (2008) to weevil infestation scores were assessed using linear regression analysis (proc REG). Relationships of peak heights and proportions of weevils responding to specific genotypes were assessed in a similar manner.

Glucosinolate characterization

Estimates of the identities of uncharacterised glucosinolates from Shaw (2008) associated with cauline leaves (inversely correlated with susceptibility to weevil attack) and seeds (correlated with susceptibility to weevil attack) were performed using retention time shifts corrected by linear interpolation (as per Gong et al. 2004). Results of High Performance Liquid Chromatography – Mass Spectrometry (HPLC-MS) analysis of glucosinolate calibration standards (Lee et al. 2006; Rochfort et al. 2008) were compared with the results of plant tissue

analyses of Shaw (2008). All of these studies evaluated retention times of glucosinolate compounds using reversed-phase Hypersil BDS C_{18} columns. Column dimensions were 250 mm × 4.6 mm, 5 µm for Shaw (2008), 250 mm × 4.6 mm, 5 µm for Lee et al. (2006), and 150 mm × 2.1 mm, 3 µm for Rochfort et al. (2008); flow rates were 1.0 mL min^{-1}, and 0.2 mL min^{-1} for Lee et al. (2006) and Rochfort et al. (2006), respectively. Flow rate was not indicated by Shaw (2008). For Shaw (2008) and Lee et al. (2006), mobile phase consisted of a gradient of 30 mmol L^{-1} ammonium acetate (component A) and methanol (component B); Rochfort et al. (2008) used a mobile phase gradient of 0.1% ammonium acetate in water (component A) and 0.1% ammonium acetate in methanol (component B). Formic acid was used by Shaw (2008) and Lee et al. (2006) to achieve a pH 5.0 in component A; pH was not indicated by Rochfort et al. (2008). Correlation analysis (proc CORR) was conducted to assess the validity of interpolated retention times associated with known compounds from Lee et al (2206) and Rochfort et al. (2008) and to make estimates of the identities of unknown compounds from Shaw (2008).

Results

Comparing responses to whole plants and flowering racemes

Significant differences in the responses of females of the overwintered generation to genotypes were apparent when flowering racemes and whole plants were tested

(χ^2 = 63.82; *df* = 6; *P* < 0.0001). Similar responses to the susceptible genotypes Q2, 154 S and 171 S were apparent (*P* > 0.05 for all comparisons); these were greater than those associated with the resistant genotypes *S. alba*, 173R, 121 R, and 276 R (*P* < 0.007 for all comparisons). There were no significant differences in the responses of overwintered-generation females to resistant genotypes (*P* > 0.05 for all comparisons). Females responded similarly to whole plants and flowering racemes (χ^2 = 0.02; *df* = 1; *P* = 0.8953). No interaction of flowering racemes by whole plant and genotype was apparent (χ^2 = 8.50; *df* = 6; *P* = 0.2036).

Significant differences in the responses of overwintered generation males to genotypes were also apparent (χ^2 = 28.84; *df* = 6; *P* < 0.0001). Responses of males to tested genotypes were similar to those of females. There were no significant differences in the responses of males of the overwintered generation to whole plants or flowering racemes (χ^2 = 0.03; *df* = 1; *P* = 0.8613) or interaction of flowering racemes by whole plant and genotype (χ^2 = 4.73; *df* = 6; *P* = 0.5792).

Responses to flowering racemes at different points in the growing season

Significant differences in the responses of females to the tested genotypes were apparent (χ^2 = 92.92; *df* = 6; *P* < 0.0001). Responses to the susceptible genotypes Q2, 154 S and 171 S were similar (*P* > 0.05 for all comparisons) and greater than those associated with the resistant genotypes *S. alba*, 173R, 121 R, or 276 R (*P* < 0.01 for all comparisons) (Table 4.1). There were no significant differences in the responses of females to resistant genotypes (*P* > 0.05 for all comparisons). The

effect of date was significant (χ^2 = 8.83; *df* = 2; *P* = 0.0181). Responses of females were greater in June and August than July (χ^2 = 5.72; *df* = 1; *P* = 0.0168 and X^2 = 6.40; *df* = 1; *P* = 0.0114, respectively). Differences between June and August were not significant (χ^2 = 0.04; *df* = 1; *P* = 0.8508). No significant interaction of testing date and genotype was apparent (χ^2 = 6.42; *df* = 12; *P* = 0.8937), suggesting similarities in the relative responses of female *C. obstrictus* to these genotypes throughout the field season.

Significant differences in the responses of males to these genotypes were also apparent (χ^2 = 71.72; *df* = 6; *P* < 0.0001). Responses of males were similar to those of females (Table 4.1). No significant effects of testing date (χ^2 = 2.55; *df* = 2; *P* = 0.2791) or interaction of testing date and genotype (χ^2 = 10.57; *df* = 12; *P* = 0.5664) were detected suggesting similar responses of males to olfactory cues associated with test genotypes in June, July and August.

Responses to cauline leaves by weevil generation

Females responded similarly to macerated and excised cauline leaves (χ^2 = 0.95; *df* = 1; *P* = 0.3288). A significant effect of genotype was apparent (χ^2 = 64.55; *df* = 6; *P* < 0.0001). Responses to the susceptible genotypes 154 S, 171 S and Q2 were similar (*P* > 0.05 for all comparisons) and significantly greater than those associated with 276R, 173 R or *S. alba*; 154 S and 171 S were more attractive than 121 R; 121 R was more attractive than *S. alba* (*P* < 0.01 for all comparisons) (Table 4.2). The effect of generation was also significant (χ^2 = 6.55; *df* = 1; *P* = 0.0105); responses were greater for overwintered than new generation weevils.

No interactions of generation by genotype, preparation technique by genotype, generation by preparation technique, or generation by preparation technique by genotype were apparent for females ($P > 0.05$ for all).

Macerated cauline leaves were more attractive to males than excised leaves ($\chi^2 = 8.68$; $df = 2$; $P = 0.0032$). A significant genotype effect was also apparent ($\chi^2 = 43.27$; $df = 6$; $P < 0.0001$). Responses to Q2, 171 S and 154 S were greater than those associated with *S. alba*, 121 R or 173 R; responses to Q2 and 154 S were greater than those to 276 R ($P < 0.01$) (Table 4.2). Male responses did not differ by generation ($\chi^2 = 0.14$; $df = 1$; $P = 0.7073$). No interactions of generation by genotype, preparation technique by genotype, generation by preparation technique, or generation by preparation technique by genotype were apparent for males ($P > 0.05$ for all).

Responses of *C. obstrictus* females to reconstituted freeze-dried cauline leaves indicated differences among genotypes ($\chi^2 = 18.30$; $df = 4$; $P = 0.0011$) (Table 4.3). No effects of heat treatment or significant interaction of heat treatment by genotype were apparent ($\chi^2 = 0.01$; $df = 1$; $P = 0.9116$, and $\chi^2 = 7.74$; $df = 4$; $P = 0.1014$, respectively). However, pair-wise comparisons indicated significant differences in the responses of females to genotypes without heat treatment: 154 S and 171 S were more attractive than *S. alba*; 171S was also more attractive than 121R to females ($P < 0.01$ for all comparisons). No significant differences in responses of females to heat-treated leaves were detected ($P > 0.05$ for all comparisons) (Table 4.3).

Responses of males to reconstituted freeze-dried cauline leaves did not differ among genotypes (χ^2 = 8.82; *df* = 4; *P* = 0.0656) and no significant heat treatment effect was detected (χ^2 = 1.88; *df* = 1; *P* = 0.1702). However, a significant interaction of heat treatment and genotype was apparent (χ^2= 10.01; *df* = 4; *P* = 0.0403) and pair-wise comparisons indicated that 154 S and 171 S without heat treatment were more attractive than *S. alba* or 173 R without heat treatment (*P* < 0.01 for all comparisons) (Table 4.3). No significant differences in responses of males to heat-treated leaves were detected (*P* > 0.05 for all comparisons) (Table 4.3).

Comparing responses to flowers and cauline leaves

Comparison of the responses of females of *C. obstrictus* of the new generation to *S. alba*, 121 R, 173 R, 154 S and 171 S flowers, excised, macerated and freeze-dried cauline leaves indicated a significant genotype effect (χ^2 = 43.13; *df* = 4; *P* < 0.0001); susceptible genotypes attracted similar numbers of weevils (*P* > 0.05 for all comparisons) and significantly more than resistant genotypes (*P* < 0.01 for all comparisons). A significant effect of plant part or preparation technique was also apparent (χ^2 = 43.83; *df* = 4; *P* < 0.0001). No significant interactions of genotype and plant part or preparation technique were apparent (χ^2 = 14.34; *df* = 16; *P* = 0.5735). Racemes attracted significantly more females than macerated, excised, freeze-dried and reconstituted or freeze-dried, heat-treated and reconstituted cauline leaves (*P* < 0.01 for all comparisons). Macerated and excised cauline leaves attracted similar proportions of females (χ^2 = 0.01; *df* = 1; *P* = 0.8405);

both treatments resulted in greater proportions attracted than for freeze-dried cauline leaves ($P < 0.01$ for all comparisons). Freeze-dried and reconstituted and freeze-dried, heat-treated and reconstituted cauline leaves attracted similar proportions of females ($\chi^2 = 0.01$; $df = 1$; $P = 0.9118$).

Responses of males indicated a significant genotype effect ($\chi^2 = 90.01$; $df = 4$; $P < 0.0001$); susceptible genotypes attracted similar numbers of weevils ($P > 0.05$ for all comparisons) and significantly more than resistant genotypes ($P < 0.01$ for all comparisons). A significant effect of plant part or preparation technique was also apparent ($\chi^2 = 28.59$; $df = 4$; $P < 0.0001$), although no significant interaction of genotype and plant part or preparation technique was apparent ($\chi^2 = 22.28$; $df = 16$; $P = 0.1343$). Racemes attracted significantly more males than macerated, excised, freeze-dried and reconstituted and freeze-dried, heat-treated and reconstituted cauline leaves ($P < 0.05$ for all comparisons). Unlike females, males responded more strongly to macerated than excised cauline leaves ($\chi^2 = 4.66$; $df = 1$; $P = 0.0309$). Males were also more attracted to excised than freeze-dried cauline leaves ($P < 0.05$ for both comparisons). Freeze-dried and freeze-dried and heat-treated cauline leaves attracted similar proportions of males ($\chi^2 = 1.90$; $df = 1$; $P = 0.1682$).

Relationships of glucosinolate content and weevil responses

Significant negative relationships of female responses to whole plants, flowers, macerated, excised and freeze-dried and reconstituted cauline leaves and mean

peak heights associated with the uncharacterised cauline leaf glucosinolate (Shaw 2008) were detected ($P < 0.05$ for all assessments). However, no relationship of female responses to freeze-dried, heat-treated and reconstituted cauline leaves and this glucosinolate was detected ($F_{1, 14} = 4.11$; $R^2 = 0.1718$; $P = 0.0621$). Similar trends were detected for males: responses to whole plants, flowers, macerated, excised and freeze-dried cauline leaves were strongly and negatively associated with this glucosinolate ($P < 0.01$ for all assessments). No relationship of male responses to heat-treated freeze-dried cauline leaves and this glucosinolate was detected ($F_{1, 14} = 1.41$; $R^2 = 0.0918$; $P = 0.2541$).

Relationships between female responses and peak heights associated with the uncharacterised glucosinolate detected by Shaw (2008) from seeds of these genotypes were demonstrated for whole plants and flowering racemes ($F_{1, 21} = 13.08$; $R^2 = 0.3544$; $P = 0.0016$ and $F_{1, 72} = 90.05$; $R^2 = 0.5495$; $P < 0.0001$, respectively). Similar relationships were demonstrated for males ($F_{1, 21} = 11.26$; $R^2 = 0.3181$; $P = 0.0030$ and $F_{1, 72} = 80.62$; $R^2 = 0.5217$; $P < 0.0001$, respectively).

Glucosinolate characterization

Comparisons of HPLC results from Shaw (2008) and other studies (Lee et al. 2006; Rochfort et al. 2008) by linear interpolation indicate that the uncharacterised glucosinolate (retention time 20.5 ± 0.01 minutes) detected from seeds of immature pods and positively correlated with weevils scores and responses is likely 2-phenylethyl glucosinolate. Linear interpolation predicted the

retention time of 2-phenylethyl glucosinolate at 20.6 minutes. Cauline leaves, mature foliage and seeds also exhibited a peak at 17 minutes. This second peak was likely associated with 3-butenyl glucosinolate and was relatively consistent among resistant and susceptible lines (Shaw 2008). The peak associated with cauline leaves and negatively correlated with weevil scores is likely 1-methoxy-3-indolylmethyl glucosinolate. Mean retention time associated with this compound was 21.4 ± 0.03 minutes; linear interpolation predicts a retention time of 21.4 minutes for 1-methoxy-3-indolylmethyl glucosinolate (Table 4.4). Correlation analysis of the results of retention time shifts of known standards from Lee et al. (2006) and Rochfort et al. (2008) corrected by linear interpolation and retention times associated with uncharacterised peaks from Shaw (2008) indicated a significant relationship ($R = 0.9998$; $P = 0.0121$).

Discussion

Results of this study indicate differences among the responses of *C. obstrictus* to olfactory cues associated with *B. napus* and *S. alba* and accessions obtained by crosses of these species. Odours from whole flowering plants, flowering racemes and cauline leaves of the susceptible genotypes *B. napus* Q2, 171 S and 154 S were significantly more attractive than resistant genotypes to both male and female weevils. Similarities in weevil responses among Q2, 171S and 154 S suggest similarities in the profiles of the volatile compounds that stimulate electrophysiological and/or behavioural activity in the weevil. Behavioural

responses to resistant lines were similar. Differences among responses of weevils to resistant and susceptible introgressed lines indicate chemical differences.

Although differences in susceptibilities of *S. alba*, *B. napus*, and *B. rapa* to insect herbivores have also been attributed to *p*-hydroxybenzyl glucosinolate content (Bodnaryk 1991), the major hydrolysis product of sinalbin is the highly unstable *p*-hydroxybenzyl isothiocyanate (Vaughn and Berhow 2005). As *p*-hydroxybenzyl isothiocyanate breaks down, a quinone that hydrolyzes to SCN^- is formed (Borek and Morra 2005). Given that the half-life of *p*-hydroxybenzyl isothiocyanate is ca. 6 min at physiological pH (Borek and Morra 2005), its influence on long-distance olfactory responses in *C. obstrictus* seems unlikely. Differences in the attractiveness of odours from whole plants, flowering racemes and cauline leaves of *S. alba* and those of the susceptible genotypes in this study are likely associated with a lack of stimulatory kairomones (as per McCaffrey et al. 1999).

Ceutorhynchus obstrictus is attracted to volatile compounds associated with *B. napus* (Free and Williams 1978; Bartlet et al. 1993). Initial responses to host odours include positive anemotaxis (Evans and Allen-Williams 1998) consistent with responses of weevils in the olfactometer used in this study. Visser (1986) suggested that compounds specific to Brassicaceae should act as host association cues to crucifer specialists; these compounds include glucosinolates and their hydrolysis products. Hydrolysis of glucosinolates in plants results from the action of specific myrosinases (*β*-thioglucosidases) that facilitate the irreversible hydrolysis of the thioglucosidic bond, liberating the D-glucose and

aglycone moieties; unstable isothiocyanates, epithionitriles and nitriles are the typical products (Rask et al. 2000 and references therein). Because myrosinase and glucosinolates are compartmentalized in the Capparales including the Brassicaceae, volatile hydrolysis products of glucosinolates are generally produced only when tissues and thus myrosin and sulphur rich cells are damaged (Rask et al. 2000; Andréasson et al. 2001). However, allyl, 3-butenyl, 4-pentenyl, and 2-phenylethyl isothiocyanate have been detected in headspace volatiles of undamaged *B. napus*, and specific olfactory cells tuned to these compounds have been detected in *C. obstrictus* (Blight et al. 1995).

Of these isothiocyanates, only derivatives of 2-phenylethyl glucosinolate (2-phenylethyl isothiocyanate) elicited strong responses in one study (Blight et al. 1995). In another study, 3-butenyl and 4-pentenyl isothiocyanate elicited the strongest electroantennogram responses (Evans and Allen-Williams 1992). Smart and Blight (1997) found that each of these compounds was attractive to *C. obstrictus*, and recommended baiting traps with 2-phenylethyl isothiocyanate for monitoring spring populations. This compound is also attractive to *Ceutorhynchus napi* (Gyllenhal) and *C. pallidactylus* (Marsham) (Walczak et al. 1998). Examination of foliar glucosinolates indicated no 3-butenyl, 4-pentenyl or 2-phenylethyl glucosinolate in *S. alba* although these compounds were detected in *B. napus* (McCloskey and Isman 1993). Shaw (2008) determined that Q2 and the susceptible and resistant *S. alba* x *B. napus* lines tested in this study differed in the amounts of an uncharacterised glucosinolate in seeds of immature pods. Peak height was correlated with weevil scores and, on average, was 3.5 times greater in

susceptible than resistant lines (Shaw 2008). Genotype-specific peak heights associated with this compound were also correlated with male and female weevil olfactometer responses in this study. I found, based on estimates of uncharacterised glucosinolates from Shaw (2008) by retention time shifts corrected by linear interpolation (as per Gong et al. 2004), that the identity of this compound was likely 2-phenylethyl glucosinolate.

Glucosinolate profiles can vary among plant tissues (for example, Porter et al. 1991). However, examination of results from Shaw (2008) indicates that the peak putatively associated with 2-phenylethyl glucosinolate is also present in mature foliage and is consistently greater in susceptible than resistant lines. Another peak corresponding to 3-butenyl glucosinolate was also associated with seeds and mature foliage for all genotypes except *S. alba* (Shaw 2008). The additive or synergistic effects of 3-butenyl and 2-phenylethyl isothiocyanates on *C. obstrictus* responses have been demonstrated in olfactometer studies (Bartlet et al. 1993). Smart and Blight (1997) also found that a mixture of 3-butenyl, 4-pentenyl, 2-phenylethyl, and allyl isothiocyanates were highly attractive to *C. assimilis* in trapping studies. In an examination of naturalized *Brassica oleracea* L. and *Brassica nigra* L. populations in England, Moyes and Raybould (2001) found greater *C. obstrictus* oviposition in plant populations that expressed higher levels of 3-butenyl glucosinolate. Greater seed and foliar expression of 2-phenylethyl- and 3-butenyl glucosinolates by susceptible genotypes explains differences in responses of *C. obstrictus* among susceptible and resistant genotypes detected in this study.

I propose that the uncharacterised peak associated with cauline leaves and inversely correlated with weevil scores and olfactometer responses was 1-methoxy-3-indolylmethyl glucosinolate. This compound has been shown by other researchers to increase in brassicaceous host plants in response to insect herbivore attack (Birch et al. 1992; Bodnaryk 1992, 1994; Doughty et al. 1995). Local increases in 1-methoxy-3-indolylmethyl glucosinolate concentration occur in *B. napus* in response to mechanical damage and *Phyllotreta cruciferae* (Goeze) (Coleoptera; Chrysomelidae) feeding on cotyledons (Bodnaryk 1992), and exogenous application of methyl jasmonate and jasmonic acid (Bodnaryk 1994; Doughty et al. 1995). Birch et al. (1992) reported that 1-methoxy-3-indolylmethyl glucosinolate content in the Brassicaceae they tested (these included a *B. napus* oilseed variety) increased systemically as much as 17-fold in response to *Delia floralis* (Fallén) (Diptera: Anthomyiidae) attack; this increase was the greatest of any individual glucosinolate.

Moyes and Raybould (2001) reported that 1-methoxy-3-indolylmethyl glucosinolate was present in all *B. oleracea* tested but that no relationship was evident between population-wide content of this compound and *C. obstrictus* oviposition. However, mean constitutive population-wide concentrations of 1-methoxy-3-indolylmethyl glucosinolate were significantly higher on one site than at the four other sites tested by Moyes et al. (2000); plants from this site supported fewer *C. obstrictus* larvae than sites characterised by plants with lower levels of this glucosinolate in one study year (Moyes and Raybould 2001). Although Shaw (2008) did not measure concentrations of individual compounds in the plant

tissues tested in this study, putative 1-methoxy-3-indolylmethyl glucosinolate HPLC peaks that were, on average 3.5 times greater in resistant than susceptible lines derived from *S. alba* x *B. napus* indicate differences comparable to those detected by Moyes et al. (2000); their results indicated as much as 4.8- fold differences in plant population-wide concentrations of 1-methoxy-3-indolylmethyl glucosinolate content between sites.

Hydrolysis products of 1-methoxy-3-indolylmethyl glucosinolate include indole isothiocyanates, indole-cyanides, indolyl-3-carbinol, thiocyanate and possibly phytoalexins and auxins (Mithen 1992; Mewis et al. 2002). Indole isothiocyanates are unstable but the slightly volatile indole-cyanides are relatively stable and prevalent in *B. rapa* ssp. *chinensis* cv. Joi Choi, Black Behi and Bai Tsai (Mewis et al. 2002). Indole isothiocyanates, particularly 1-methoxy-3-indolylmethyl glucosinolate, are suspected of contributing to reduced oviposition by the oligophagous butterfly *Hellula undalis* (Fabricius) (Lepidoptera: Pyralidae) (Mewis et al. 2002). Moreover, increased levels of indole glucosinolates and greater overall glucosinolate levels greatly reduced *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae) feeding on cotyledons of induced plants (Bartlet et al. 1999).

Cook et al. (2006) found that *B. rapa* was more attractive to *C. obstrictus* than a high indolyl/ low alkenyl glucosinolate *B. napus* genotype, and in olfactometer trials responses to a high alkenyl-low indolyl genotype were similar to those of *B. rapa*. However, given the slight volatility of indole-cyanides, their role in long-distance olfactory responses of *C. obstrictus* seems unlikely.

Although their effects in this study cannot be discounted, behavioural responses of weevils to 1-methoxy-3-indolylmethyl glucosinolate hydrolysis products are likely limited to more intimate ranges. Responses of *C. obstrictus* to volatile hydrolysis products of indolyl glucosinolates require more rigorous testing.

Responses of both males and females to racemes were greater than to cauline leaves. In addition to the glucosinolates associated with cauline leaves, flowering racemes also exude compounds such as (*E*,*E*,)-α-farnesene (Evans and Allen-Williams 1992); (*E*,*E*,)-α-farnesene is a major component of *B. napus* floral volatiles (Blight et al. 1995) but is exuded in much smaller amounts from *S. alba* (Tollsten and Bergstrom 1988). Evans and Allen-Williams (1992) found that it was the only volatile compound they detected from *B. napus* flowers that elicited strong electroantennogram responses from *C. obstrictus* at relatively low doses. Attractive glucosinolate hydrolysis products and (*E*,*E*,)-α-farnesene have also been suspected to have a synergistic attractive effect on *C. obstrictus* behaviour (Evans and Allen-Williams 1992, 1998). Omitting α-farnesene from artificial rape odour reduced *C. obstrictus* electroantennogram responses and attractiveness of odours in wind tunnel tests (Evans and Allen-Williams 1992, 1998).

Responses of both males and females were greater to fresh (macerated or excised) cauline leaf material than to freeze-dried and reconstituted material. Maceration, excision and rending cauline leaves into powder would damage tissues and allow release of glucosinolate hydrolysis products. Differences between preparation techniques are likely due to the presentation of lesser amounts of freeze-dried material. Although a significant effect of heat treatment

after freeze-drying was not detected, samples without heat treatment elicited different behavioural responses; tissue from susceptible genotypes was more attractive. These effects were not seen with heat-treated samples. These results support the conclusion that differences in *C. obstrictus* responses to resistant and susceptible genotypes are associated with hydrolysis products of glucosinolates acting as attractive kairomones (as per McCaffrey et al. 1999).

Males respond more strongly to macerated than intact cauline leaf material. Females respond similarly to these odour sources. The green leaf volatiles *cis*-3-hexen-l-ol and *cis*-3-hexenyl acetate have been detected from *B. napus* headspace and male weevils are more sensitive to these compounds than females (Evans and Allen-Williams 1992). These compounds comprise 90% of the odour from macerated *B. napus* leaves but less than 15 % of crop odour (Evans and Allen-Williams 1992) and likely influenced male response in this study.

Female response to flowering racemes from susceptible genotypes was greater in June than in July. A similar response was detected in June and August. Bartlet et al. (1993) found that pre-diapause weevils (corresponding to August/new generation weevils in this study) were unresponsive to floral odours if they had been field-collected but responsive if reared from pods. Bartlet et al. (1993) suggested that the reduced response in field-collected specimens was associated with satiation of weevils that had fed in preparation for diapause. Responses of August females to host plant odours in this study suggest that these *C. obstrictus* had not yet completed feeding before diapause. They may have also

been influenced by acclimation to 12:12 (L:D) (Chapter 3). Post-diapause weevils (collected in April in England and roughly corresponding to June weevils in this study) were responsive to olfactory cues associated with susceptible genotypes. This attraction waned as this weevil cohort aged. Increased responses of gravid female *C. obstrictus* to visual cues as the season progressed have been demonstrated in a trapping study and are adaptive if these cues are used for oviposition site location (Chapter 2). Reductions in attractiveness of olfactory cues, however, may be subject to another mechanism. Reduced chemoreceptor sensitivity in insects is well documented and influenced by age; sensory input to the central nervous system may decrease as sensillae become inoperative (Schoonhoven 1969; Blaney et al. 1986). For instance, the sensitivity of boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae), chemoreceptors is greatest in the period coinciding with mating and host location (Dickens and Moorman 1990).

Fluorescent lighting can influence insect behaviours (Shields 1980). For example, gathering behaviour of the honeybee *Apis mellifera* (Hymenoptera: Apidae) is reduced in a room illuminated by fluorescent tubes (Renner 1957). Insect flicker fusion frequencies (FFF: the minimum number of flashes per second that are no longer resolved as continuous light) range from 20 to 300 cycles per second (Miall 1978). Fluorescent lamps that use standard ballasts flicker at 120 cycles per minute (Shields 1989). Although the potential effect of flicker on *C. obstrictus* behaviours is as yet unknown, all tests in the current study were conducted under the same lighting conditions.

Results of this study offer insights into the differences in susceptibilities of novel canola germplasm and suggest potential strategies for resistance breeding. Restraining expression of attractive volatile cues associated with hydrolysis products of 3-butenyl and 2-phenylethyl glucosinolates and/or encouraging production of indole glucosinolates may facilitate production of canola genotypes that are less attractive to *C. obstrictus*. Deployment of less attractive germplasm coupled with a highly attractive trap crop such a *B. rapa* may prove to be an effective strategy for concentrating and controlling weevil populations (as per Cárcamo et al. 2007). Examination of the responses of these weevils to current commercial cultivars that express varying proportions of alkenyl and indolyl glucosinolates is required and may allow this strategy to be used effectively with existing germplasm. Although olfactory cues are clearly important to *C. obstrictus* host location, it should be noted that visual cues are also essential to this interaction. Smart et al. (1997) found that traps baited with a mixture of allyl, 3-butenyl, 4-pentenyl and 2-phenylethyl isothiocyanate were more attractive if held vertically than at a 45° angle; traps baited with isothiocyanates alone were not attractive.

Tables

Table 4.1. Mean proportions of *C. obstrictus* (S.E.) responding to racemes of *Brassica napus* var. Q2, *Sinapis alba* var. AC Pennant and several lines derived from *S. alba* x *B. napus* in a Y-tube olfactometer in mid June, mid July and mid August, 2008. There are no significant differences ($P < 0.007$) by Wald chi-square tests among like lettered groups. The 'S' associated with genotype designations denotes a susceptible genotype; 'R' represents resistant.

Genotype	*n*	Proportion of total responding		Proportion responding = female		Proportion responding = male	
Brassica napus var. Q2	280	0.83 (0.03)	a	0.43 (0.02)	a	0.40 (0.02)	a
171 S	240	0.75 (0.03)	a	0.35 (0.02)	a	0.40 (0.02)	a
154 S	260	0.75 (0.03)	a	0.37 (0.02)	a	0.38 (0.02)	a
173 R	220	0.38 (0.03)	b	0.15 (0.03)	b	0.24 (0.02)	b
121 R	280	0.37 (0.03)	b	0.19 (0.02)	b	0.18 (0.02)	b
276 R	200	0.40 (0.04)	b	0.18 (0.03)	b	0.22 (0.02)	b
Sinapis alba var. AC Pennant	220	0.43 (0.03)	b	0.21 (0.03)	b	0.22 (0.02)	b

Table 4.2. Mean proportions of *C. obstrictus* (S.E.) responding to pooled data associated with macerated and excised cauline leaves of *Brassica napus* var. Q2, *Sinapis alba* var. AC Pennant and several lines derived from *S. alba* x *B. napus* in a Y-tube olfactometer in mid June and mid August, 2008. There are no significant differences ($P < 0.007$) by Wald chi-square tests among like lettered groups. The 'S' associated with genotype designations denotes a susceptible genotype; 'R' represents resistant.

Genotype	*n*	Proportion of total responding		Proportion responding = female		Proportion responding = male	
Brassica napus var. Q2	340	0.55 (0.04)	a	0.27 (0.03)	ab	0.28 (0.03)	a
171 S	320	0.54 (0.04)	a	0.31 (0.03)	a	0.23 (0.03)	ab
154 S	320	0.53 (0.04)	a	0.30 (0.03)	a	0.25 (0.03)	a
173 R	320	0.31 (0.04)	b	0.17 (0.03)	bc	0.14 (0.03)	bc
121 R	320	0.33 (0.04)	b	0.18 (0.03)	b	0.14 (0.03)	bc
276 R	280	0.33 (0.04)	b	0.17 (0.03)	bc	0.16 (0.03)	bc
Sinapis alba var. AC Pennant	300	0.26 (0.04)	b	0.10 (0.03)	c	0.16 (0.03)	c

Table 4.3. Mean proportions of *C. obstrictus* (S.E.) responding to freeze-dried and reconstituted (with water) cauline leaves of *Sinapis alba* var. AC Pennant and several lines derived from *S. alba* x *B. napus* with and without heat treatment to denature myrosinases in a Y-tube olfactometer. Tests were conducted in mid June and mid August, 2008. There are no significant differences ($P < 0.01$) by Wald chi-square tests among like lettered groups. The 'S' associated with genotype designations denotes a susceptible genotype; 'R' represents resistant.

Genotype	*n*	Proportion of total responding		Proportion responding = female		Proportion responding = male	
Native							
171 S	80	0.43 (0.05)	a	0.24 (0.04)	a	0.19 (0.02)	a
154 S	80	0.38 (0.05)	a	0.19 (0.04)	ab	0.19 (0.02)	a
173 R	80	0.14 (0.05)	b	0.11 (0.04)	abc	0.03 (0.02)	b
121 R	80	0.21 (0.04)	b	0.10 (0.03)	bc	0.11 (0.02)	ab
Sinapis alba var. AC Pennant	80	0.06 (0.05)	b	0.03 (0.04)	c	0.05 (0.02)	b
Denatured							
171 S	80	0.20 (0.05)	a	0.15 (0.04)	a	0.05 (0.02)	a
154 S	80	0.19 (0.05)	a	0.11 (0.04)	a	0.06 (0.02)	a
173 R	80	0.15 (0.05)	a	0.08 (0.04)	a	0.08 (0.02)	a
121 R	80	0.10 (0.05)	a	0.03 (0.04)	a	0.06 (0.03)	a
Sinapis alba var. AC Pennant	60	0.15 (0.05)	a	0.12 (0.04)	a	0.03 (0.03)	a

Table 4.4. Retention times of glucosinolates; some with electrophysiological and/behavioural effects on *C. obstrictus*: † represents unidentified peaks from cauline leaves and negatively correlated with weevil responses (Shaw 2008); †† represents uncharacterised peaks from seeds and foliage; *represents uncharacterised peaks correlated with weevil responses (Shaw 2008). HPLC results: *a* –estimates of unidentified glucosinolates from Shaw (2008) by retention time shifts corrected by linear interpolation (as per Gong et al. 2004); *b*- Shaw (2008); *c*- Lee et al. (2006); *d* - Rochfort et al. (2008).

Trivial Name	Side Chain, *R*-		HPLC Retention Time (min)			
		source	*a*	*b*	*c*	*d*
Glucoiberin	3-Methylsulphinylpropyl-		-	5.8	4.8	3.0
Glucocheirolin	3-Methylsulfonylpropyl-		-	6.6	5.2	-
Sinigrin	Allyl / 2-Propenyl-		-	8.1	6.2	-
Glucosinalbin	*p*-Hydroxybenzyl-		-	16.6	11.2	6.3
Gluconapin	3-Butenyl-		17.1	17.0††	11.9	-
Glucotropaeolin	Benzyl-		-	19.8	15.8	16.8
Glucoerucin	4-Methylthiolbutyl-		-	19.9	16.2	18.4
Gluconasturtiin	2-Phenylethyl-		20.6	20.5††,*	19.1	-
Neoglucobrassican	1-Methoxy-3-indolylmethyl-		21.4	21.4†*	-	34.2

Figure

Figure 4.1. Y-tube olfactometer.

References

Agriculture and Agri-Food Canada. 2002. Soilless growth medium for plants: preparation. SOP code: 120.0000.00. 6 pp.

Andréasson, E., L.B. Jorgensen, A.S. Hoglund, L. Rask, and J. Meijer. 2001. Different myrosinase and idioblast distribution in *Arabidopsis* and *Brassica napus*. Plant Physiology 127: 1750–1763.

Bartlet, E., G. Kiddle, I. Williams, and R. Wallsgrove. 1999. Wound induced increases in the glucosinolate content of oilseed rape and their effect on subsequent herbivory by a crucifer specialist. Entomologia Experimentalis et Applicata 91: 163–167.

Bartlet, E., M.M. Blight, A.J. Hick, and I.H. Williams. 1993. The responses of the cabbage seed weevil (*Ceutorhynchus assimilis*) to the odour of oilseed rape (*Brassica napus*) and some volatile isothiocyanates. Entomologia Experimentalis et Applicata 68: 295-302.

Bartlet, E., M.M. Blight, P. Lank, and I.H. Williams. 1997. The responses of the cabbage seed weevil *Ceutorhynchus assimilis* to volatile compounds from oilseed rape in a linear track olfactometer. Entomologia Experimentalis et Applicata 85: 257-262.

Birch, A.N.E., D.W. Griffiths, R.J. Hopkins, W.H. Macfarlane Smith, and R.G. McKinlay. 1992. Glucosinolate responses of swede, kale, forage and oilseed rape to root damage by turnip root fly (*Delia floralis*) larvae. Journal of the Science of Food and Agriculture 60: 1-9.

Blaney, W.M., L.M. Schoonhoven, and M.S.J. Simmonds. 1986. Sensitivity variations in insect chemoreceptors; a review. Cellular and Molecular Life Sciences 42: 13-19.

Blight, M.M., J.A. Pickett, L.J. Wadhams, and C. M. Woodcock. 1995. Antennal perception of oilseed rape *Brassica napus* (Brassicaceae) volatiles by the cabbage seed weevil, *Ceutorhynchus assimilis* (Coleoptera: Curculionidae). Journal of Chemical Ecology 21: 1649–1664.

Bodnaryk, R.P. 1991. Developmental profile of sinalbin (*p*-hydroxybenzyl glucosinolate) in mustard seedlings, *Sinapis alba* L., and its relationship to insect resistance. Journal of Chemical Ecology 17: 1543–1556.

Bodnaryk, R.P. 1992. Effects of wounding on glucosinolates in the cotyledons of oilseed rape and mustard. Phytochemistry 31: 2671–2677.

Bodnaryk, R.P. 1994. Potent effect of jasmonates on indole glucosinolates in oilseed rape and mustard. Phytochemistry 35: 301–305.

Bonnemaison, L. 1957. Le charançon des siliques (*Ceuthorhynchus assimilis* Payk.), biologie et méthodes de lutte. Annales des Épiphytes 4: 387-543.

Boodly, J.W., and R. Sheldrake. 1982. Cornell peat-lite mixes for commercial plant growing. New York State College of Agriculture and Life Sciences, Cornell University, Ithaca, New York.

Borek, V., and M.J. Morra. 2005. Ionic thiocyanate (SCN-) production from 4-hydroxybenzyl glucosinolate contained in *Sinapis alba* seed meal. Journal of Agricultural and Food Chemistry 53: 8650-8654.

Brodeur J., L. Leclerc, M. Fournier, and M. Roy. 2001. Cabbage seedpod weevil (Coleoptera: Curculionidae) a new pest of canola in northeastern North America. The Canadian Entomologist 133: 709–711.

Buntin, G. D., J.P. McCaffrey, P.L. Raymer, and J. Romero. 1995. Quality and germination of rapeseed and canola seed damaged by adult cabbage seedpod weevil, *Ceutorhynchus assimilis* (Paykull) [Coleoptera: Curculionidae]. The Canadian Journal of Plant Science 75: 539-541.

Cárcamo, H.A., L. Dosdall, M. Dolinski, O. Olfert, and J.R. Byers. 2001. The cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae) - a review. Journal of the Entomological Society of British Columbia 98: 201-210.

Cárcamo, H.A., R. Dunn, L.M. Dosdall, and O. Olfert. 2007. Managing cabbage seedpod weevil in canola using a trap crop – a commercial field-scale study in western Canada. Crop Protection 26: 1325-1334.

Cole, R.A. 1976. Isothiocyanates, nitriles, and thiocyanates as products of autolysis of glucosinolates in Cruciferae. Phytochemistry 15: 759-762.

Dickens. J.C., and E.E. Moorman. 1990. Maturation and maintenance of electroantennogram responses to pheromone and host odors in boll weevils fed their host plant or an artificial diet. Zeitschrift fur Angewandte Entomologie 109: 470-480.

Dmoch, J. 1965. The dynamics of a population of the cabbage seedpod weevil (Ceutorhynchus assimilis Payk.) and the development of winter rape. Part I. Ekologia Polska Ser. A 13: 249–287.

Dosdall L.M., and L.S. Kott. 2006. Introgression of resistance to cabbage seedpod weevil to canola from yellow mustard. Crop Science 46: 2437-2445.

Dosdall L.M., D. Moisey, H. Cárcamo, and R. Dunn. 2001. Cabbage seedpod weevil factsheet. Alberta Agriculture, Food and Rural Development Agdex 4: 622-624.

Dosdall, L.M., R.M. Weiss, O. Olfert, and H.A. Cárcamo. 2002. Temporal and geographical distribution patterns of cabbage seedpod weevil (Coleoptera: Curculionidae) in canola. The Canadian Entomologist 134: 403-418.

Dosdall L.M., and D.W.A. Moisey. 2004. Developmental biology of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae), in spring canola, *Brassica napus*, in western Canada. Annals of the Entomological Society of America 97: 458-465.

Doughty, K.J., G.A. Kiddle, B.J. Pye, R.M. Wallsgrove, and J.A. Pickett. 1995. Selective induction of glucosinolates in oilseed rape leaves by methyl jasmonate. Phytochemistry 38: 347-350.

Evans, K.A., and L.J. Allen-Williams. 1992. Electroantennogram responses of the cabbage seed weevil, *Ceutorhynchus assimilis*, to oilseed rape, *Brassica napus* ssp. *oleifera*, volatiles. Journal of Chemical Ecology 18: 1641-1659.

Evans, K.A., and L. J. Allen-Williams. 1993. Distant olfactory response of the cabbage seed weevil, *Ceutorhynchus assimilis*, to oilseed rape odour in the field. Physiological Entomology 18: 251-256.

Evans, K.A., and L.J. Allen-Williams. 1998. Response of cabbage seed weevil (*Ceutorhynchus assimilis*) to baits of extracted and synthetic host-plant odor. Journal of Chemical Ecology 24: 2101-2114.

Feeny, P. P. 1976. Plant apparency and chemical defence. Recent Advances in Phytochemistry 10: 1-40.

Fox, A.S., and L.M. Dosdall. 2003. Reproductive biology of *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae) on wild and cultivated Brassicaceae in southern Alberta. Journal of Entomological Science 38: 365-376.

Free, J.B., and I.H. Williams. 1978. The responses of the pollen beetle, *Meligethes aeneus,* and the seed weevil, *Ceutorhynchus assimilis,* to oilseed rape, *Brassica napus,* and other plants. Journal of Applied Ecology 15: 761-764.

Gong, F., Y. Lian, Y. Fung, and F. Chau. 2004. Correction of retention time shifts for chromatographic fingerprints of herbal medicine. Journal of Chromatography A 1029: 173-183.

Harper, F.R., and B. Berkenkamp. 1975. Revised growth-stage key for *Brassica campestris* and *B. napus*. The Canadian Journal of Plant Science 55: 657–658.

Hopkins, R.J., B. Ekbom, and L. Henkow. 1998. Glucosinolate content and susceptibility for insect attack of three populations of *S. alba*. Journal of Chemical Ecology 24: 1203–1216.

Kalischuk, A.R., and L.M. Dosdall. 2004. Susceptibilities of seven Brassicaceae species to infestation by the cabbage seedpod weevil (Coleoptera: Curculionidae). The Canadian Entomologist 136: 265-276.

Lee, K.C., M.W. Cheuk, W. Chan, A.W.M. Lee, Z.Z. Zhao, Z.H. Jiang, and Z. Cai. 2006. Determination of glucosinolates in traditional Chinese herbs by high-performance liquid chromatography and electrospray ionization mass spectrometry. Analytical and Bioanalytical Chemistry 386: 2225-2232.

Mason, P.G., T. Baute, O. Olfert, and M. Roy. 2004. Cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae) in Ontario and Quebec. Journal of the Entomological Society of Ontario 134: 107–113.

McCaffrey, J. P. 1992. Review of the U.S. canola pest complex: cabbage seedpod weevil, pp. 140-143. *In* Proceedings, 1992 U.S. Canola Conference, 5-6 March 1992. Ameri-Can Pedigreed Seed Company, Memphis, TN.

McCaffrey, J.P., B.L. Harmon, J. Brown, A.P. Brown, and J.B. Davis. 1999. Assessment of *Sinapis alba*, *Brassica napus* and *S. alba* x *B. napus* hybrids for resistance to cabbage seedpod weevil, *Ceutorhynchus assimilis* (Coleoptera: Curculionidae). Journal of Agricultural Science 132: 289-295.

McCloskey, C., and M.B. Isman. 1993. Influence of foliar glucosinolates in oilseed rape and mustard on feeding and growth of the bertha armyworm, *Mamestra configurata* Walker. Journal of Chemical Ecology 19: 249-266.

McLeod, J.H. 1962. Cabbage seedpod weevil – *Ceutorhynchus assimilis* (Payk.) Curculionidae. *In*: A Review of the Biological Control Attempts Against Insects and Weeds in Canada. Blackwell Publishing.

Mewis, I., C. Ulrichs, W.H. Schnitzler. 2002. Possible role of glucosinolates and their hydrolysis products in oviposition and host-plant finding by cabbage

webworm, *Hellula undalis*. Entomologia Experimentalis et Applicata 105: 129–139.

Miall, R.C. 1978. The flicker fusion frequencies of six laboratory insects, and the response of the compound eye to mains fluorescent 'ripple'. Physiological Entomology 3: 99-106.

Mithen, R. 1992. Leaf glucosinolate profiles and their relationship to pest and disease resistance in oilseed rape. Euphytica 63: 71–83.

Moyes, C.L., and A.F. Raybould. 2001. The role of spatial scale and intraspecific variation in secondary chemistry in host-plant location by *Ceutorhynchus assimilis* (Coleoptera: Curculionidae). Proceedings of the Royal Society of London 268: 1567-1573.

Moyes C.L., H.A. Collin, G. Britton, and A.F. Raybould. 2000. Glucosinolates and differential herbivory in wild populations of *Brassica oleracea*. Journal of Chemical Ecology 26: 2625-2641.

Porter, A.J.R., A.M. Morton, G. Kiddle, K.J. Doughty, and R.M. Wallsgrove. 1991. Variation in the glucosinolate content of oilseed rape (*Brassica napus* L.) leaves. I, Effect of leaf age and position. Annals of Applied Biology 118: 461-467.

Rask, L., E. Andréasson, B. Ekbom, S. Eriksson, B. Pontoppidan, and J. Meijer. 2000. Myrosinase: Gene family evolution and herbivore defence in Brassicaceae. Plant Molecular Biology 42: 93-113.

Renner, M. 1957. Neue Versuche uber den Zeitsinn der Honigbiene. Zeitschrift für Vergleichende Physiologie 40: 40-85.

Rochfort, S.J., V.C. Trenerry, M. Imsic, J. Panozzo, and R. Jones. 2008. Class targeted metabolomics: ESI ion trap screening methods for glucosinolates based on MS^n fragmentation. Phytochemistry 69: 1671–1679.

Ross, D., J. Brown, J. McCaffrey, and J.B. Davis. 2006. Cabbage seedpod weevil resistance in canola (*Brassica napus* L.), yellow mustard (*Sinapis alba* L.) and canola x yellow mustard hybrids. Proceedings of the American Society of Agronomy-Crop Science Society of America –Soil Science Society of America International Annual Meetings. Indianapolis, Indiana. November 12-16, 2006.

SAS Institute. 2005. SAS, Version 9.1. SAS Institute, Inc., Cary, North Carolina.

Schoonhoven, L.M. 1969. Sensitivity changes in some insect chemoreceptors and their effect on food selection behavior. Proceedings of the Section of Sciences, Koninklijke Akademie van Wetenschappen te Amsterdam C 72: 491-498.

Shaw, E. 2008. The detection of biochemical markers for cabbage seedpod weevil (*Ceutorhynchus obstrictus*) resistance in *Brassica napus* L. x *Sinapis alba* L. germplasm. M.Sc. Thesis, University of Guelph, Guelph, ON. 155 pp.

Shields, E.J. 1980. Locomotory Activity of *Orius tristicolor* Under Various Intensities of Flickering and Non-Flickering Light. Annals of the Entomological Society of America 73: 74-77.

Shields, E.J. 1989. Artificial Light: Experimental Problems with Insects. Bulletin of the Entomological Society of America 35: 40-45.

Smart, L.E., and M.M. Blight. 1997. Field discrimination of oilseed rape, *Brassica napus* volatiles by cabbage seed weevil, *Ceutorhynchus assimilis*. Journal of Chemical Ecology 23: 2555-2567.

Smart, L. E., M.M. Blight, and A.J. Hick. 1997. The effect of visual cues and a mixture of isothiocyanates on trap capture of cabbage seed weevil, *Ceutorhynchus assimilis.* Journal of Chemical Ecology 23: 889-902.

Tansey, J.A., S.D. Noble, L.M. Dosdall, and B.A. Keddie. 2008. Visual cues influence host choices of the cabbage seedpod weevil. Proceedings of the Entomological Society of Alberta, 56th annual meeting. Edmonton, Alberta. November 6-8, 2008.

Tollsten L., and G. Bergstrom. 1988. Headspace volatiles of whole plant and macerated plant parts of *Brassica* and *Sinapis*. Phytochemistry 27: 4013–4018.

Ulmer, B.J., and L.M. Dosdall. 2006. Spring emergence biology of the cabbage seedpod weevil (Coleoptera: Curculionidae). Journal of Applied Entomology 99: 64-69.

Vaughn, S. F., and M.A. Berhow. 2005. Glucosinolate hydrolysis products from various plant sources: pH effects, isolation, and purification. Industrial Crops and Products 21: 193-202.

Visser, J.H. 1986. Host odor perception in phytophagous insects. Annual Review of Entomology 31: 121-144.

Walczak, B., M. Kelm, Z. Klukowski, L.E. Smart, A.W. Ferguson, and I.H. Williams. 1998. The effect of trap design and 2-phenylethyl isothiocyanate

on catches of stem weevils (*Ceutorhynchus pallidactylus* Marsh. and *C. napi* Gyll.) in winter oilseed rape. OILB WPRS Bulletin. 21: 141–146.

Chapter 5

Antixenosis and antibiosis modes of resistance to *Ceutorhynchus obstrictus* of novel *S. alba* x *B. napus* germplasm

Introduction

The cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (syn. *C. assimilis* (Paykull)) (Coleoptera: Curculionidae), is a serious pest of canola, *Brassica napus* L. and *Brassica rapa* L., and mustard, *Brassica juncea* (L.) Czern. in Europe and North America (McCaffrey 1992; Buntin et al. 1995; Dosdall et al. 2001). This European native is invasive in North America and was first reported in south-western British Columbia in 1931 (McLeod 1962). Its current North American range extends throughout continental U.S.A. (McCaffrey 1992); the weevil was documented near Lethbridge, Alberta in 1995 (Cárcamo et al. 2001); it was subsequently found in Québec and Ontario (Brodeur et al. 2001; Mason et al. 2004). Western North American populations are likely introduced from source locations in western or northern Europe, whereas north-eastern North American populations are introduced separately from Scandinavia or Russia (Laffin et al. 2005). *Ceutorhynchus obstrictus* is dispersing north and east from southern Alberta at approximately 55 km per year and is predicted to eventually occupy all western Canadian canola production regions (Dosdall et al. 2002).

Ceutorhynchus obstrictus is univoltine and overwinters as an adult in the soil below leaf litter (Dmoch 1965; Ulmer and Dosdall 2006a). Newly emerged adults feed initially on brassicaceous plants near their overwintering sites (Bonnemaison 1957); these plants provide sites for aggregation and mating and nutrients for sustenance and ovarian development (Fox and Dosdall 2003). Oviposition occurs in developing siliques; suitable hosts for larval development include *B. napus*, *B. rapa*, *Raphanus* spp. and to a lesser extent *Sinapis arvensis*

L. (Dmoch 1965). Larvae feed within pods during three instars and may consume 5-6 seeds (Dmoch 1965). Mature third instar larvae chew holes in pod walls through which they emerge and fall to the ground; pupation occurs in earthen cells. A period of 31-58 d is required for development from egg to adult in western Canadian spring canola (Dosdall and Moisey 2004).

White mustard, *Sinapis alba* L., is resistant to infestation by *C. obstrictus* (Doucette 1947; Kalischuk and Dosdall 2004), and consequently has been used as a source of host resistance in the development of intergeneric hybrid germplasm. Several lines developed through *S. alba* x *B. napus* that have proven resistant to *C. obstrictus* in field trials and laboratory experiments (Dosdall and Kott 2006). Mechanisms of resistance include variable responses to floral visual and olfactory cues (Tansey et al. 2008; Chapters 2, 3, 4). In a no-choice situation, *C. obstrictus* will oviposit and can complete larval development in *S. alba* (Dosdall and Kott 2006), but when given choices, weevils select species of more suitable hosts (Moyes and Raybould 2001; Kalischuk and Dosdall 2004). Non-preference or antixenosis and antibiosis resistance modes as per Painter (1951) and Kogan and Ortman (1978) have also been reported for *S. alba* and several novel *S. alba* x *B. napus* genotypes. Fewer eggs are deposited and *C. obstrictus* larvae experience increased development times and have lowered body weights in resistant plants (McCaffrey et al. 1999; Dosdall and Kott 2006).

Mixes of hydrolysis products of 3-butenyl, 4-pentenyl and 2-phenylethyl glucosinolates are attractive to *C. obstrictus* during their crop colonizing migration (Smart et al. 1997). Isothiocyanate hydrolysis products of these

compounds were individually attractive to *C. obstrictus* in a trapping study (Smart and Blight 1997). Although differences in oviposition and feeding preferences have been attributed in part to varying amounts of these attractive compounds, mechanisms of antibiosis resistance demonstrated for novel *S. alba* x *B. napus* germplasm (Dosdall and Kott 2006) remain unclear. McCaffrey et al. (1999) attributed prolonged development and reduced larval weights in *S. alba* to *p*-hydroxybenzyl glucosinolate (sinalbin). Differences in susceptibilities of *S. alba*, *B. napus*, and *B. rapa* to herbivores have been attributed to this compound (Bodnaryk 1991). However, introgressed genotypes tested by Dosdall and Kott (2006) did not express detectable amounts of sinalbin. These results indicate that another factor is involved in antibiosis and probably antixenosis resistance to *C. obstrictus*.

Shaw (2008) used high performance liquid chromatography (HPLC) analysis to examine upper cauline leaves of resistant and susceptible *S. alba* x *B. napus* lines. A peak was associated with an as yet uncharacterised compound (retention time 21.4 ± 0.03 min) that was determined, though myrosinase degradation, to be a glucosinolate. Peak height was inversely correlated with weevil scores (the mean numbers of larvae per pod from genotypes in replicated field trials) and, on average, the peak was 3.5 times larger in resistant than susceptible lines (Shaw 2008). This compound was later putatively identified through comparisons of HPLC data by retention time shifts corrected by linear interpolation (as per Gong et al. 2004) as 1-methoxy-3-indolylmethyl glucosinolate (Chapter 4). This compound is subject to induction in response to

herbivore feeding and methyl jasmonate and jasmonic acid application in Brassicaceae (Birch et al. 1992; Bodnaryk 1992, 1994; Doughty et al. 1995; Bartlet et al. 1999). Shaw (2008) also detected differences among resistant and susceptible *S. alba* x *B. napus* lines in the amounts of another as yet unidentified glucosinolate (retention time 20.5 ± 0.01 min) in seeds of immature pods; its peak height was correlated with weevil scores and, on average, the peak was 3.5 times greater in susceptible than resistant lines (Shaw 2008). This compound was later proposed to be 2-phenylethyl glucosinolate (Chapter 4).

Here, I present results of laboratory and greenhouse assessments of the feeding and oviposition preferences of *C. obstrictus* for resistant and susceptible novel *S. alba* x *B. napus* germplasm and the parental genotypes, *B. napus* and *S. alba.* I examined the development times and associated biomass of larvae that developed on these genotypes. Tests also included assessments of oocyte development of post-diapause, springtime adult females on resistant and susceptible novel germplasm, parental genotypes and an early-season food host, *Thlaspi arvense* L. (Dmoch 1965), that is resistant to another Brassicaceae oligophage, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae) (Palaniswamy et al. 1997; Gavloski et al. 2000). Potential effects of glucosinolate hydrolysis products were used to make inferences regarding the influence of detected glucosinolate polymorphisms (Shaw 2008) on feeding and oviposition preferences, larval development and oocyte development. Potential courses for resistance breeding and deployment strategies for novel germplasm are also discussed.

Methods and Materials

Plants and insects

Seed for test plants included *B. napus* var. Q2 (hereafter referred to as Q2), *S. alba* var. AC Pennant (hereafter referred to as *S. alba*), and several *C. obstrictus*-resistant and susceptible *S. alba* x *B. napus* genotypes. *Thlaspi arvense* seed was obtained from field-collected plants in central Alberta. Plants were propagated in a soilless growth medium consisting of a modified Cornell mix based on the recipe of Boodly and Sheldrake (1982) (Agriculture and Agri-Food Canada 2002) in a greenhouse chamber and maintained at 16:8 (L:D) and 60% relative humidity.

Adults of *C. obstrictus* for laboratory arena and larval development trials were captured using sweep nets in a commercial *B. napus* field near Lethbridge (49° 41′ 39″ N, 112° 49′ 58″ W). Weevils for arena trials were maintained for no more than two days on a diet of 10% sucrose solution and wild mustard flowers in 2006. Weevils for larval development assessments in 2006-2008 and weevils for laboratory arena trials in 2007 and 2008 were maintained on caged, potted *B. napus*. Weevils were maintained at 20 ± 1°C in all three years. Adult weevils for oocyte development assessments were captured using pyramidal wooden-framed mesh cages (1 m^2 base, 1 m high, following the design of Dosdall et al. (1996)) placed on overwintering sites in early May, 2007.

Laboratory Arena Trials

Approximately eight weeks after planting, pods 40 to 60 mm long were excised from plants. Female weevils were separated using a binocular microscope, and

pods of *B. napus* were used to confirm their ovipositional status prior to commencing the experiments (as per Harmon and McCaffrey 1997).

The experimental setup was similar to that of Harmon and McCaffrey (1997). All tests were replicated four times and used a pod to weevil ratio of 6:1. In 2006, each cage (18.5 cm x 18.5 cm x 8 cm high) held evenly spaced pods of seven genotypes in a randomized complete block design with eight blocks (56 pods per cage). The genotypes tested included Q2, *S. alba*, 171 S, 154 S, 145 MS, 173 R and 121 R: 'S' denotes a susceptible genotype, as determined in field assessments similar to those of Dosdall and Kott (2006), 'R' denotes a resistant genotype and 'MS' denotes moderate susceptibility. In 2007, two tests were conducted: six genotypes in a randomized complete block design with eight blocks were assessed in Test 1, and five genotypes in a randomized complete block design with eight blocks were assessed in Test 2. These included Q2, *S. alba*, 171 S, 154 S, 276 R and 214 R in Test 1 and Q2, *S. alba*, 145 MS, 149 R and 121 R in Test 2. In 2008, eight genotypes were assessed in a Latin square design (each genotype randomly represented once in each row and column). Tested genotypes included Q2, *S. alba*, 171 S, 154 S, 145 MS, 173 R, 276 R and 121 R.

In all tests, pods were removed after 24 h, dissected under a binocular microscope and numbers of weevil eggs and feeding marks per pod were counted. ANOVA (SAS proc MIXED) was used to assess differences in oviposition and feeding among genotypes in the randomized complete block and Latin square designs (SAS Institute 2005). Block was considered a random factor. Egg and

feeding mark numbers were $\log_{10}(x+0.1)$ transformed to achieve a normal distribution as necessary. Comparisons of Tukey-adjusted least squared means were conducted using the LSMEANS statement with the PDIFF option in proc MIXED (SAS Institute 2005).

Relationships of glucosinolate content, oviposition and feeding

Relationships between numbers of eggs deposited and feeding marks per pod by genotype and genotype-specific peak height associated with the uncharacterised glucosinolates detected by Shaw (2008) from seeds and cauline leaves were assessed using proc CORR (SAS Institute 2005). Relationships between mean numbers of eggs and mean numbers of feeding marks were also assessed using proc CORR (SAS Institute 2005). Relationships were assessed for genotypes Q2, 171 S, 154 S, 276 R, 214 R, 173 R, 149 R and 121 R.

Larval development times and weights

The experimental setup was similar to that of Dosdall and Kott (2006). Groups of 60 *C. obstrictus* adults (30 males: 30 females) per plant were confined in a no-choice situation to caged plants with abundant pods of 40 to 60 mm in length in a greenhouse chamber under similar conditions to those under which plants were grown. Weevils were introduced to caged plants in mid-August 2006 and mid-July 2007 and 2008. Genotypes tested included Q2, *S. alba*, 171 S, 154 S, 173 R, 276 R and 121 R in all three years. In 2006, genotypes tested also included 127 S, 145 MS, 276 R, 255 R, 214 R, 152 R, 149 R, 139 R, 137 R and 116 R, and in

2007 145 MS, 214 R, 149 R, and 139 R were also tested. Each genotype was replicated three to five times per year. After 24 h, weevils were removed and plants were transferred to a growth chamber maintained at 22 ± 1°C, 16:8 (L:D) and 60% relative humidity. In 2006 and 2007, 30-cm-diameter paper traps with 20 cm raised edges were fitted to plants ensuring a tight seal between stem and trap. In 2008, rectangular cardboard traps were fitted to plants. These were approximately 2700 cm^2 and had raised edges of 8 cm. Traps caught larvae as they fell from pods at the beginning of pre-puparial wandering. Larvae were removed, counted and frozen daily and later freeze-dried and weighed. ANOVA (SAS proc MIXED) was used to assess differences in larval development times (days) and weights of larvae reared from plants of different genotypes (SAS Institute 2005). Emergence times were $\log_{10}(x+0.1)$ transformed to achieve a normal distribution. Comparisons of Tukey-adjusted least squared means were conducted using the LSMEANS statement with the PDIFF option in proc MIXED (SAS Institute 2005).

Relationships of glucosinolate content, larval development times and larval weights

Relationships between mean larval weights and development times associated with each host genotype and host genotype-specific peak height associated with uncharacterised glucosinolates detected by Shaw (2008) from seeds and cauline leaves and relationships between mean larval weights and development times

were assessed using proc CORR (SAS Institute 2005). Relationships were assessed for genotypes Q2, 171 S, 154 S, 276 R, 214 R, 173 R, 149 R and 121 R.

Oocyte development

Groups of 30 randomly selected female weevils were introduced to mesh cages containing Q2, 171 S, 121 R, 145 MS, *S. alba* or *T. arvense* on 15 May 2007. Females were introduced directly from emergence cages and, as no potential host plants were present in emergence cages, not yet fed sufficiently on early season hosts to allow ovary development (Ni et al. 1990). Cages were replicated four times and set in four blocks along a greenhouse bench. Groups of 10 females were removed from each cage 30 May 2007, and stored in Kahle's solution for later dissection (as per Borror et al. 1981). Status of the most advanced of developing oocytes were assessed using a rating system modified from Bonnemaison (1957) and Fox and Dosdall (2003): 1, little oocyte development detected; 2, length of oocyte comparable to its width; 3, length of oocyte greater than its width; 4, oocyte swollen with dense yolk; 5, mature chorion in ovarioles or lateral oviducts. ANOVA (SAS proc MIXED) was used to assess differences among mean oocyte development ratings associated with weevils reared from each genotype in the randomized complete block design (SAS Institute 2005). Block and sample nested in cage were considered random factors. Comparisons of Tukey-adjusted least squared means were conducted using the LSMEANS statement with the PDIFF option in proc MIXED.

Results

Laboratory Arena Trials

In 2006, significant differences in *C. obstrictus* egg numbers per pod were detected among host plant genotypes ($F_{6, 42} = 50.87$; $P < 0.0001$) (Table 1). Oviposition was greater in pods of Q2 and all *S. alba* x *B. napus* lines than in *S. alba* ($P < 0.0001$ for all comparisons). There were no significant differences among Q2 and *S. alba* x *B. napus* lines ($P > 0.05$ for all comparisons). Significant differences in numbers of feeding marks were also apparent among host plant genotypes ($F_{6, 42} = 104.64$; $P < 0.0001$) (Table 5.1). Greater numbers of feeding marks per pod were observed on Q2 and all *S. alba* x *B. napus* lines than on *S. alba* ($P < 0.001$ for all comparisons).

In 2007, Test 1 indicated significant differences in levels of oviposition among genotypes ($F_{5, 35} = 95.91$; $P < 0.0001$) (Table 5.1). Oviposition was greater in pods of Q2 and all *S. alba* x *B. napus* lines than in *S. alba* and greater in Q2 and 154 S than in 214 R or 276 R ($P < 0.05$ for all comparisons). Test 1 also indicated significant differences in the numbers of feeding marks associated with the genotypes: ($F_{5, 35} = 308.90$; $P < 0.0001$) (Table 5.1). Weevils fed more frequently on Q2 and all *S. alba* x *B. napus* genotypes than on *S. alba* ($P < 0.05$ for all comparisons). More feeding marks were detected on Q2 than on 276 R or 214 R pods ($P < 0.05$).

Test 2 in 2007 indicated significant differences in egg deposition among host plant genotypes ($F_{4, 28} = 44.31$; $P < 0.0001$) (Table 1). Oviposition was more frequent in Q2 and 145 MS than the other genotypes tested, and

significantly more eggs were deposited on 121 R and 149 R than on *S. alba* ($P < 0.05$ for all comparisons). Test 2 also indicated significant differences in the numbers of feeding holes by genotype ($F_{4, 28} = 32.06$; $P < 0.0001$) (Table 5.1). Greater numbers of feeding marks were found on Q2 and 145 S than the other genotypes tested, and more feeding marks were recorded on 121 R and 149 R than on *S. alba* ($P < 0.05$ for all comparisons).

In 2008, significant differences in *C. obstrictus* egg numbers were also found among genotypes ($F_{6,174} = 16.63$; $P < 0.0001$) (Table 1). Weevils oviposited more frequently on Q2 and all *S. alba* x *B. napus* genotypes than on *S. alba* ($P < 0.001$ for all comparisons), and more frequently on Q2 and 154 S than on pods of 276 R ($P < 0.01$ for both comparisons). Similar numbers of eggs were deposited in Q2, 154 S, 171 S, 121 R and 173 R ($P > 0.05$ for all comparisons). There were also significant differences in the numbers of feeding holes associated with genotypes ($F_{6,174} = 45.08$; $P < 0.0001$) (Table 5.1). Weevils fed more frequently on pods of Q2 and all *S. alba* x *B. napus* genotypes than on *S. alba* ($P < 0.01$ for all comparisons), and more frequently on Q2, 171 S and 154 S than on 276 R ($P < 0.01$ for all comparisons) (Table 5.1). Similar numbers of feeding holes were detected in Q2, 154 S, 171 S, 173 R and 121 R pods ($P >0.05$ for all comparisons).

Relationships of glucosinolate content, oviposition and feeding

A significant correlation of oviposition and genotype-specific peak height associated with the unknown glucosinolate detected by Shaw (2008) from

immature seeds and later proposed to be 2-phenylethyl glucosinolate (Chapter 4) was detected (R= 0.7318; P = 0.0391). However, no relationship of numbers of feeding marks and peak height associated with this glucosinolate was detected (R= 0.5766; P = 0.1346). A negative relationship between unknown cauline leaf glucosinolate content (as per Shaw 2008), later proposed to be 1-methoxy-3-indolylmethyl glucosinolate (Chapter 4), and oviposition was significant (R= -0.9057; P = 0.0020). A relationship of peak height associated with this glucosinolate and mean feeding marks was also detected (R= -0.8165; P = 0.0134). Mean numbers of eggs and mean numbers of feeding marks were correlated (R= 0.9117; P = 0.0016). Excluding *B. napus* from analysis indicated no relationships of 2-phenylethyl glucosinolate content and eggs (R= 0.6757; P = 0.0957) or feeding marks (R= 0.5124; P = 0.2397). Negative relationships were apparent between 1-methoxy-3-indolylmethyl glucosinolate content and eggs (R= -0.8888; P = 0.0075) and between this glucosinolate and feeding marks (R= -0.7828; P = 0.0374).

Larval development times and weights

In 2006 significant differences were observed in the development times of *C. obstrictus* larvae reared from different host plant genotypes ($F_{15, 504}$ = 5.63; P < 0.0001) (Table 5.2). Larval development times were less for larvae reared on Q2, 171 S, 154 S, 145 MS and 116 R than for 214 R or *S. alba* (P < 0.05). Similar development times were detected for larvae reared from 127 S, 276 R, 255 R, 214 R, 173 R, 152 R, 149 R, 139 R, 137 R, 121 R and *S. alba* (Table 5.2). Difference

in larval weights among genotypes were also apparent ($F_{15, 504} = 3.67$; $P < 0.0001$). No significant differences were detected among larvae reared from Q2 and any of the *S. alba* x *B. napus* genotypes ($P > 0.05$), but larvae reared on plants of Q2, 171 S, 154 S, 127 S, 255 R and 173 R were significantly heavier than those from *S. alba* ($P < 0.05$). Similar weights were observed for larvae reared on the other genotypes and *S. alba* (Table 5.2).

Evaluations in 2007 also indicated significant differences among larval development times by host plant genotype ($F_{10, 182} = 4.64$; $P < 0.0001$). Development times were greatest for larvae reared from *S. alba* ($P < 0.05$). Development times for larvae reared on 139 R and 121 R were approximately three days longer than those associated with Q2, 171 S, 154 S, and 145 MS ($P < 0.05$) (Table 5.2). Larval weights also differed by host plant genotype ($F_{10, 182} = 4.57$; $P < 0.0001$). Weights of larvae reared on *S. alba* were significantly less than for larvae reared on all other host plant genotypes ($P < 0.05$). Larvae from Q2, 154 S, 171 S, 145 MS, 214 R, 149 R and 139 R were heavier than those from 276 R or 121 R ($P < 0.05$) (Table 5.2).

Evaluations in 2008 also indicated significant differences in the development times of *C. obstrictus* larvae among host plant genotypes ($F_{6, 938} = 46.36$; $P < 0.0001$). All genotypes produced third-instar larvae approximately seven to 10 days sooner than *S. alba* ($P < 0.05$). Development was approximately three days more rapid in Q2 and all other *S. alba* x *B. napus* genotypes than in 121 R ($P < 0.05$). Development was more rapid in 171 S than 173 R ($P < 0.05$) (Table 5.2). Larval weights also differed by genotype ($F_{6, 938} = 15.61$; $P < 0.0001$).

Weights of larvae reared on Q2, 154S and 171 S were greater than those reared on 276 R, 121 R or *S. alba*; weights of larvae reared on 173 R, 121 R and 276 R were greater than weights of larvae reared on *S. alba* ($P < 0.05$) (Table 5.2).

Relationships of glucosinolate content, larval development times and larval weights

A significant correlation of larval weights and genotype-specific peak height associated with the unknown glucosinolate detected by Shaw (2008) from immature seeds and inferred as 2-phenylethyl glucosinolate (Chapter 4) was detected ($R = 0.8915$; $P = 0.0029$). A significant negative correlation of larval development time and peak height was also evident ($R = -0.8863$; $P = 0.0034$). A negative correlation between unknown cauline leaf glucosinolate content (as per Shaw 2008), proposed to be 1-methoxy-3-indolylmethyl glucosinolate (Chapter 4), and larval weight was significant ($R = -0.7404$; $P = 0.0356$). A relationship of peak height associated with this glucosinolate and mean larval development time was also observed ($R = 0.8152$; $P = 0.0137$). Mean larval weights and development times were also negatively correlated ($R = -0.6233$; $P = 0.0173$). Excluding *B. napus* data from the analysis yielded similar results. Larval development times were related to 2-phenylethyl glucosinolate ($R = -0.8866$; $P = 0.0078$) and 1-methoxy-3-indolylmethyl glucosinolate ($R = 0.8018$; $P = 0.0301$). However, larval weight was related to 2-phenylethyl glucosinolate ($R = 0.8605$; $P = 0.0301$) but not 1-methoxy-3-indolylmethyl glucosinolate ($R = -0.6548$; $P = 0.1105$).

Oocyte development

Significant differences were observed in mean ovary development ratings among females reared on different host plant genotypes ($F_{5, 54}$ = 30.80; $P < 0.0001$). Faster ovarian development was detected in females associated with Q2, 171 S than 214 R, *S. alba* or *T. arvense* ($P < 0.05$) (Table 5.3). Ovarian development of females associated with 214 R exceeded that of females on *S. alba* and *T. arvense*, and ovarian development on *S. alba* significantly exceeded that of *T. arvense* ($P < 0.05$) (Table 3).

Discussion

Introgression of *S. alba* x *B. napus* produced several genotypes that demonstrated resistance to *C. obstrictus* in similar replicated field assessments conducted in Alberta (Dosdall and Kott 2006; Shaw 2008) and Ontario (Shaw 2008). Responses of *C. obstrictus* have been shown to vary among genotypes in behavioural assessments based on vision and olfaction (Tansey et al. 2008; Chapters 3, 4). Results of the current study indicate that mechanisms of resistance also include antibiosis and non-preference or antixenosis modes as defined by Painter (1951) and Kogan and Ortman (1978). The greatest differences in oviposition and feeding preferences among the host plant genotypes tested in laboratory arena trials were between susceptible genotypes and *S. alba*. Differences in oviposition and feeding were also demonstrated between resistant and susceptible lines. Although these genotypes were less preferred than

susceptible lines, they were still selected as oviposition sites and fed upon more frequently than *S. alba.*

McCaffrey et al. (1999) suggested that differences in *C. obstrictus* colonisation of *B. napus* and *S. alba* could be attributed in part to differences in attractive kairomones. Shaw (2008) determined that susceptible and resistant *S. alba* x *B. napus* lines tested in this study differed in the amounts of an uncharacterised glucosinolate detected in seeds of immature pods. Peak heights were correlated with weevil scores and, on average, were 3.5 times greater in susceptible than resistant lines (Shaw 2008). The identity of this compound was likely 2-phenylethyl glucosinolate (Chapter 4). Another peak proposed to correspond to 3-butenyl glucosinolate (Chapter 4) was also associated with seeds of all genotypes except *S. alba* (Shaw 2008). The additive or synergistic effects of 3-butenyl and 2-phenylethyl isothiocyanates on *C. obstrictus* responses have been demonstrated (Bartlet et al. 1993). A significant correlation of oviposition and levels of 2-phenylethyl glucosinolate suggest that differences in oviposition preferences detected in this study can be attributed in part to differences in 2-phenylethyl-glucosinolate content among test genotypes. However, there was no correlation of feeding and levels of 2-phenylethyl glucosinolate detected. This result indicates that oviposition behaviour may be more sensitive to the attractive or stimulant effects of this compound and/or feeding behaviour is more strongly influenced by a deterrent factor(s).

Effects of deterrent compounds likely also account for differences in weevil feeding and oviposition preferences detected in this study. Differences in

susceptibilities of *S. alba*, *B. napus*, and *B. rapa* to other insect herbivores have been attributed to *p*-hydroxybenzyl glucosinolate (sinalbin) content (Bodnaryk 1991). However, because differences in *C. obstrictus* oviposition between *S. alba* varieties that differed greatly in *p*-hydroxybenzyl glucosinolate were not detected, Ulmer and Dosdall (2006b) concluded that the demonstrated antixenosis could not be attributed to this compound. Sinalbin was not detected in any tissues of introgressed genotypes examined by Shaw (2008) and assessed in this study, so is not associated with differences in weevil feeding or oviposition preferences among these genotypes. However, significant negative correlations between peak heights of an uncharacterised cauline leaf glucosinolate (Shaw 2008), later proposed to be 1-methoxy-3-indolylmethyl glucosinolate (Chapter 4), and oviposition and feeding were detected. This correlation suggests that both behaviours are strongly influenced by this compound and/or its hydrolysis products.

Hydrolysis products of 1-methoxy-3-indolylmethyl glucosinolate include indole-isothiocyanates, indole-cyanides, indolyl-3-carbinol, thiocyanate and possibly phytoalexins and auxins (Mithen 1992; Mewis et al. 2002). Indole isothiocyanates, particularly 1-methoxy-3-indolylmethyl isothiocyanate, are suspected to contribute to reduced oviposition by the Brassicaceae oligophage *Hellula undalis* (Fabricius) (Lepidoptera: Pyralidae) (Mewis et al. 2002). Given the slight volatility of indole-cyanides, their role in long-distance olfactory responses of *C. obstrictus* seems unlikely and responses are likely limited to more

intimate ranges. These responses are apparently reflected in the differences in oviposition and feeding preferences among genotypes detected in this study.

Differences in adult feeding and oviposition preferences may also be associated with induced qualitative defences. Induction of 1-methoxy-3-indolylmethyl glucosinolate has been detected in *B. napus* in response to *P. cruciferae* feeding on cotyledons (Bodnaryk 1992) and exogenous application of methyl jasmonate and jasmonic acid (Doughty et al. 1995). *Delia floralis* (Fallén) (Diptera: Anthomyiidae) attack resulted in great systemic increases in 1-methoxy-3-indolylmethyl glucosinolate content in a *B. napus* oilseed variety (Birch et al. 1992). Induction of indole glucosinolates was also associated with reduced *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae) feeding on *B. napus* cotyledons (Bartlet et al. 1999).

A correlation of peak height associated with 1-methoxy-3-indolylmethyl glucosinolate (inferred identity of this compound as per Chapter 4) and weevil scores was not detected for seeds or pericarp tissue (Shaw 2008). However, correlations between levels detected in cauline leaf tissue and weevil responses observed in this study are likely not coincidental. Processing of seeds for chemical analysis, particularly heating samples to denature myrosinases, would not elicit the same responses as *C. obstrictus* feeding and/or oviposition; consequently induced responses may not have been detected by Shaw (2008). Induction of qualitative defences in plants has been shown to vary by mode of attack and herbivore species. Agrawal (2000) found that responses of two specialist and two generalist Lepidoptera larvae on *Raphanus sativus* L.

(Brassicaceae) differed after exposure to generalist or specialist herbivores. Schmelz et al. (2003) found that *Spodoptera exigua* (Hibner) (Lepidoptera: Noctuidae) feeding and exogenous application of volicitin (*N*-(17-hydroxylinolenoyl)-L-glutamine) from insect oral secretions to wounded plants resulted in greater induction of volatile emissions than mechanical damage alone. Differences in induced responses of these genotypes associated with different challenges and under different conditions require testing.

Laboratory assessments were conducted with excised pods. Induction of glucosinolates can be influenced by translocation among tissues. Although grafting experiments by Lein (1972) indicated that pericarp tissue is the major site of seed glucosinolate synthesis, provision of precursors for glucosinolate biosynthesis is associated in part with translocation from other tissues (Billsborough et al. 1993). Induction of indolyl glucosinolates and so differences in responses of *C. obstrictus* could have been affected by pod excision. However, Kim and Jander (2007) found induced increases in indole glucosinolates in response to *Myzus persicae* Sulzer (Homoptera: Aphididae) feeding to be similar in intact and excised *Arabidopsis thaliana* L. foliar tissue.

Significant differences in oviposition and feeding among Q2 and introgressed genotypes were not detected in 2006 (Table 1). Tests in 2006 were conducted in mid-August; those in 2007 and 2008 were conducted in mid-July. A lack of demonstrated feeding and oviposition preferences among introgressed genotypes and Q2 in 2006 was likely associated with physiological state/age of weevils. Reduced chemoreceptor sensitivity in insects is influenced by age;

sensillae become inoperative and sensory input to the central nervous system may decrease (Schoonhoven 1969; Blaney et al. 1986). Boll weevil, *Anthonomus grandis* (Boheman) (Coleoptera: Curculionidae), antennal chemoreceptors are most sensitive in the period coinciding with mating and host location (Dickens and Moorman 1990). For *C. obstrictus*, this period coincides with flowering and pod elongation in *B. napus* crops in mid June to mid July in western Canada (Dosdall and Moisey 2004).

Biomass of larvae associated with some resistant genotypes was significantly less than biomass associated with Q2 and some susceptible lines; these larvae were also delayed in their development. The significant correlation of larval weight and development time suggest that these measures are subject to the same factor(s). The correlations of 2-phenylethyl glucosinolate content and weevil scores (Shaw 2008), amounts of this glucosinolate in seeds and feeding, and oviposition preferences and larval weights implicate this compound as a feeding stimulant for larvae. The negative correlation of larval development times and 2-phenylethyl glucosinolate content also indicate its role as a feeding stimulant. However, 2-phenylethyl glucosinolate has also been detected in *S. alba* seed (Lim et al. 2008) again suggesting the importance of feeding deterrents and/or toxins in this interaction.

Greater development times and reduced larval weights associated with *S. alba* have been attributed to *p*-hydroxybenzyl glucosinolate content (McCaffrey et al. 1999; Dosdall and Kott 2006). Because *p*-hydroxybenzyl glucosinolate was not detected in any tissue examined in either Q2 or the introgressed genotypes

(Shaw 2008), another factor must be influencing larval development in resistant introgressed *S. alba* x *B. napus* lines. The potential effects of induced responses on larval feeding may also contribute to reductions in larval weights and prolonged development times demonstrated in this study and by Dosdall and Kott (2006). The correlation detected for peak heights associated with cauline leaves and larval development times and the inverse relationship of these peak heights and larval weights suggest the importance of this compound in antibiosis resistance demonstrated for some *S. alba* x *B. napus* genotypes. Prolonged *Pieris rapae* L. (Lepidoptera: Pieridae) development times were associated with *Brassica oleracea* L. expressing greater levels of 1-methoxy-3-indolylmethyl glucosinolate (Gols et al. 2008).

Sinalbin is the predominant glucosinolate in *S. alba* and has been reported to be subject to induction by insect feeding (Koritsas et al. 1991). Mechanical damage and *P. cruciferae* feeding resulted in reduced palatability of *S. alba* to these beetles (Palaniswamy and Lamb 1993). Indolyl glucosinolates, including 1-methoxy-3-indolylmethyl glucosinolate were also detected in all tissues of three cultivars of *S. alba* examined by Hopkins et al. (1998) and in all Brassicaceae tribes examined by Bäuerle et al. (1986) except Drabeae. Koritsas et al. (1991) reported that 1-methoxy-3-indolylmethyl glucosinolate levels increased more than 10-fold (from < 0.01 to 0.10 mg g^{-1}) in response to mechanical damage in *S. alba*. Antibiosis in *S. alba* may be subject to *p*-hydroxybenzyl glucosinolate levels (as per McCaffrey et al. 1999), 1-methoxy-3-indolylmethyl glucosinolate and a possible interaction of these compounds and their hydrolysis products. Additive

effects or an interaction would account for antibiosis demonstrated for *S. alba* in this study and others (McCaffrey et al. 1999; Dosdall and Kott 2006).

Induced responses can include transient and more long-lasting effects. Birch et al. (1992) found that indole-based glucosinolates were greatly increased in roots of oilseed and forage *B. napus* genotypes after *D. floralis* attack and peaked four weeks after first colonization. A similar, sustained induction of indolyl glucosinolates in the seed and pods of introgressed lines tested in this study could account for reduced feeding and so reduced weights and prolonged development times. Long-lasting effects of induction can also include abnormal tissue growth. Tryptophan-derived indolyl glucosinolates can act as precursors to auxins; galls, cankers and twisted leaves are associated with increased occurrence of these substances (Mithen 1992). Mithen (1992) suggested that these structures may be associated with reducing the spread of *Plasmodiophora brassicae* Woronin (Plasmodiophoraceae) or *Leptosphaeria maculans* (Desm.) Ces. and de Not. through the production of galls and stem cankers, respectively. The production of abnormal tissue growth within pods in response to *C. obstrictus* larvae has also been documented. Dosdall et al. (2001) describe the distorted appearance of infested pods in the field; they attribute this to compensatory growth of remaining seeds. *Ceutorhynchus obstrictus* eggs and larvae can be isolated from developing seeds by proliferating tissue in *B. napus* (Fox and Dosdall 2003). Comparisons of the occurrence of abnormal growth in susceptible and resistant introgressed genotypes are required.

Differences in oocyte development among the susceptible and resistant lines tested can also likely be attributed in part to combined effects of the same variable attractive and deterrent factors that influence feeding and oviposition. Ni et al. (1990) reported that food quality and post-diapause feeding duration influence egg development but that some ovarian development is possible on a diet of green host-plant material. An almost complete lack of oocyte development on *T. arvense* and reduced development on *S. alba* indicate strong antifeedant or potential toxic effects for *C. obstrictus* adults. I observed considerable activity of weevils caged on *T. arvense* within a day of introduction. Weevils were observed on cage walls rather than on plants (data not shown). This behaviour was unlike that of weevils in cages containing *B. napus*, introgressed genotypes or *S. alba*. Palaniswamy et al. (1997) reported profound antixenosis resistance of *T. arvense* to *P. cruciferae*. Many arthropods respond to the initial stages of starvation by increasing locomotory activity (Tanaka and Ito 1982 and references therein). This seems a likely scenario for *C. obstrictus* on *T. arvense*, given its high levels of activity and low levels of oocyte development on this host. Although Dmoch (1965) indicated that *T. arvense* is a good early-season food host for *C. obstrictus* in Europe, these results suggest this relationship may not apply to *C. obstrictus* and *T. arvense* populations in western Canada.

Potential antifeedant and toxic factors in *T. arvense* include flavone glycosides (Onyilagha et al. 2003), 1-methoxy-3-indolylmethyl glucosinolate hydrolysis products (Mewis et al. 2002) and substances associated with indolyl glucosinolate synthesis. Cadmium exposure induces a systemic shift from alkenyl

glucosinolate to indolyl glucosinolate in *T. arvense* (Tolrà et al. 2006). Because jasmonate is involved in the signal transduction pathway of cadmium (Xiang and Oliver 1998) and is a strong elicitor of indolyl glucosinolate synthesis in *A. thaliana* (Jost et al. 2005; Mewis et al. 2005), this response may be similar to induction associated with herbivory. Camalexin is a Brassicaceae phytoalexin that shares the first steps of synthesis with indolyl glucosinolates and is induced in *A. thaliana* in response to *Brevicoryne brassicae* L. (Homoptera: Aphididae) feeding and reduces aphid fecundity (Kuśnierczyk et al. 2008). Increased levels of *p*-hydroxybenzyl glucosinolate have also been detected in *T. arvense* in response to stress (Tolrà et al. 2006) and may be induced in response to herbivory.

Greater levels of feeding by both *C. obstrictus* adults and larvae and greater oviposition are likely attributable in part to greater amounts of attractive and lesser amounts of repellent and/or toxic glucosinolates and their hydrolysis products. Results of the current study indicate that both antibiosis and antixenosis resistance are expressed in some resistant introgressed lines but that levels of these resistance modes are relatively low. Although profound effects on weevil control attributable to these modes of resistance are unlikely, effects do occur and will have benefits for crop protection. Additionally, selective pressures associated with resistant genotypes should be relatively low and facilitate maintenance of these resistance traits. However, it will still be important to deploy this and other resistant germplasm in conjunction with susceptible refugia to maintain these traits. These results also suggest directions for further work. Rigorous assessment of inducibility, particularly of indolyl glucosinolates, and its effects on *C.*

obstrictus are essential. Combined effects of reduced levels of attractive glucosinolates (and subsequent hydrolysis products) and potential induced responses contributing to antixenosis and antibiosis resistance to this weevil and other insects should be exploited in further breeding efforts. Induction of indolyl glucosinolates is also influenced by pathogens. This suggests the potential to develop resistance to broad assemblages of commercially important organisms in Brassicaceous oilseed crops. In addition, assessments of *T. arvense* as a potential oilseed crop have recently been undertaken in North America (e.g. Isbell 2009). Factors favouring commercial exploitation of this species include much greater resistance than *B. napus* to *C. obstrictus* and other insect pests including *P. cruciferae* (Palaniswamy et al. 1997; Gavloski et al. 2000).

Tables

Table 5.1. Mean eggs per pod (S.E.) and mean feeding marks per pod (S.E.) associated with genotypes tested in laboratory arena trials August 2006, and July 2007 and 2008. 'S' denotes a susceptible *S. alba* x *B. napus* genotype, 'R' denotes a resistant genotype and 'MS' denotes moderate susceptibility. Different letter designations indicate significant differences in Tukey adjusted means detected by ANOVA.

Genotype	**2006**		**2007 Test 1**		**2007 Test 2**		**2008**	
Mean Eggs per Pod (S.E.)								
B. napus cv. Q2	3.50 (0.29)	a	4.06 (0.42)	a	2.44(0.36)	a	3.07 (0.45)	a
171 S	2.72 (0.65)	a	3.15 (0.78)	ab	-	-	3.32 (0.79)	ab
154 S	2.44 (0.66)	a	4.16 (0.21)	a	-	-	4.39 (0.71)	a
145 MS	3.03 (0.34)	a	-	-	2.16 (0.29)	a	-	-
173 R	2.84 (0.23)	a	-	-	-	-	2.18 (0.52)	ab
121 R	3.03 (0.21)	a	-	-	0.50 (0.25)	b	2.21 (0.45)	ab
276 R	-	-	2.41 (0.80)	b	-	-	1.64 (0.45)	b
214 R	-	-	2.19 (0.35)	b	-	-	-	-
149 R	-	-	-	-	0.93 (0.34)	b	-	-
S. alba cv. AC Pennant	0.00 (0.00)	b	0.00 (0.00)	c	0.00 (0.00)	c	0.04 (0.04)	c
Mean Feeding Marks per Pod (S.E.)								
B. napus cv. Q2	8.81 (1.30)	a	9.50 (1.28)	a	5.06 (0.54)	a	12.14 (1.43)	a
171 S	6.53 (1.07)	a	6.66 (1.46)	ab	-	-	11.96 (1.09)	a
154 S	6.19 (1.07)	a	6.75 (0.66)	ab	-	-	10.46 (0.78)	a
145 MS	6.47 (1.28)	a	-	-	4.84 (0.45)	a	-	-
173 R	7.47 (0.78)	a	-	-	-	-	9.04 (1.10)	ab
121 R	5.94 (0.19)	a	-	-	2.03 (0.35)	b	8.89 (0.85)	ab
276 R	-	-	5.91 (1.71)	b	-	-	5.82 (0.79)	b
214 R	-	-	5.45 (0.45)	b	-	-	-	-
149 R	-	-	-	-	2.91 (0.35)	b	-	-
S. alba cv. AC Pennant	0.34 (0.22)	b	0.03 (0.03)	c	0.03 (0.03)	c	1.54 (0.57)	c

Table 5.2. Mean larval dry weights and development time from egg to emergence of third instar larvae (days) (S.E.) associated with genotypes tested in July 2006, 2007 and 2008. 'S' denotes a susceptible *S. alba* x *B. napus* genotype, 'R' denotes a resistant genotype and 'MS' denotes moderate susceptibility. Different letter designations indicate significant differences in Tukey adjusted means detected by ANOVA.

Genotype	***n***	**Mean weight (mg) (S.E.)**		**Mean days to emergence (S.E.)**	
			2006		
B. napus cv. Q2	64	1.57 (0.05)	a	15.83 (0.28)	a
171 S	57	1.50 (0.05)	a	15.02 (0.43)	a
154 S	24	1.53 (0.08)	a	15.29 (0.46)	a
127 S	14	1.53 (0.10)	a	17.29 (0.60)	ab
145 MS	39	1.35 (0.06)	ab	16.18 (0.36)	a
276 R	4	1.13 (0.19)	ab	18.75 (1.12)	ab
255 R	19	1.48 (0.09)	a	16.95 (0.51)	ab
214 R	17	1.36 (0.09)	ab	18.06 (0.54)	b
173 R	46	1.41 (0.05)	a	17.00 (0.33)	ab
152 R	53	1.29 (0.05)	ab	16.75 (0.31)	ab
149 R	44	1.30 (0.06)	ab	17.45 (0.43)	ab
139 R	30	1.21 (0.07)	ab	17.17 (0.41)	ab
137 R	27	1.39 (0.07)	ab	16.59 (0.43)	ab
121 R	52	1.26 (0.05)	ab	16.21 (0.31)	ab
116 R	27	1.27 (0.07)	ab	15.19 (0.43)	a
S. alba cv. AC Pennant	3	0.97 (0.21)	b	20.33 (1.30)	b
			2007		
B. napus cv. Q2	37	1.68 (0.06)	a	17.46 (0.39)	a
171 S	39	1.59 (0.04)	a	16.82 (0.36)	a
154 S	29	1.65 (0.06)	a	17.10 (0.42)	a
145 MS	18	1.44 (0.08)	a	17.11 (0.80)	a
276 R	3	1.20 (0.19)	b	17.67 (0.67)	ab
214 R	5	1.38 (0.15)	a	17.60 (0.40)	ab
173 R	11	1.36 (0.06)	ab	18.09 (0.49)	ab
149 R	10	1.36 (0.11)	a	18.10 (0.67)	ab
139 R	17	1.38 (0.09)	a	20.82 (1.23)	b
121 R	20	1.30 (0.09)	b	20.05 (0.75)	b
S. alba cv. AC Pennant	4	0.98 (0.17)	c	24.00 (0.70)	c
			2008		
B. napus cv. Q2	264	1.45 (0.02)	a	17.24 (0.18)	ab
171 S	207	1.38 (0.02)	a	16.50 (0.20)	a
154 S	87	1.35 (0.04)	a	17.69 (0.31)	ab
276 R	179	1.22 (0.03)	b	17.07 (0.22)	ab
173 R	47	1.23 (0.05)	ab	18.43 (0.42)	b
121 R	146	1.21 (0.03)	b	20.45 (0.24)	c
S. alba cv. AC Pennant	15	0.93 (0.09)	c	27.00 (0.76)	d

Table 5.3. Mean *Ceutorhynchus obstrictus* oocyte development ratings on susceptible and resistant host plants. 'S' denotes a susceptible *S. alba* x *B. napus* genotype, 'R' denotes a resistant genotype and 'MS' denotes moderate susceptibility. Oocyte development was rated on a five point scale: 1, little oocyte development detected; 2, length of oocyte comparable to its width; 3, length of oocyte greater than its width; 4, oocyte swollen with dense yolk; 5, mature egg in ovarioles or lateral oviducts. Different letter designations indicate significant differences in Tukey adjusted means detected by ANOVA.

Genotype	***n***	**Mean ovary development rating (S.E.)**	
B. napus cv. Q2	40	4.08 (0.17)	a
171 S	40	3.73 (0.17)	a
145 MS	30	3.63 (0.19)	ab
214 R	40	3.21 (0.17)	b
S. alba cv. AC Pennant	35	2.26 (0.18)	c
Thlaspi arvense	29	1.38 (0.20)	d

References

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

Agriculture and Agri-Food Canada. 2002. Soilless growth medium for plants: preparation. SOP code: 120.0000.00. 6 pp.

Bartlet, E., M.M. Blight, A.J. Hick, and I.H. Williams. 1993. The responses of the cabbage seed weevil (*Ceutorhynchus assimilis*) to the odour of oilseed rape (*Brassica napus*) and some volatile isothiocyanates. Entomologia Experimentalis et Applicata 68: 295-302.

Bartlet E., G. Kiddle, I. Williams, and R. Wallsgrove. 1999. Wound induced increases in the glucosinolate content of oilseed rape and their effect on subsequent herbivory by a crucifer specialist. Entomologia Experimentalis et Applicata 91: 163–167.

Bäuerle, R., H. Wagner, and H. Schraudolf. 1986. Distribution of 4-methoxy-3-indolylmethyl-glucosinolate (4-methoxy-glucobrassicin) in Brassicaceae. Cellular and Molecular Life Sciences 42: 86.

Billsborough, P.E., E.J. Evans, F. Murray, and F.J. Zhao. 1993. Glucosinolate changes in developing pods of single and double low varieties of autumn-sown oilseed rape (*B. napus*). Annals of Applied Biology 122: 135-143.

Birch, A.N.E., D.W. Griffiths, R.J. Hopkins, W.H. MacFarlane Smith, and R.G. McKinlay. 1992. Glucosinolate responses of swede, kale, forage and oilseed

rape to root damage by turnip root fly (*Delia floralis*) larvae. Journal of the Science of Food and Agriculture 60: 1-9.

Blaney, W.M., L.M. Schoonhoven, and M.S.J. Simmonds. 1986. Sensitivity variations in insect chemoreceptors; a review. Cellular and Molecular Life Sciences 42: 13-19.

Bodnaryk, R.P. 1991. Developmental profile of sinalbin (*p*-hydroxybenzyl glucosinolate) in mustard seedlings, *Sinapis alba* L., and its relationship to insect resistance. Journal of Chemical Ecology 17: 1543–1556.

Bodnaryk, R.P. 1992. Effects of wounding on glucosinolates in the cotyledons of oilseed rape and mustard. Phytochemistry 31: 2671–2677.

Bodnaryk, R.P. 1994. Potent effect of jasmonates on indole glucosinolates in oilseed rape and mustard. Phytochemistry 35: 301–305.

Bonnemaison, L. 1957. Le charançon des siliques (Ceutorhynchus assimilis Payk.) biologie et méthode de lutte. Annales Épiphytes 4: 387–543.

Boodly, J.W., and R. Sheldrake. 1982. Cornell Peat-Lite Mixes for Commercial Plant Growing. An extension publication of the New York State College of Agriculture and Life Sciences, a statutory college of the state university, at Cornell University, Ithaca, New York.

Borror, D. J., D. M. De Long, and C. A. Triplehorn. 1981. An Introduction to the Study of Insects. 5th ed. Philadelphia: Saunders College Publishing.

Brodeur J., L. Leclerc, M. Fournier, and M. Roy. 2001. Cabbage seedpod weevil (Coleoptera: Curculionidae) a new pest of canola in northeastern North America. The Canadian Entomologist 133: 709–711.

Buntin, G. D., J.P. McCaffrey, P.L. Raymer, and J. Romero. 1995. Quality and germination of rapeseed and canola seed damaged by adult cabbage seedpod weevil, *Ceutorhynchus assimilis* (Paykull) [Coleoptera: Curculionidae]. The Canadian Journal of Plant Science 75: 539-541.

Dickens, J.C., and E.E. Moorman. 1990. Maturation and maintenance of electroantennogram responses to pheromone and host odors in boll weevils fed their host plant or an artificial diet. Zeitschrift fur Angewandte Entomologie 109: 470-480.

Cárcamo, H.A., L. Dosdall, M. Dolinski, O. Olfert, and J.R. Byers. 2001. The cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae) - a review. Journal of the Entomological Society of British Columbia 98: 201-210.

Dmoch, J. 1965. The dynamics of a population of the cabbage seedpod weevil (Ceutorhynchus assimilis Payk.) and the development of winter rape. Part I. Ekologia Polska. Ser. A 13: 249–287.

Dosdall, L.M., and L.S. Kott. 2006. Introgression of resistance to cabbage seedpod weevil to canola from yellow mustard. Crop Science 46: 2437-2445.

Dosdall, L. M., M. J. Herbut, N. T. Cowle, and T. M. Micklich. 1996. The effect of tillage regime on emergence of root maggots (*Delia* spp.) (Diptera: Anthomyiidae) from canola. The Canadian Entomologist 128: 1157-1165.

Dosdall, L.M., D. Moisey, H. Cárcamo, and R. Dunn. 2001. Cabbage seedpod weevil factsheet. Alberta Agriculture, Food and Rural Development Agdex 4: 622-624.

Dosdall, L.M., R.M. Weiss, O. Olfert, and H.A. Cárcamo. 2002. Temporal and geographical distribution patterns of cabbage seedpod weevil (Coleoptera: Curculionidae) in canola. The Canadian Entomologist 134: 403-418.

Dosdall L.M., and D.W.A. Moisey. 2004. Developmental biology of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae), in spring canola, *Brassica napus*, in western Canada. Annals of the Entomological Society of America 97: 458-465.

Doucette, C.F. 1947. Host plants of the cabbage seedpod weevil. Journal of Economic Entomology 40: 838-840.

Doughty, K.J., G.A. Kiddle, B.J. Pye, R.M. Wallsgrove, and J.A. Pickett. 1995. Selective induction of glucosinolates in oilseed rape leaves by methyl jasmonate. Phytochemistry 38: 347-350.

Fox, A. S., and L. M. Dosdall. 2003. Reproductive biology of *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae) on wild and cultivated Brassicaceae in southern Alberta. Journal of Entomological Science 38: 533-544.

Gavloski, J.E., U. Ekuere, A. Keddie, L. Dosdall, and L .Kott. 2000. Identification and evaluation of flea beetle (*Phyllotreta cruciferae*) resistance within Brassicaceae. The Canadian Journal of Plant Science 80: 881-887.

Gols, R., R. Wagenaar, T. Bukovinszky, N. M. van Dam, M. Dicke, J.M. Bullock, and J.A. Harvey. 2008. Genetic variation in defense chemistry in wild cabbages affects herbivores and their endoparasitoids. Ecology 89: 1616-1626.

Gong, F., Y. Lian, Y. Fung, and F. Chau. 2004. Correction of retention time shifts for chromatographic fingerprints of herbal medicine. Journal of Chromatography A 1029: 173-183.

Harmon, B.L., and J.P. McCaffrey. 1997. Laboratory bioassay to assess *Brassica* spp. germplasm for resistance to the cabbage seedpod weevil (Coleoptera Curculionidae). Journal of Economic Entomology 90: 1392–1399.

Hopkins, R.J., B. Ekbom, and L. Henkow. 1998. Glucosinolate content and susceptibility for insect attack of three populations of *Sinapis alba*. Journal of Chemical Ecology 24: 1203–1216.

Isbell, T. 2009. U.S. effort in the development of new crops (Lesquerella, Pennycress and Cuphea). AFECG Journee Chevreul Conference, 4/7/09, Paris, France, p. 7.

Jost, R., L. Altschmied, E. Bloem, J. Bogs, J. Gershenzon, U. Hahnel, R. Hansch, T. Hartmann, S. Kopriva, C. Kruse, R.R. Mendel, J. Papenbrock, M. Reichelt, H. Rennenberg, E. Schnug, A. Schmidt, S. Textor, J. Tokuhisa, A. Wachter, M. Wirtz, T. Rausch, and R. Hell. 2005. Expression profiling of metabolic genes in response to methyl jasmonate reveals regulation of genes of primary and secondary sulphur related pathways in *Arabidopsis thaliana*. Photosynthesis Research 86: 491–508.

Kalischuk, A.R., and L.M. Dosdall. 2004. Susceptibilities of seven Brassicaceae species to infestation by the cabbage seedpod weevil (Coleoptera: Curculionidae). The Canadian Entomologist 136: 265-276.

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

Kogan, M., and E.F. Ortman. 1978. Antixenosis—A new term proposed to define Painter's 'non preference' modality of resistance. Bulletin of the Entomological Society of America 24: 175–176.

Koritsas, V.M, J. A. Lewis, and G. R. Fenwick. 1991. Glucosinolate responses of oilseed rape, mustard and kale to mechanical wounding and infestation by cabbage stem flea beetle (*Psylliodes chrysocephala*). Annals of Applied Biology 118: 209 – 221.

Kuśnierczyk, A., P. Winge, T.S. Jørstad, J. Troczynska, J.T. Rossiter, and A.M. Bones. 2008. Towards global understanding of plant defence against aphids – timing and dynamics of early *Arabidopsis* defence responses to cabbage aphid (*Brevicoryne brassicae*) attack. Plant, Cell and Environment 31: 1097–1115.

Laffin, R.D., L.M. Dosdall, and F.A.H. Sperling. 2005. Population structure of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae): Origins of North America introductions. Environmental Entomology 34: 504-510.

Lein, K.A. 1972. Genetische und physiologische Untersuchungen zur Bildung Von Glucosinolaten in Rapssamen: Lokalisierung des Hauptbiosyntheseortes durch Pfropfung. Zeitschrift für Pflanzenphysiologie 67: 333-342.

Lim, J.H., H.W. Kim, J.H. Jeon, and H.S. Lee. 2008. Acaricidal constituents isolated from *Sinapis alba* L. seeds and structure−activity relationships. Journal of Agricultural and Food Chemistry 56: 9962-9966.

Mason, P.G., T. Baute, O. Olfert, and M. Roy. 2004. Cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae) in Ontario and Quebec. Journal of the Entomological Society of Ontario 134: 107–113.

McCaffrey, J. P. 1992. Review of the U.S. canola pest complex: cabbage seedpod weevil, pp. 140-143. *In* Proceedings, 1992 U.S. Canola Conference, 5-6 March 1992. Ameri-Can Pedigreed Seed Company, Memphis, TN.

McCaffrey, J.P., B.L. Harmon, J. Brown, A.P. Brown, and J.B. Davis. 1999. Assessment of *Sinapis alba*, *Brassica napus* and *S. alba* x *B. napus* hybrids for resistance to cabbage seedpod weevil, *Ceutorhynchus assimilis* (Coleoptera: Curculionidae). Journal of Agricultural Science 132: 289-295.

McLeod, J.H. 1962. Cabbage seedpod weevil – *Ceutorhynchus assimilis* (Payk.) Curculionidae. *In*: McLeod, J.H., McGugan, B.M., and Coppel, H.C. (eds.), A review of the biological control attempts against insects and weeds in Canada. Commonwealth Agricultural Bureau (CAB), Farnham Royal, Bucks, UK, pp. 5-6.

Mewis, I., C. Ulrichs, and W.H. Schnitzler. 2002. Possible role of glucosinolates and their hydrolysis products in oviposition and host-plant finding by cabbage webworm, *Hellula undalis*. Entomologia Experimentalis et Applicata 105: 129–139.

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

Mithen, R. 1992. Leaf glucosinolate profiles and their relationship to pest and disease resistance in oilseed rape. Euphytica 63: 71–83.

Moyes, C.L., and A.F. Raybould. 2001. The role of spatial scale and intraspecific variation in secondary chemistry in host-plant location by *Ceutorhynchus assimilis* (Coleoptera: Curculionidae). Proceedings of the Royal Society of London 268: 1567-1573.

Ni, X.Z., J.P. McCaffrey, R.L. Stoltz, and B.L. Harmon. 1990. Effects of postdiapause adult diet and temperature on oogenesis of the cabbage seedpod weevil (Coleoptera: Curculionidae). Journal of Economic Entomology 83: 2246–2251.

Onyilagha, J., A. Bala, R. Hallett, M. Gruber, S. Soroka, and N. Westcott. 2003. Leaf flavonoids of the cruciferous species, *Camelina sativa*, *Crambe* spp., *Thlaspi arvense* and several other genera of the family Brassicaceae. Biochemical Systematics and Ecology 31: 1309-1322.

Painter, R.H. 1951. Insect Resistance in Crop Plants. University of Kansas, Lawrence, KS.

Palaniswamy, P., and R.J. Lamb. 1993. Wound-induced antixenotic resistance to flea beetles, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae), in crucifers. The Canadian Entomologist 125: 903-912.

Palaniswamy, P., R.J. Lamb, and R.P. Bodnaryk. 1997. Antibiosis of preferred and non-preferred host-plants for the flea beetle, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae). The Canadian Entomologist 129: 43-49.

SAS Institute. 2005. SAS, Version 9.1. SAS Institute, Inc., Cary, North Carolina.

Schmelz, E.A., H.T. Alborn**,** and J.H. Tumlinson. 2003**.** Synergistic interactions between volicitin, jasmonic acid and ethylene mediate insect-induced volatile emission in *Zea mays*. Physiologia Plantarum 117: 403-412.

Schoonhoven, L.M. 1969. Sensitivity changes in some insect chemoreceptors and their effect on food selection behavior. Proceedings of the Section of Sciences, Koninklijke Akademie van Wetenschappen te Amsterdam C 72: 491-498.

Shaw, E. 2008. The detection of biochemical markers for cabbage seedpod weevil (*Ceutorhynchus obstrictus*) resistance in *Brassica napus* L. x *Sinapis alba* L. germplasm. M.Sc. Thesis, University of Guelph, Guelph, ON. 155 pp.

Smart, L.E., and M.M. Blight. 1997. Field discrimination of oilseed rape, *Brassica napus* volatiles by cabbage seed weevil, *Ceutorhynchus assimilis*. Journal of Chemical Ecology 23: 2555-2567.

Smart, L. E., M.M. Blight., and A.J. Hick. 1997. The effect of visual cues and a mixture of isothiocyanates on trap capture of cabbage seed weevil, *Ceutorhynchus assimilis.* Journal of Chemical Ecology 23: 889-902.

Tanaka, K., and Y. Ito. 1982. Decrease in respiratory rate on a spider, *Pardosa astigera* (Koch), under starvation. Research in Population Ecology 24: 360–374.

Tansey, J.A., S.D. Noble, L.M. Dosdall, and B.A. Keddie. 2008. Visual cues influence host choices of the cabbage seedpod weevil. Proceedings of the Entomological Society of Alberta, 56th annual meeting. Edmonton, Alberta. November 6-8, 2008.

Tolrà, R., P. Pongrac, C. Poschenrieder, K. Vogel-Mikus, M. Regvar, and J. Barceló. 2006. Distinctive effects of cadmium on glucosinolate profiles in Cd hyperaccumulator *Thlaspi praecox* and non-hyperaccumulator *Thlaspi arvense*. Plant and Soil 288: 333–341.

Ulmer B.J., and L.M. Dosdall. 2006a. Spring emergence biology of the cabbage seedpod weevil (Coleoptera: Curculionidae). Annals of the Entomological Society of America 99: 64-69.

Ulmer, B.J., and L.M. Dosdall. 2006b. Glucosinolate profile and oviposition behaviour in relation to the susceptibilities of different Brassicaceae to the

cabbage seedpod weevil. Entomologia Experimentalis et Applicata 121: 203-213.

Xiang, C.B., and B.J. Oliver. 1998. Glutathione metabolic genes co-ordinately respond to heavy metals and jasmonic acid in *Arabidopsis*. Plant Cell 10: 1539–1550.

Chapter 6

Incorporation of novel *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae)-resistant canola genotypes into mixed cropping strategies and its effects on weevil spatial dynamics

Introduction

The cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (syn. *C. assimilis* (Paykull)) (Coleoptera: Curculionidae), is a serious alien invasive pest of brassicaceous oilseed crops in North America, especially canola, *Brassica napus* L. and *Brassica rapa* L., and mustard, *Brassica juncea* (L.) Czern. (McCaffrey 1992; Buntin et al. 1995; Dosdall et al. 2001). It is native to Europe and was first reported in south western British Columbia in 1931 (McLeod 1962). It has since dispersed or was accidentally introduced throughout continental U.S.A. (McCaffrey 1992), and was recently found in Québec and Ontario (Brodeur et al. 2001; Mason et al. 2004). In the Canadian prairies, *C. obstrictus* was first reported near Lethbridge, Alberta in 1995 (Cárcamo et al. 2001), and is dispersing north and east from southern Alberta at approximately 55 km per year. The weevil reached western Saskatchewan by 2000 and is predicted to eventually occupy all western Canadian canola production regions (Dosdall et al. 2002).

Crosses of *Sinapis alba* L. x *B. napus* have produced several lines that are resistant to *C. obstrictus* in field trials and laboratory experiments (Dosdall and Kott 2006; Chapter 5). Mechanisms of resistance include antixenosis and antibiosis in resistant genotypes, resulting in fewer eggs deposited and increased development times of *C. obstrictus* larvae (McCaffrey et al. 1999; Dosdall and Kott 2006; Chapter 5). Reduced apparency of resistant lines has also been demonstrated; *C. obstrictus* adults are less responsive to visual and olfactory cues associated with resistant genotypes (Tansey et al. 2007, 2008; Chapters 2, 3). Deployment strategies for resistant genotypes require examination. Because toxic

cultivars are not evolutionarily sustainable in monoculture (Bernal et al. 2004), incorporation of susceptible refugia has been proposed for these genotypes as a means of reducing the potential evolution of search efficiency mechanisms and selective pressures associated with antibiosis (Tansey et al. 2008).

Polycultures of plants that vary in resistance to a particular pest have been employed by traditional and subsistence farmers for millennia as a means of stabilizing yields and reducing pest impacts. For instance, Mediterranean Bronze Age (ca. 2000 BC) farmers employed polycultures of olive, grape and wheat to increase production (Renfrew 1972). According to reviews by Elton (1958) and Pimentel (1961), arthropod pest outbreaks are more prevalent in monocultures than polycultures. Diversified agroecosystems tend to support lower densities of individual herbivore species than monocultures (Risch et al. 1983). Specialist herbivores immigrate less and emigrate more from polycultures than monocultures (Kareiva 1983; Elmstrom et al. 1988). Although most agroecosystem diversification has involved use of different plant species through intercropping, trap cropping and border cropping, varietal mixtures can also influence insect pest populations. Varietal mixture is a traditional practice employed by cassava, *Manihot esculenta* Crantz (Euphorbiaceae), farmers in Latin America and is associated with lowered herbivore populations (Lozano et al. 1980). Although detailed examinations of the use of mixed varieties or genotypes to control insect populations are relatively rare, those that have been conducted suggest their potential for reducing the impacts of insect pests. Mixed populations of glabrous and hirsute cotton genotypes incurred less damage by the

fleahopper, *Pseudatomoscelis seriatus* Reuter (Hemiptera: Miridae), and yielded more than either line grown alone (Benedict et al. 1986). Such reductions in herbivore feeding associated with genetic or taxonomic diversity are consistent with associational resistance (Tahvanainen and Root 1972).

Herbivorous insects use both visual and olfactory cues for host location and can demonstrate great selectivity among visual cues (Prokopy and Owens 1983). Host location can follow a stepwise process that incorporates directed flight in response to olfactory cues, visual responses to reflected spectral properties of potential host plants at relatively close range and chemical and tactile cues at intimate ranges (Kennedy 1965). Directed flight in response to visual cues has also been proposed (Moericke 1952). Variability among factors used by *C. obstrictus* as host association cues demonstrated for these *S. alba* x *B. napus* genotypes (Chapters 2, 3, 4) could prove influential in genotypically heterogeneous landscapes.

Site diversification may have other benefits. Maintaining a resistance trait depends primarily on mediating the selective pressures associated with the mechanisms of resistance (Gould 1998). Maintaining refuges of susceptibility can be an effective means of minimizing selective pressures and maintaining the efficacy of resistance traits in transgenic crops (Gould 1998; Bernal et al. 2004; Smith et al. 2007). Antibiosis resistance to *C. obstrictus* provides strong selective pressure on herbivorous insects (Bernal et al. 2004). Mixing resistant and susceptible lines may have added benefits of disrupting host association cues and providing refugia for insects that are susceptible to antibiosis resistance.

Here I present results of a small plot field study examining the effects of mixing *C. obstrictus*-susceptible and resistant genotypes in alternating strips in a region inhabited by a large, naturalized *C. obstrictus* population. Monocultures of susceptible and resistant genotypes and mixes with various proportions of resistant: susceptible genotypes. These planting arrangements were assessed for susceptibilities to infestation by *C. obstrictus* and to test the hypothesis that mixtures of genotypes deter attack by the weevil on susceptible host plant germplasm (associational resistance). I also discuss potential mechanisms of associational resistance in light of recent developments that indicate differences among host plant genotypes in visual and olfactory characteristics, and antixenosis and antibiosis resistance modes. Results of this study suggest resistant genotype deployment strategies that may best exploit these factors.

Methods and Materials

Plants and field setup

Seed was obtained from the germplasm collection of Dr. Laima S. Kott, University of Guelph, Guelph, ON. Host plant genotypes evaluated included the susceptible *B. napus* var. Q2 (hereafter referred to as Q2) and two *C. obstrictus*-resistant *S. alba* x *B. napus* genotypes: Accessions 173R and 121R. 'R' denotes resistant genotypes; these will hereafter be referred to as 173R and 121R.

Test plots were seeded near Lethbridge, Alberta (49° 41′ 39″ N, 112° 49′ 58″ W) within a 2.0 ha *B. napus* field in 2007 and an irrigated 10.7 ha *B. napus* field in 2008. Plots were surrounded by borders of *B. rapa.* Plots were 4 x

6 m in 2007 and 4 x 4 m in 2008 and seeded at a rate of 5.0 kg ha^{-1} (ca. 0.8 and 0.5 g of seed per row per plot, respectively); row spacing of 20 cm was used in both years. In 2007, planting arrangements included monocultures of *B. napus* Q2 and 121R, and mixes of 25, 50 and 75% Q2 and 75, 50 and 25% 121R, respectively. In 2008, monocultures of *B. napus* Q2, 121R, and 173R, and mixes of 25 and 75% Q2 interspersed with 173R and 25% Q2 interspersed with 121R were planted. The resistant line 173R was included in 2008 due to a shortage of 121R seed in that year. Genotypes were seeded in alternating rows for 50% mixes and evenly distributed, genotype-specific rows for 25 and 75% Q2 mixes. Planting arrangements were set in a four-block randomized complete block design (Figure 6.1).

Exit-hole counts

Entire, mature plants were removed on 25 August 2007 and 15 August 2008. In 2007, 10 plants per row from three rows in Q2 and 121R monocultures and six rows in mixed-genotype plots were sampled. In 2008, 7 to 10 entire plants per row from three rows per plot were sampled (2 rows of R in 25 and 50% S:R mixes; 2 rows of S in 75% S:R mixes). The numbers of larval exit-holes from all pods on these plants were recorded. In 2007, the numbers of larval exit-holes from 26,843 pods from 960 plants were assessed. In 2008, 46,818 pods from 687 plants were examined.

Weevil Counts

Four yellow, plastic bowl traps, half-filled with a 1:1 solution of water: propylene glycol antifreeze (Prestone Low Tox, Danbury, CT) were set 1 m, 45^{o} from the outside corners of each plot in both years. Trap dimensions were 15 cm in diameter and 9 cm deep, and were attached by a metal bracket to a metal post. The height of each bracket was adjusted on the post so the trap could be kept just above the top of the crop canopy throughout the study. Traps were sampled approximately weekly from 30 May to 5 August 2007 and 1 June to 10 August 2008. Weevils were preserved in 70% ethanol. Preserved *C. obstrictus* were dissected in the laboratory to determine sex and gravid status of females. Gravid status was determined by the presence or absence of chorionated oocytes in the ovarioles or lateral oviducts.

All analyses of *C. obstrictus* exit-hole and capture counts were conducted with SAS version 9.1 (SAS Institute 2005). Larval exit-hole counts (expressed as the number of holes per pod by genotype in individual plots) were compared among planting arrangements in both years using analysis of variance of the randomized complete block design (proc MIXED). Plant genotype and planting arrangement were considered fixed effects. Pair-wise *t*-tests ($P = 0.05$) with a pooled variance estimate from the ANOVA (PDIFF option in Proc MIXED Procedure) were used when significant factor effects or interactions of factors were apparent. For all comparisons, block was considered a random factor and treatment effects were considered significant at $P \leq 0.05$. Weekly weevil counts (total weevils, males, total and gravid females) from each bowl trap were

analyzed using a repeated measures analysis with Poisson generalized estimating equations (proc GENMOD). An exchangeable correlation structure was used for these and weekly weevil count analyses to account for correlation of counts from each trap. Pair-wise comparisons for weekly total *C. obstrictus*, males, total females and gravid females in catches were made using a Wald chi-square test (LSMEANS statement with DIFF option in proc GENMOD) (SAS Institute 2005).

Weevil Spatial Distribution

Spatial distributions of *C. obstrictus* adults collected at each sampling interval and exit-holes at the end of the season were calculated using Spatial Analysis by Distance IndicEs (SADIE) software (Perry 1998). Using tests of randomization, this analysis package performs permutations of observed insect counts among sampling units and assesses arrangements in species count data. Areas of clustering or gaps are identified by assigning an index that quantifies the contribution of sample counts to population clustering. The total distance that individuals must be moved between sampling locations to achieve as close to a regular distribution as possible are calculated (Perry 1998). Spatial patterns associated with weevil captures throughout the growing season and larval exit-holes at the end of the season were determined using the main SADIE index, I_a , the subsidiary index, J_a , and the distance, δ. Values of I_a near 1.0 indicate random distributions; values greater than 1.0 indicate aggregated distributions over the entire sample area (Perry and Klukowski 1997). I_a is defined as D/E_a where E_a is

the arithmetic mean distance to regularity for the randomized samples, and *D* is the minimum total distance that individuals need to move to achieve an identical number of individuals in all units (Perry 1998). The index, J_a, is used to assess differences among patterns. Values of J_a greater than 1.0 indicate a single major cluster; values less than 1.0 indicate two or more clusters. The distance from the centroid of the counts (*C*) to the centroid of the sample units (*P*) is calculated and denoted δ; this quantifies the degree to which individuals occupy the edge or the centre of the area defined by the sample units (Perry and Klukowski 1997). Clustering or non-randomness is estimated by the distance to regularity in rearrangements of the observed data (Perry 1998). Analyses used the maximum number of randomizations possible within the SADIE program. Spatial associations among male and female weevils and exit-hole distributions were calculated using similarities in the clustering indices of two sets of data (Winder et al. 2001; Perry and Dixon 2002); individual sample units are assigned specific aggregation measures allowing quantification of the contribution of a particular unit toward the distribution. Associations between capture counts of male, total female and gravid female *C. obstrictus* and mean exit-holes per pod per plot were evaluated by comparing local cluster indices associated with each data set. The SADIE index, *X*, represents the correlation coefficient associated with comparisons of clustering indices of two data sets. $X > 0$ for positive spatial association, $X = 0$ when association is random and $X < 0$ are negatively associated. The formal test of significance in spatial associations among data sets is according to the randomization method of Perry and Dixon (2002).

Results

2007 Exit-Hole Counts

Of the 26,632 pods examined, 12,622 had at least one larval emergence hole. No significant effect of planting arrangement was apparent ($F = 1.07$; df = 4, 85; $P = 0.3785$). The effect of plant genotype was significant ($F = 20.50$; df = 1, 85; $P < 0.0001$). Although the interaction of planting arrangement and genotype was not significant ($F = 1.01$; df = 2, 85; $P = 0.3675$), differences in the numbers of holes per pod associated with Q2 and 121R in different planting arrangements were detected. Similar numbers of holes per pod were detected for 121R in all planting arrangements ($P > 0.05$ or all comparisons). Significantly greater proportions of holes per pod were associated with Q2 than 121R in all planting arrangements ($P < 0.01$ for all comparisons) except for Q2 in 75% 121R plots; these had similar numbers of holes per pod as 121R in monocultures ($t = 1.24$; df = 1, 85; $P = 0.2196$), 121R in 75% 121R plots ($t = 1.54$; df = 1, 85; $P = 0.1275$), and 121R in 25% 121R plots ($t = 1.88$; df = 1, 85; $P = 0.0640$). Greater numbers of holes per pod were associated with Q2 in monocultures than in 75% 121R plots ($t = 2.12$; df = 1, 85; $P = 0.0369$) (Figure 6.2).

2008 Exit-hole Counts

Of the 46,818 pods examined, 14,510 had at least one larval emergence hole. No effect of planting arrangement was apparent ($F = 1.67$; df = 5, 60.1; $P = 0.1558$), but a significant effect of plant genotype was observed ($\chi^2 = 22.12$; df = 2, 60.5; $P < 0.0001$). Although the interaction of planting arrangement and genotype was

not significant (F = 0.20; df = 2, 62.6; P = 0.6559), pair-wise comparisons indicated differences in the numbers of holes per pod associated with Q2, 173R and 121R in different planting arrangements. Similar numbers of exit-holes per pod were detected for 121R and 173R in all planting arrangements ($P > 0.05$ for all comparisons). Significantly greater numbers of exit-holes per pod were associated with Q2 than either 121R or 173R in any planting arrangement ($P < 0.01$ for all comparisons). Fewer exit-holes per pod were associated with Q2 in 75% 121R plots than in monocultures (t = 2.03; df = 1, 60.5; P = 0.0464). Although differences were not significant, fewer holes per pod were associated with Q2 in 75% 173R plots than in monocultures (t = 1.69; df = 1, 60.5; P = 0.0961). Similar numbers of holes per pod were detected for Q2 in all other planting arrangements ($P > 0.05$ for all comparisons) (Figure 6.2).

2007 Weevil Counts

Total *C. obstrictus*

A total of 12,849 *C. obstrictus* were collected in bowl traps in 2007. Of these, 5,873 were male and 6,872 were female. Of the females, 3,859 were gravid. Significant differences in the total numbers of *C. obstrictus* captured by date were apparent (χ^2 = 6263; df = 8; $P < 0.0001$). The greatest number of weevils were captured 29 June when significantly more *C. obstrictus* were captured than on any other date ($P < 0.0001$ for all comparisons). A significant effect of planting arrangement was also apparent (χ^2 = 11.02; df = 4; P = 0.0264). Significantly more *C. obstrictus* were captured in Q2 monocultures than any other planting

arrangement ($P < 0.05$ for all comparisons). Although reduction in capture occurred with increasing 121R in the planting arrangement, significant differences among these arrangements were not detected ($P > 0.05$ for all comparisons) (Figure 6.3). No significant interaction of sampling date and planting arrangement was observed ($\chi^2 = 42.16$; df = 32; $P = 0.1080$).

Male *C. obstrictus*

A significant effect of date was detected ($\chi^2 = 58.07$; df = 8; $P < 0.0001$). Significantly more males were captured on 29 June 2007 than on any other sampling date ($P < 0.0001$ for all comparisons), and a second peak in male captures was detected on 10 August. Although a significant effect of planting arrangement was not detected ($\chi^2 = 8.34$; df= 4; $P = 0.0798$), pair-wise comparisons indicated that more males were captured in Q2 monocultures than in 121R monocultures or plots seeded with 50% 121R ($\chi^2 = 10.15$; df = 1; $P = 0.0014$, and $\chi^2 = 6.99$; df = 1; $P = 0.0082$, respectively) (Figure 6.3). No significant interaction of sampling date and planting arrangement was observed ($\chi 2 = 37.20$; df = 32; $P = 0.2419$).

Total Female *C. obstrictus*

The effect of date was significant ($\chi 2 = 64.11$; df= 8; $P < 0.0001$). Most females were captured on 29 June 2007. Although capture numbers were significantly greater on this sampling date than any other ($P < 0.0001$ for all comparisons), a second peak in female captures was also detected 10 August. The effect of

planting arrangement was also significant (χ^2 = 43.63; df = 4; P = 0.0340). More females were captured by traps in Q2 monocultures than in 100%, 75%, or 50% 121R plots (P < 0.001 for all comparisons) (Figure 6.3). No significant interaction of sampling date and planting arrangement was observed (χ^2 = 42.22; df= 32; P = 0.1068).

Gravid Female *C. obstrictus*

A significant effect of date was detected (χ^2 = 67.54; df= 8; P < 0.0001). More gravid females were captured on 29 June than any other date (P < 0.0001 for all comparisons). The effect of planting arrangement was also significant (χ^2= 20.04; df= 4; P = 0.0005). Significantly more gravid female *C. obstrictus* were captured in Q2 monocultures than in any other planting arrangement (P < 0.001 for all comparisons). Traps in plots with 25% 121R captured more gravid females than traps in 100% 121R plots (χ^2 = 4.18; df= 1; P = 0.0408) (Figure 6.3). No significant interaction of planting arrangement and sampling date was detected (χ^2 = 38.84; df= 32; P = 0.1887).

2008 Weevil Counts

Total *C. obstrictus*

A total of 10,993 *C. obstrictus* were collected in bowl traps in 2008. Of these, 4,896 were male and 6,057 were female. Of the females, 3,684 were gravid. Significant differences in the numbers of total *C. obstrictus* captured by date were apparent (χ^2 = 93.19; df = 9; P < 0.0001). Significantly more *C. obstrictus* were

captured on 18 June than any other date ($P < 0.0001$ for all comparisons). Although a significant effect of planting arrangement was not apparent ($\chi^2 = 10.74$; df = 5; $P = 0.0568$), pair-wise comparisons indicated differences. More *C. obstrictus* were captured in Q2 monocultures than in 75% 173R plots or 121R or 173R monocultures ($P < 0.05$ for all comparisons). Fewer *C. obstrictus* were captured in 75% 173R than 75% 121R plots ($\chi^2 = 5.92$; df = 1; $P = 0.0150$) (Figure 6.3). A significant interaction of sampling date and planting arrangement was also apparent ($\chi^2 = 61.69$; df= 45; $P = 0.0497$). More weevils were captured in susceptible monocultures on 18 June, 24 June and 1 July than in resistant monocultures.

Male *C. obstrictus*

A significant date effect was apparent ($\chi^2 = 88.22$; df= 9; $P < 0.0001$). Significantly more males were captured on 18 June than any other sampling date ($P < 0.0001$ for all comparisons). Although a significant effect of planting arrangement was not observed ($\chi^2 = 10.64$; df= 5; $P = 0.0591$), significantly more males were captured in Q2 monocultures than in 121R monocultures or 75% 173R plots; traps in 75% 121R plots captured more than those in 121R monocultures, or 75% 173R plots ($P < 0.05$ for all comparisons). A significant interaction of sampling date and planting arrangement was also apparent ($\chi^2 = 70.26$; df= 45; $P = 0.0094$) (Figure 6.3). More males were captured in susceptible monocultures on 18 June, 24 June, 1 July, 8 July, and 20 July than in resistant monocultures.

Total Female *C. obstrictus*

A significant date effect on total female captures was apparent (χ^2 = 86.16; df= 9; $P < 0.0001$). Significantly more females were captured on 18 June than any other sampling date ($P < 0.0001$ for all comparisons). Although no significant effect of planting arrangement was apparent (χ^2 = 6.39; df= 5; P = 0.2703), pair-wise comparisons indicated differences. Significantly more gravid females were captured in Q2 monocultures than in 75% 173R, 25% 173R or 121R monocultures. A significant interaction of sampling date and planting arrangement was also apparent (χ^2 = 63.00; df= 45; P = 0.0393) (Figure 6.3). More females were captured in susceptible monocultures 18 June, 24 June, and 20 July than in resistant monocultures. More females were captured 1 July in Q2 monocultures than in any other planting arrangement.

Gravid Female *C. obstrictus*

Gravid females were captured on all sampling dates. A significant date effect was apparent (χ^2 = 83.49; df = 9; $P < 0.0001$) as was an effect of planting arrangement (χ^2 = 16.62; df = 5; P = 0.0053). Significantly more gravid females were captured on 1 July than any other sampling date ($P < 0.0001$ for all comparisons). Significantly more females were captured in Q2 monocultures than in any other arrangement ($P < 0.01$ for all comparisons). A significant interaction of sampling date and planting arrangement was also apparent (χ^2 = 67.42; df= 45; P = 0.0168) (Figure 6.3). More gravid females were captured in susceptible than resistant

monocultures on 20 July. More gravid females were captured in susceptible monocultures than any other planting arrangement on 18 June and 1 July.

Weevil Spatial Distribution: 2007

The index I_a and its associated *P*-values indicated that *C. obstrictus* males were significantly aggregated in early June, before plants had produced buds or flowers ($I_a = 1.416$; $P = 0.022$). Males were also aggregated on 15 July, in late flowering to pod enlargement ($I_a = 1.416$; $P = 0.022$) and 10 August when pods were mature and few flowers were present ($I_a = 1.509$; $P = 0.012$). Females were aggregated on 15, 22 and 29 June, in early to mid-flowering ($I_a = 1.601$; $P = 0.004$, $I_a = 1.385$; $P = 0.029$, and $I_a = 1.503$; $P = 0.013$, respectively), on 15 July during late flowering to pod enlargement ($I_a = 1.361$; $P = 0.033$), and 10 August when pods were mature and few flowers were present ($I_a = 1.326$; $P = 0.045$). Gravid females were also aggregated on 15, 22 and 29 June ($I_a = 1.331$; $P = 0.047$, $I_a = 1.326$; $P = 0.047$, and $I_a = 1.544$; $P = 0.008$, respectively), on 15 July ($I_a = 1.387$; $P = 0.025$) and 10 August ($I_a = 1.459$; $P = 0.014$). The index J_a was not significantly different from unity (at $\alpha = 0.05$) on any date for males or total females indicating that weevils were distributed in multiple clusters. For gravid females, J_a significantly exceeded unity on 15 July ($J_a = 1.089$; $P = 0.010$) indicating that their distribution was characterised by a single aggregation on this date and multiple smaller aggregations on the other dates when distributions were aggregated. The values of δ associated with each date were consistently 0 to 2 m, indicating that aggregations occurred within individual 4 by 4 m plots. Values of δ, I_a, J_a and

associated *P*-values for male, total and gravid female *C. obstrictus* for all sampling dates in 2007 are presented in Table 6.1.

Distributions of males and females were significantly associated on all sampling dates examined ($P < 0.01$ for all associations). Male distributions and the mean values of larval exit-holes per plot were significantly associated on 15 June, during early flowering ($X = 0.3390$; $P = 0.0008$). Female distributions and mean values of larval exit-holes per plot were significantly associated on 15 and 29 June and on 15 July 2007 ($P < 0.025$ for all associations). Gravid female distributions and mean values of larval exit-holes per plot were also significantly associated 15 and 29 June and 15 July 2007 ($P < 0.025$ for all associations). Correlations among male, total and gravid female *C. obstrictus* and exit-hole counts for all sampling dates in 2007 are presented in Table 6.2.

Weevil Spatial Distribution: 2008

The index I_a and its associated *P*-values indicated that *C. obstrictus* males were again aggregated in late May, before most plants had germinated on this site ($I_a = 1.473$; $P = 0.020$) and 10, 18 and 24 June and 1 and 8 July, in early to late-flowering. They were also aggregated on 20 July during pod enlargement, and 5 August when pods were mature and very few flowers remained ($I_a = 1.458$; $P = 0.018$, and $I_a = 1.412$; $P = 0.025$, respectively). Females were aggregated on 10, 18 and 24 June in early to mid-flowering, and again on 29 July and 5 August when pods were mature and few flowers were present ($I_a = 2.085$; $P = 0.002$, and $I_a = 1.810$; $P = 0.001$, respectively). Gravid females were also aggregated on 18

and 24 June ($I_a = 1.535$; $P = 0.013$, and $I_a = 1.944$; $P = 0.002$, respectively) and again on 29 July and 4 August. The distributions of gravid females were not assessed on 30 May and 4 June due to low numbers in captures. The index J_a was not significantly different than unity (at $\alpha = 0.05$) on any date for males or total females indicating distribution of weevils into multiple clusters. For gravid females, J_a significantly exceeded unity 29 July ($J_a = 1.034$; $P = 0.044$) indicating that their distribution was characterised by a single aggregation on this date and multiple smaller aggregations on the other dates when distributions were found to be aggregated. The values of δ associated with each date were consistently on the order of 0 to 2 m, indicating that small aggregations occurred within individual plots. The greatest value of δ was associated with gravid females on 29 July (2.467 m). Values of δ, I_a, J_a and associated *P*-values for male, total and gravid female *C. obstrictus* for all sampling dates in 2008 are presented in Table 6.3.

Males and females were significantly associated on all of the sampling dates examined ($P < 0.01$ for all associations), except 30 May and 5 August. Male distributions and the mean values of larval exit-holes per plot were significantly associated 4 and 18 June, 1 and 8 July during early to mid-flowering ($P < 0.05$ for all associations). Female distributions and mean values of larval exit-holes per plot were significantly associated 18 June and 1 and 20 July 2008 ($P < 0.05$ for all associations). Dissociation of male and female counts and exit-holes was apparent 24 June ($X = -0.2328$; $P = 0.9880$ and $X = -0.3337$; $P = 0.9990$, respectively). Gravid female distributions and mean values of larval exit-holes per plot were also significantly associated 18 June and 1 July 2008 ($P <$

0.025 for both). Associations among male, total and gravid female *C. obstrictus* distributions and exit-hole counts for all sampling dates in 2008 are presented in Table 6.4.

Discussion

The resistant genotypes 121R and 173R had fewer larval emergence holes per pod than Q2 in all planting arrangements in 2008 and in all but 75% 121R plots in 2007; reduced numbers of larvae were found from Q2 in 75% 121R mixes. Not only do these results indicate that resistance in these novel genotypes is maintained regardless of planting arrangement, but they also suggest protective effects of resistant on susceptible genotypes in mixed planting arrangements. This conclusion is supported by reduced numbers of larval emergence holes per pod from Q2 associated with 75% 121R and reduced (albeit not significantly) numbers from Q2 associated with 75% 173R. Chemical or visual traits associated with individual Q2 plants would not be influenced by intermixed plantings with resistant genotypes. This means that the presence of resistant genotypes intermixed at moderately high densities with susceptible Q2 has a disruptive effect on *C. obstrictus* host choices and lessens the attractiveness of Q2 in these planting arrangements. In another study, a resistant variety of cassava planted as a mixed crop with a susceptible variety reduced *Trialeurodes variabilis* (Quaintance) (Hemiptera: Aleyrodidae) feeding on a susceptible variety under outbreak conditions in Columbia (Gold et al. 1989). These and the findings of the current study are consistent with associational resistance; reductions in herbivore

feeding on otherwise suitable plants when they are associated with genetically or taxonomically diverse co-habitants (Tahvanainen and Root 1972). However, associational resistance is not consistently detected in evaluations of mixes of susceptible and resistant hosts. For example, Smith et al. (2007) found that densities of mature *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae) larvae were proportional to refuge size in interspersed refugia associated with resistant germplasm expressing the antibiotic resistance gene *Sm1*.

Mechanisms proposed for reductions in herbivore occurrence and/or damage with increased plant diversity include increased competition among herbivorous species, increased natural enemy assemblages and reduced apparency of susceptible plants (Root 1973; Risch et al. 1983; Baliddawa 1985; Andow 1990). Other *B. napus* herbivores collected in this study were not abundant at the time of weevil oviposition (data not shown) so interspecific competition likely had little influence on results. Similarly parasitism of *C. obstrictus* larvae by Chalcidoidea (Hymenoptera) has remained at relatively low levels at this site, usually less than 15% (data not shown), so increased natural enemy populations were also unlikely to have affected weevil densities in the plots.

Plant apparency addresses host finding efficiency and is related to plant life-history (Feeny 1976). Given that these genotypes were seeded and matured simultaneously, effects of life history-associated factors can be discounted. However, differences in responses of *C. obstrictus* to visual cues associated with resistant and susceptible *S. alba* x *B. napus* lines have recently been demonstrated (Tansey et al. 2008). Differences in responses to visual cues were attributed

primarily to the interaction of 580 and 350 nm light reflected from flowers (Chapter 3). Wehner (1981) indicated that many insects respond to objects based on conformation to a specific predetermined template and ignore less accordant stimuli. Reduced responses of *C. obstrictus* indicate that, in a context of a relatively finely defined template of stimuli that elicit responses, less attractive genotypes are less apparent (as per Feeny 1976). Reduced weevil captures in mixes and resistant monocultures suggest that the attractive effects of light reflected from susceptible genotype flowers may be obscured by proximity to resistant and less attractive genotypes.

Host apparency may also be influenced by genotypic differences in olfactory cues produced by potential host plants (Prokopy and Owens 1983). It has been suggested that movement of *C. assimilis* from overwintering sites to crops is influenced by odour-mediated anemotaxis in response to host-plant odour (Evans and Allen-Williams 1993). The effective range of odour-mediated upwind anemotaxis to *B. napus* floral and foliar extracts was found to be at least 20 m for *C. obstrictus* (Evans and Allen-Williams 1993). Differences in responses of *C. obstrictus* in linear track and Y-tube olfactometer tests have been detected; responses to Q2 were greater than to the resistant genotypes 121R and 173R (Chapter 3). In an examination of naturalized *B. oleracea* L. and *Brassica nigra* (L.) populations in England, Moyes and Raybould (2001) found greater *C. obstrictus* oviposition in individual plants within populations characterised by higher levels of 3-butenyl glucosinolate. *Ceutorhynchus obstrictus* population densities can vary between sites but generally not between habitats within sites

(Moyes et al. 2000). The average glucosinolate profile of populations, rather than that of individual plants, was suggested to be the primary factor influencing oviposition (Moyes and Raybould 2001).

Fewer gravid female *C. obstrictus* captured in all mixes of susceptible and resistant genotypes and resistant monocultures than in susceptible monocultures in both years and reductions in male captures, relative to susceptible monocultures, were demonstrated for resistant monocultures and all mixes suggest reduced attraction of *C. obstrictus* to plots with greater proportions of resistant genotypes. Gravid females are apparently more sensitive to reduced attractiveness associated with mixes of resistant and susceptible genotypes than total females; females are apparently more sensitive than males. Female *C. obstrictus* are more sensitive to the attractive effects of visual cues associated with host plant flowers than males (Tansey et al. 2008), but both responded similarly to olfactory cues associated with these genotypes in olfactometer trials (Chapter 4) and to *B. napus* extracts in trapping tests (Evans and Allen-Williams 1993).

Relative similarities in the patterns of responses by males and females to mixes and monocultures were demonstrated in this study. However, greater selectivity by females than males among mixes and monocultures despite similarities among responses to olfactory cues indicate that the attractive effects of olfactory cues are attenuated more strongly by visual cues in females. Female *Anthonomus pomorum* L. (Coleoptera: Curculionidae) were more responsive to ultraviolet, green, and blue light; Hausmann et al. (2004) attributed this difference to greater affinity of females than males to host plant-associated visual cues.

Greater proportions of *C. obstrictus* males than females captured in a greater variety of trap colours were attributed to greater discrimination among, and greater affinity of, females for visual cues (Chapter 2). Greater selectivity by gravid females in this study also suggests greater sensitivity to visual cues and the potential role of factors that are influential at intimate ranges.

Females were aggregated in mid to late June during the bud to early flowering stages of crop development in both years and again in mid July 2007 during late flowering. These results suggest an affinity of females to susceptible genotypes within mixed plots and to susceptible monocultures. This conclusion is supported by a correlation of female weevil captures and exit-hole counts during these periods. However, no correlation of exit-holes and gravid female captures and a significant and negative correlation of total female captures and exit-holes were detected 24 June 2008. More gravid females were captured in 75% resistant genotypes than susceptible monocultures in late June 2008, in contrast to planting arrangement-capture associations detected for the majority of the season. No correlation of total female captures and exit-holes was detected late June 2007. These results suggest departure from sites where females had previously aggregated and presumably oviposited. Correlation of total female captures with exit-holes was apparent again in early July in both years suggesting that their affinity for attractive host cues returned. Causes of this apparent abandonment of aggregation sites were not tested but may include an epideictic substance produced by female weevils.

Females brush an epideictic substance onto pods after oviposition that deters conspecific females from ovipositing (Kozlowski et al. 1983; Ferguson and Williams 1991). Application of epideictic substances reduces repeated oviposition by individuals and conspecifics into limited larval food sources (Prokopy et al. 1984). Deterrence by epideictic substances promotes uniform distribution of progeny and efficient resource partitioning among larvae, an adaptive strategy given the limited carrying capacity of pods in brassicaceous plants (Mudd et al. 1997). Deposition of this substance may also influence spatial distribution of adults. However, the behavioural effects of oviposition-deterrent pheromone last only about 2 h (Ferguson and Williams 1991). Other means for females to detect developing larvae in pods may exist but have not yet been examined.

Attraction of both sexes to yellow traps prior to crop germination, and so in the absence of attractive volatile cues, supports the importance of visual cues. Males were aggregated very early in the season in both years before crop germination, although they demonstrated no preferences for planting arrangements. This result is somewhat surprising. There is no evidence as yet of a male-produced aggregation pheromone in *C. obstrictus*, though such compounds are produced by males of several other weevil species (e.g., Rochat et al. 1991). I detected random distributions of females at this time in both years. Weevils have been observed hovering above yellow objects in the absence of olfactory cues; they disperse once physical contact with objects is made (J. Tansey, unpublished data). Detection of aggregated distributions at this time may also be a product of invasion of the site from its periphery. Dosdall et al. (2006) reported that patterns

of weevil invasions of spring canola in western Canada were similar to those observed in winter canola in Europe, with greater numbers of weevils aggregated on crop edges and subsequent dispersal to more homogeneous distributions as the season progressed (Risbec 1952; Free and Williams 1979; Ferguson et al. 1999). Plots in the current study were situated 10 m from the eastern boundary of the crop; the greatest numbers of weevils were captured in traps on the eastern and northern boundary of the plots early in the season (data not shown). Male spring emergence precedes that of females in Europe (Bonnemaison 1957, Dmoch 1965) and in western Canada (Ulmer and Dosdall 2006); their greater localized numbers in traps on the eastern and northern edges of the study area are consistent with invasion from crop edges and offer an explanation of aggregated distributions detected early in the season. Although aggregations were detected, distributions of males, and total and gravid females were more uniform than at the first sampling interval throughout the plots through the remainder of both growing seasons.

Apparency theory predicts reduced colonization of less apparent (resistant) lines (Feeny 1976; Rhoades and Cates 1976; Bernal et al. 2004). Although initial resistance of novel host plant genotypes may be enhanced by being less apparent, herbivore search efficiency evolves after colonization of novel hosts regardless of apparency (Parmesan 1991). Although antibiosis, antixenosis and reduced apparency have been demonstrated for resistant genotypes, all of the lines tested can support larval development and were colonized in this study. Because toxic cultivars are not evolutionarily sustainable in monoculture (Bernal et al. 2004),

incorporation of susceptible refugia as a means of reducing the potential evolution of search efficiency and selective pressures associated with antibiosis has been proposed for these genotypes (Tansey et al. 2008).

Results of this study indicate that not only is resistance maintained by resistant lines accompanied by susceptible genotypes, but that susceptible lines are somewhat protected by association with resistant genotypes. These results are consistent with associational resistance (Tahvanainen and Root 1972). However, associational resistance may confound a goal of incorporating susceptible refugia: slowing evolution of virulence in the pest. These results also suggest that disruptive effects associated with mixes may be achieved with relatively low proportions of resistant genotypes but that relatively high proportions are required for protection of susceptible members of the plant population from *C. obstrictus*. These results will contribute to the optimal design of refuges to maintain resistance of novel germplasm to *C. obstrictus.*

Tables

Table 6.1. Mean total (S.E.), male (S.E.), total (S.E.) and gravid female (S.E.) *Ceutorhynchus obstrictus* from weekly samples 4 June to 10 August 2007 near Lethbridge, AB. One trap was set in each corner of replicated plots seeded to 100% *Brassica napus* var. Q2, 100% *Sinapis alba* x *B. napus* 121 R and mixes of Q2 and resistant genotypes planted in genotype-specific rows. Spatial distribution indices calculated with the SADIE procedure are presented for each group. Values of $I_a > 1$ (at $\alpha = 0.05$) indicate aggregation within the sample area; values of $J_a \leq 1$ indicate multiple clusters when $I_a > 1$.

Date (2007)	Sex	Mean per trap (S.E.)	δ (m)	I_a[1]	*P*	J_a[2]	*P*
June 1	male	6.613 (0.596)	0.667	1.416	**0.022**	0.091	0.100
	female	4.538 (0.415)	0.457	1.225	0.094	0.896	0.999
June 8	male	4.325 (0.537)	0.325	0.952	0.547	0.097	0.751
	female	2.950 (0.358)	0.267	0.897	0.710	0.962	0.802
June 15	male	3.400 (0.286)	0.351	1.228	0.086	0.109	0.551
	female	3.875 (0.379)	0.732	1.601	**0.004**	1.002	0.475
	gravid female	1.413 (0.284)	1.063	1.331	**0.047**	0.962	0.688
June 22	male	9.937 (1.007)	0.500	1.193	0.123	1.001	0.492
	female	11.313 (1.201)	0.596	1.385	**0.029**	1.046	0.111
	gravid female	6.213 (0.699)	0.653	1.326	**0.047**	1.011	0.396
June 29	male	24.613 (1.516)	0.175	1.115	0.212	0.992	0.630
	female	33.675 (1.862)	0.319	1.503	**0.013**	0.963	0.968
	gravid female	23.46 (1.575)	0.476	1.544	**0.008**	1.001	0.459
July 7	male	8.175 (0.629)	0.195	0.902	0.692	1.014	0.307
	female	8.675 (0.601)	0.296	1.078	0.261	1.003	0.445
	gravid female	7.100 (0.536)	0.306	1.053	0.305	1.011	0.355
July 15	male	3.763 (0.306)	0.528	1.453	**0.016**	0.997	0.527
	female	4.463 (0.398)	0.458	1.361	**0.033**	1.063	0.413
	gravid female	2.025 (0.399)	0.456	1.387	**0.025**	1.089	**0.010**
July 27	male	3.950 (0.319)	0.111	0.898	0.711	0.966	0.818
	female	7.100 (0.518)	0.252	0.963	0.516	0.995	0.511
	gravid female	2.162 (0.526)	0.500	1.114	0.208	0.973	0.718
Aug 10	male	8.588 (0.836)	0.726	1.509	**0.012**	1.017	0.322
	female	9.225 (0.631)	0.477	1.326	**0.045**	0.995	0.556
	gravid female	5.825 (0.526)	0.666	1.459	**0.014**	0.946	0.947

Table 6.2. Associations among *Ceutorhynchus obstrictus* and mean exit- holes per pod per plot near Lethbridge, AB, 2007. The SADIE association index, $X > 0$ indicates association, $X = 0$ random and $X < 0$ dissociation. For a two-tail test $\alpha <$ 0.025 for association and > 0.975 for dissociation.

Date (2007)	Association	*X*	*P*
June 1	males-females	0.9443	< **0.0001**
	males-exit-holes	-0.0791	0. 7499
	females-exit-holes	-0.0827	0. 7634
June 8	males-females	0.9094	< **0.0001**
	males-exit-holes	-0.1066	0.8035
	females-exit-holes	-0.0708	0.7056
June 15	males-females	0.3990	**0.0008**
	males-exit-holes	0.3111	**0.0136**
	females-exit-holes	0.3774	**0.0029**
	gravid-exit-holes	0.4533	**0.0001**
June 22	males-females	0.8207	< **0.0001**
	males-exit-holes	0.1137	0.1585
	females-exit-holes	0.2559	**0.0188**
	gravid-exit-holes	0.4015	**0.0028**
June 29	males-females	0.6715	< **0.0001**
	males-exit-holes	0.2135	0.0316
	females-exit-holes	0.2297	**0.0218**
	gravid-exit-holes	0.4216	< **0.0001**
July 7	males-females	0.5867	< **0.0001**
	males-exit-holes	0.2507	**0.0148**
	females-exit-holes	0.1886	0.0518
	gravid-exit-holes	0.1298	0.1265
July 15	males-females	0.6038	< **0.0001**
	males-exit-holes	0.1681	0.1137
	females-exit-holes	0.3219	**0.0037**
	gravid-exit-holes	0.5040	**0.0004**
July 27	males-females	0.7223	< **0.0001**
	males-exit-holes	0.0539	0.3210
	females-exit-holes	0.2666	**0.0178**
	gravid-exit-hole	0.1261	0.1534
August 10	males-females	0.7032	< **0.0001**
	males-exit-holes	0.0610	0.2987
	females-exit-holes	0.1032	0.2095
	gravid-exit-holes	-0.1809	0.9254

Table 6.3. Mean total (S.E.), male (S.E.), total (S.E.) and gravid female (S.E.) *Ceutorhynchus obstrictus* from weekly samples 30 May to 5 August 2008 near Lethbridge, AB. One trap was set in each corner of replicated plots seeded to 100% *Brassica napus* var. Q2, 100% *Sinapis alba* x *B. napus* 121 R, 100% *S. alba* x *B. napus* 173R and mixes of Q2 and resistant genotypes planted in genotype-specific rows. Spatial distribution indices, calculated with the SADIE procedure, are presented for each group. Values of $I_a > 1$ (at $\alpha = 0.05$) indicate aggregation within the sample area; values of $J_a \leq 1$ indicate multiple clusters when $I_a > 1$.

Date (2008)	Sex	Mean per trap (S.E.)	δ (m)	I_a^1	*P*	J_a^2	*P*
May 30	male	1.198 (0.167)	1.130	1.473	**0.020**	0.939	0.871
	female	0.625 (0.094)	0.327	0.973	0.483	0.999	0.507
June 4	male	3.031 (0.277)	0.420	1.331	0.052	0.875	0.999
	female	2.615 (0.242)	0.218	1.097	0.231	0.896	0.999
June 10	male	9.442 (0.503)	0.622	1.682	**0.004**	0.942	0.992
	female	8.000 (0.354)	0.528	1.634	**0.007**	0.916	0.999
	gravid female	0.063 (0.025)	0.938	0.938	0.983	0.904	0.509
June18	male	16.285 (0.911)	0.181	1.521	**0.013**	0.934	0.999
	female	16.363 (1.724)	0.440	1.391	**0.037**	0.968	0.900
	gravid female	3.635 (0.969)	0.527	1.535	**0.013**	0.733	0.999
June 24	male	4.229 (0.296)	0.624	1.574	**0.008**	0.973	0.840
	female	6.396 (0.405)	0.473	1.412	**0.029**	0.955	0.967
	gravid female	2.573 (0.268)	0.997	1.944	**0.002**	0.907	0.993
July 1	male	6.479 (0.413)	0.399	1.396	**0.030**	0.948	0.985
	female	10.484 (0.581)	0.178	1.010	0.391	0.951	0.924
	gravid female	9.072 (0.514)	0.219	1.040	0.331	0.955	0.983
July 8	male	6.094 (0.503)	0.616	1.388	**0.034**	0.922	0.995
	female	5.177 (0.354)	0.410	1.179	0.132	0.956	0.945
	gravid female	4.469 (0.339)	0.372	1.111	0.212	0.970	0.844
July 20	male	0.500 (0.096)	1.451	1.458	**0.018**	1.042	0.301
	female	0.750 (0.186)	1.076	1.047	0.316	0.829	0.978
	gravid female	0.594 (0.121)	1.118	1.113	0.216	0.944	0.752
July 29	male	0.448 (0.085)	1.061	1.148	0.162	0.875	0.967
	female	0.646 (0.110)	2.320	2.085	**0.002**	1.097	0.977
	gravid female	0.615 (0.106)	2.467	2.175	**< 0.001**	1.134	**0.044**
August 5	male	3.302 (0.226)	0.511	1.412	**0.025**	1.027	0.168
	female	7.281 (0.457)	0.702	1.810	**< 0.001**	0.987	0.699
	gravid female	6.854 (0.438)	0.728	1.836	**0.002**	0.988	0.676

Table 6.4. Associations among *Ceutorhynchus obstrictus* and mean exit- holes per pod per plot near Lethbridge, AB, 2008. The SADIE association index, $X > 0$ indicates association, $X = 0$ random and $X < 0$ dissociation. For a two-tail test $\alpha <$ 0.025 for association and > 0.975 for dissociation.

Date (2008)	Association	*X*	*P*
May 30	males-females	0. 1704	0.0562
	males-exit-holes	-0.1228	0.8585
	females-exit-holes	0.0482	0.3220
June 4	males-females	0.5902	< **0.0001**
	males-exit-holes	0.3472	**0.0013**
	females-exit-holes	0.1026	0.1572
June 10	males-females	0.2780	0.0803
	males-exit-holes	-0.1765	0.9602
	females-exit-holes	-0.1311	0.8974
	gravid-exit-holes	0.1831	0.0431
June 18	males-females	0.5254	< **0.0001**
	males-exit-holes	0.5079	< **0.0001**
	females-exit-holes	0. 3450	**0.0006**
	gravid-exit-holes	0.4829	< **0.0001**
June 24	males-females	0.5648	< **0.0001**
	males-exit-holes	-0.2328	**0.9880**
	females-exit-holes	-0.3337	**0.9990**
	gravid-exit-holes	-0.1244	0.8523
July 1	males-females	0.6022	< **0.0001**
	males-exit-holes	0.2852	**0.0067**
	females-exit-holes	0.2780	**0.0039**
	gravid-exit-holes	0.2575	**0.0070**
July 8	males-females	0.4118	< **0.0001**
	males-exit-holes	0.2576	**0.0151**
	females-exit-holes	0.0041	0.4850
	gravid-exit-holes	-0.0507	0.6735
July 20	males-females	0.4987	< **0.0001**
	males-exit-holes	0.1331	0.1402
	females-exit-holes	0.2626	**0.0085**
	gravid-exit-holes	0.1286	0.1156
July 29	males-females	0.4261	< **0.0001**
	males-exit-holes	0.0220	0.4116
	females-exit-holes	0.1364	0.1502
	gravid-exit-holes	0.1197	0.1834
August 5	males-females	0.1434	0.0900
	males-exit-holes	0.1584	0.0845
	females-exit-holes	-0.0216	0.5711
	gravid-exit-holes	-0.0498	0.6573

Figures

2007

Block 1	**Block 2**	**Block 3**	**Block 4**
121R in rows 2, 6, 10, 14, 18	100% 121R	121R every second row	100% Q2
Q2 in rows 2, 6, 10, 14, 18	121R every second row	100% Q2	121R in rows 2, 6, 10, 14, 18
121R every second row	100% Q2	100% 121R	Q2 in rows 2, 6, 10, 14, 18
100% 121R	121R in rows 2, 6, 10, 14, 18	Q2 in rows 2, 6, 10, 14, 18	121R every second row
100% Q2	Q2 in rows 2, 6, 10, 14, 18	121R in rows 2, 6, 10, 14, 18	100% 121R

2008

Block 1	**Block 2**	**Block 3**	**Block 4**
100% Q2	Q2 in rows 2,6,10,14,18 (the rest are 173R)	173 R in rows 2,6,10,14,18	Q2 in rows 2,6,10,14,18 (the rest are 121R)
2100% 173R	173R in rows 2,6,10,14,18	Q2 in rows 2,6,10,14,18 (the rest are 121R)	100% 173R
3100% 121R	Q2 in rows 2,6,10,14,18 (the rest are 121R)	Q2 in rows 2,6,10,14,18 (the rest are 173R)	100% 121R
Q2 in rows 2,6,10,14,18 (the rest are 173R)	100% Q2	100% 173R	Q2 in rows 2,6,10,14,18 (the rest are 173R)
173R in rows 2,6,10,14,18	100% 121R	100% Q2	173R in rows 2,6,10,14,18
Q2 in rows 2,6,10,14,18 (the rest are 121R)	100% 173R	100% 121R	100% Q2

Figure 6.1. Plot plans of mixed genotype deployment assessment. Plots were 4 m x 6 m in 2007 and 4 m x 4 m in 2008; 20 rows with 20 cm between each and 2.5 m walkways between plots (in both directions).

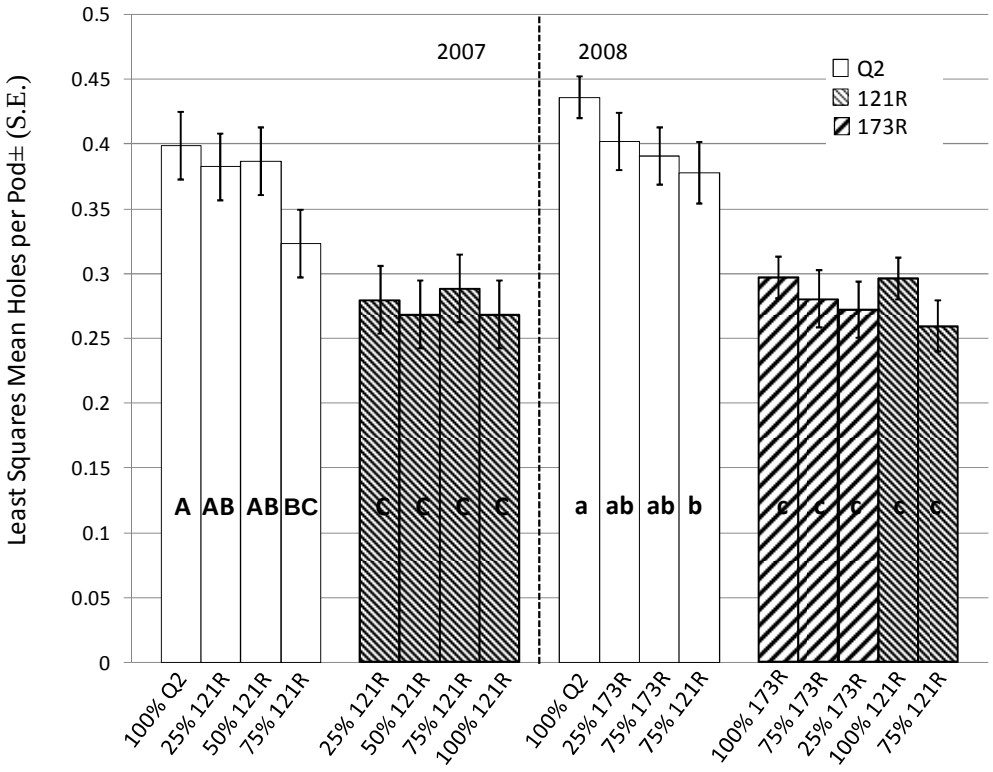

Figure 6.2. Least squares means of numbers of *Ceutorhynchus obstrictus* larval exit-holes per pod associated with *Brassica napus* var. Q2, a susceptible genotype, and the *C. obstrictus*-resistant *Sinapis alba* x *B. napus* genotypes 121R and 173R. In 2007, genotypes were planted in field plots near Lethbridge, AB in monocultures and mixes of 75% Q2: 25% 121R, 50% Q2: 50% 121R and 25% Q2: 75% 121R. In 2008, monocultures of Q2, 121R and 173R were planted and mixes of 75% Q2: 25% 173R, 25% Q2: 75% 173R and 25% Q2: 75% 121R. Different letters on bars indicate significant differences within groups by pair-wise comparison using Wald's Chi-square test ($\alpha = 0.05$).

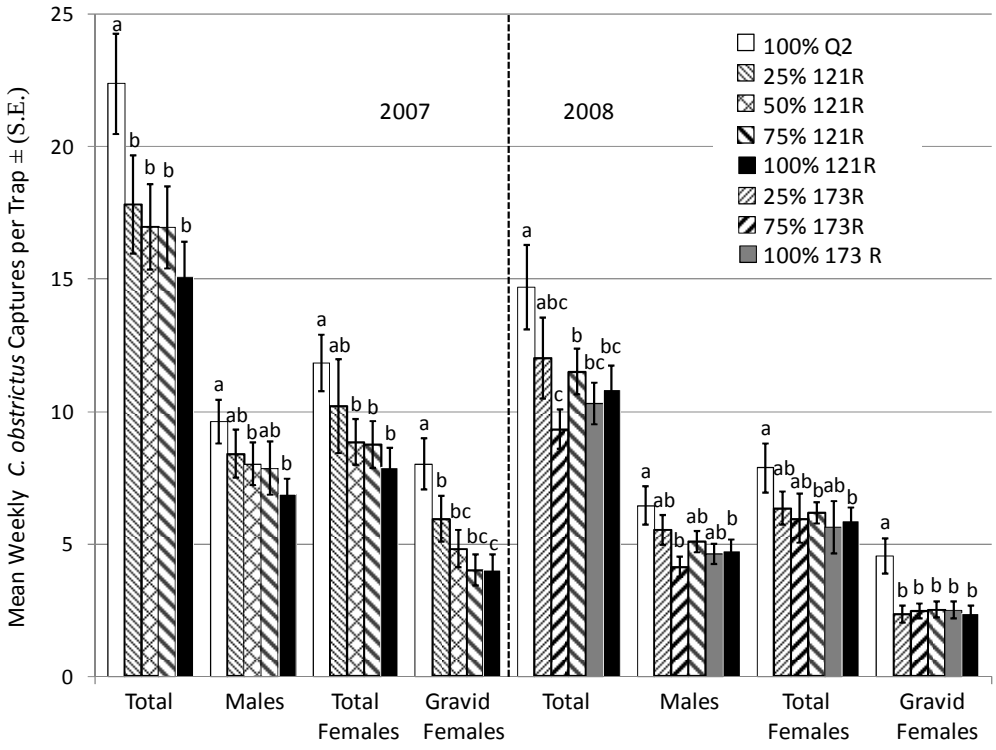

Figure 6.3. Mean total, male, total and gravid female *Ceutorhynchus obstrictus* from weekly samples 4 June to 10 August 2007 and 30 May to 5 August 2008 near Lethbridge, AB. One trap was set in each corner of replicated plots seeded to 100% *Brassica napus* var. Q2, 100% *Sinapis alba* x *B. napus* 121 R, 100% *S. alba* x *B. napus* 173R and mixes of Q2 and resistant genotypes planted in genotype-specific rows. Different letters above bars indicate significant differences within groups by pair-wise comparison using a Wald's Chi-square test ($\alpha = 0.05$).

References

Andow, D. A. 1990. Population dynamics of an insect herbivore in simple and diverse habitats. Ecology 71: 1006–1017.

Baliddawa, C.W. 1985. Plant species diversity and crop pest control. *Insect Science* and its *Application* 6: 479–487.

Benedict, J.H., M.F. Treacy, and J.C. Segers. 1986. Role of mixed plantings of susceptible and resistant phenotypes on yield and fleahopper behavior, pp. 486–488. *In* Proc. Beltwide Cotton Prod. Res. Conf., Las Vegas, NV, 4–9 Jan. 1986. Natl. Cotton Counc. Am., Memphis, TN.

Bernal, J.S., J. Prsifka, M. Sétamou, and K.M. Heinz. 2004. Transgenic insecticidal cultivars in integrated pest management: challenges and opportunities, pp. 123-145. *In* Koul, O., G.S. Dhaliwal, and G.W. Cuperus (eds.). Integrated Pest Management: Potential, Constraints and Challenges. CABI Publishing, Oxon, United Kingdom.

Bonnemaison, L. 1957. Le charançon des siliques (Ceutorhynchus assimilis Payk.) biologie et méthode de lutte. Annales des Épiphyties 4: 387–543.

Brodeur J., L. Leclerc, M. Fournier, and M. Roy. 2001. Cabbage seedpod weevil (Coleoptera: Curculionidae) a new pest of canola in northeastern North America. The Canadian Entomologist 133: 709–711.

Buntin, G. D., J.P. McCaffrey, P.L. Raymer, and J. Romero. 1995. Quality and germination of rapeseed and canola seed damaged by adult cabbage seedpod weevil, *Ceutorhynchus assimilis* (Paykull) [Coleoptera: Curculionidae]. The Canadian Journal of Plant Science 75: 539-541.

Cárcamo, H.A., L. Dosdall, M. Dolinski, O. Olfert, and J.R. Byers. 2001. The cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae) - a review. Journal of the Entomological Society of British Columbia 98: 201-210.

Dmoch, J. 1965. The dynamics of a population of the cabbage seedpod weevil (Ceutorhynchus assimilis Payk.) and the development of winter rape. Part I. Ekologiya Polska. Seria A 13: 249–287.

Dosdall, L. M., D. Moisey, H. Cárcamo, and R. Dunn. 2001. Cabbage seedpod weevil fact sheet. Alberta Agriculture, Food and Rural Development, Edmonton, AB, Canada.

Dosdall, L.M., R.M. Weiss, O. Olfert, and H.A. Cárcamo. 2002. Temporal and geographical distribution patterns of cabbage seedpod weevil (Coleoptera: Curculionidae) in canola. The Canadian Entomologist 134: 403-418.

Dosdall, L. M., and L.S. Kott. 2006. Introgression of resistance to cabbage seedpod weevil to canola from yellow mustard. Crop Science 46: 2437-2445.

Dosdall, L.M., B.J. Ulmer, G.A.P. Gibson and H.A. Cárcamo. 2006. The spatio-temporal distribution dynamics of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae), and its larval parasitoids in canola in western Canada. Biocontrol Science and Technology 16: 987-1006.

Elmstrom, K.M., D.A. Andow, and W.W. Barclay. 1988. Flea beetle movement in a broccoli monoculture and diculture. Environmental Entomology 17: 299–305.

Elton, C. S. 1958. The Ecology of Invasions by Animals and Plants. London: Methuen.

Evans, K.A., and L.J. Allen-Williams. 1993. Distant olfactory response of the cabbage seed weevil, *Ceutorhynchus assimilis*, to oilseed rape odour in the field. Physiological Entomology 18: 251-256.

Feeny, P. P. 1976. Plant apparency and chemical defence. Recent Advances in Phytochemistry 10: 1-40.

Ferguson, A.W., I.H. Williams, Z. Klukowski, B. Walczak, and J.N. Perry. 1999. Spatial population dynamics of a pest and its parasitoid in an oilseed rape crop. Aspects of Applied Biology 53: 1-6.

Ferguson, A.W., and I.H. Williams. 1991. Deposition and longevity of oviposition-deterring pheromone in the cabbage seed weevil. Physiological Entomology 1: 27-33.

Free, J.B., and I.H. Williams. 1979. The distribution of insect pests of oil-seed rape (*Brassica napu*s L.) and the damage they cause. Journal of Agricultural Science 92: 139-149.

Gold, C.G., M.A. Altieri, and A.C. Bellotti. 1989. Effects of cassava varietal mixtures on the whiteflies *Aleurotrachelus socialis* and *Trialeurodes variabilis* in Colombia. Entomologia Experimentalis et Applicata 53: 195-202.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annual Review of Entomology 47: 701–**722**.

Hausmann, C., J. Samietz, and S. Dorn. 2004. Visual orientation of overwintered Anthonomus pomorum (Coleoptera: Curculionidae). Environmental Entomology 33: 1410–1415.

Kareiva, P. M. 1983. Influence of vegetation texture on herbivore populations: resource concentration and herbivore movement, pp. 259–289. *In* Denno, R. F., and M. S. McClure (eds). Variable Plants and Herbivores in Natural and Managed Systems. Academic Press, New York.

Kennedy, J. S. 1965. Mechanisms of host plant selection. Annals of Applied Biology 56: 317 – 322.

Kozlowski, M.W., S. Lux, and J. Dmoch. 1983. Oviposition behavior and pod marking in the cabbage seed weevil, *Ceutorhynchus assimilis*. Entomologia Experimentalis et Applicata 34: 277-282.

Lozano, J., D. Byrne, and A. Bellotti. 1980. Cassava/ecosystem relationships and their influence on breeding strategy. Tropical Pest Management 26: 180-187.

Mason, P.G., T. Baute, O. Olfert, and M. Roy. 2004. Cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae) in Ontario and Quebec. Journal of Entomological Society of Ontario 134: 107–113.

McCaffrey, J. P. 1992. Review of the U.S. canola pest complex: cabbage seedpod weevil, pp. 140-143. *In* Proceedings, U.S. Canola Conference, 5-6 March 1992. Ameri-Can Pedigreed Seed Company, Memphis, TN.

McCaffrey, J.P., B.L. Harmon, J. Brown, A.P. Brown, and J.B. Davis. 1999. Assessment of *Sinapis alba*, *Brassica napus* and *S. alba* x *B. napus* hybrids for resistance to cabbage seedpod weevil, *Ceutorhynchus assimilis* (Coleoptera: Curculionidae). Journal of Agricultural Science 132: 289–295.

McLeod, J. H. 1962. Cabbage seedpod weevil – Ceutorhynchus assimilis (Payk.) Curculionidae, pp. 5-6. *In* J. H. McLeod, B. M. McGugan, and H. C. Coppel (eds.). A Review of the Biological Control Attempts Against Insects and Weeds in Canada. Commonwealth Institute of Biological Control, Trinidad.

Moericke, V. 1952. Farben als Landereize für geflügelten Blattläusen (Aphidoidea). Zeitschrift für Naturforschung 7: 304–324.

Moyes C.L., and A.F. Raybould. 2001. The role of spatial scale and intraspecific variation in secondary chemistry in host plant location by *Ceutorhynchus assimilis* (Coleoptera: Curculionidae). Proceedings of the Royal Society of London 268: 1567-1573.

Moyes, C.L., H.A. Collin, G. Britton, and A.F. Raybould. 2000. Glucosinolates and differential herbivory in wild populations of *Brassica oleracea*. Journal of Chemical Ecology 26: 2625-2641.

Mudd, A., A.W. Ferguson, M.M. Blight, I.H. Williams, P. Scubla, M. Solinas, and S.J. Clark. 1997. Extraction, isolation, and composition of oviposition-

deterring secretion of cabbage seed weevil, *Ceutorhynchus assimilis*. Journal of Chemical Ecology 23: 227-2240.

Parmesan, C. 1991. Evidence against plant "apparency" as a constraint on evolution of insect search efficiency (Lepidoptera: Nymphalidae). Journal of Insect Behavior 4: 417-430.

Perry, J.N. 1998. Measures of spatial pattern for counts. Ecology 79: 1008-1017.

Perry, J.N., and P. Dixon. 2002. A new method to measure spatial association for ecological count data. Ecoscience 9: 133-141.

Perry, J.N., and Z. Klukowski. 1997. Spatial distributions of counts at the edges of sample areas. *In* Proceedings of the VI Conference of the Biometric Society (Spanish Region) Córdoba, 21-24 September 1997, pp. 103-108.

Pimentel, D. 1961. Species diversity and insect population outbreaks. Annals of the Entomological Society of America 54: 76-86.

Prokopy, R.J., and E.D. Owens. 1983. Visual detection of plants by herbivorous insects. Annual Review of Entomology 28: 337-364.

Prokopy, R. J., B.D. Roitberg, and A.L. Averill. 1984. Resource partitioning, pp. 301-330. *In* Bell, W.J., and R.T. Cardé (eds.). Chemical Ecology of Insects. Chapman and Hall, London.

Renfrew, C. 1972. The Emergence of Civilization: The Cyclades and the Aegean in the Third Millennium B. C. Methuen, London.

Rhoades, D.F., and R.G. Cates. 1976. Toward a general theory of plant antiherbivore chemistry. Recent Advances in Phytochemistry 10: 168–213.

Risbec, J. 1952. De l'évolution des insectes nuisibles au Colza et des moyens de lutter contre eux. Comptes Rendues del' Académie d'Agriculture de France 39: 403–407.

Risch, S. J., D.A. Andow, and M.A. Altieri. 1983. Agroecosystem diversity and pest control: Data, tentative conclusions, and new research directions. Environmental Entomology 12: 625-629.

Root, R.B. 1973. Organization of a plant-arthropod association in simple and diverse habitats: The fauna of collards (*Brassica oleracea*). Ecological Monographs 43: 95-124.

Rochat, D., C. Malosse, M. Lettere, P.H. Ducrot, P. Zagatti, M. Renou, and C. Descoins. 1991. Male-produced aggregation pheromone of the American palm weevil, *Rhynchophorus palmarum* (L.) (Coleoptera: Curculionidae): Collection, identification, electrophysiological activity, and laboratory bioassay. Journal of Chemical Ecology 17: 2127-2140.

SAS Institute. 2005. SAS, Version 9.1. SAS Institute, Inc., Cary, North Carolina.

Smith, M.A., R.J. Lamb, I.L. Wise, and O.O. Olfert. 2007. An interspersed refuge for *Sitodiplosis mosellana* (Diptera: Cecidomyiidae) and a biocontrol agent *Macroglenes penetrans* (Hymenoptera: Pteromalidae) to manage crop resistance in wheat. Bulletin of Entomological Research 94: 179-88.

Tahvanainen, J. O., and R. B. Root. 1972. The influence of vegetational diversity on the population ecology of a specialized herbivore, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). Oecologia 10: 321–346.

Tansey, J.A., L.M. Dosdall, and B.A. Keddie. 2007. The influence of visual and olfactory cues on host selection by the cabbage seedpod weevil. Proceedings of the Joint meeting of the Entomological Society of Saskatchewan and the Entomological Society of Canada. Saskatoon, Saskatchewan.

Tansey, J.A., S.D. Noble, L.M. Dosdall, and B.A. Keddie. 2008. Visual cues influence host choices of the cabbage seedpod weevil. Proceedings of the Entomological Society of Alberta, 56th annual meeting. Edmonton, Alberta. 6-8 November, 2008.

Ulmer, B.J., and L.M. Dosdall. 2006. Spring emergence biology of the cabbage seedpod weevil (Coleoptera: Curculionidae). Annals of the Entomological Society of America 99: 64-69.

Wehner, R. 1981. Spatial vision in arthropods, pp. 287-616. *In*: Autrum, H., (ed.). 1981. Handbook of Sensory Physiology, Vol. 7, Sect. 6C. Springer, Berlin/New York.

Winder, L., C. Alexander, J.M. Holland, C. Woolley, and J.N. Perry. 2001. Modeling the dynamic spatio-temporal response of predators to transient prey patches in the field. Ecology Letters 4: 568-576.

Chapter 7

Conclusions

Visual responses of *Ceutorhynchus obstrictus* (Marsham) indicate that the species likely employs a visual system with three primary receptor pigments: trichromatic vision with response maxima near 250 nm, 450 nm and 550 nm (Chapter 2). Consistency between the modeled results of trapping studies (Chapter 2) and modeled responses to *Sinapis alba* L., *Brassica napus* L. and the *S. alba* x *B. napus* genotypes (Chapter 3) indicate that the interaction between 350 nm and 580 nm reflectance is important to host discrimination behaviour of this insect. Frearson et al. (2006) reported reduced attack by *C. obstrictus* on apetalous *B. napus*; significant contributions to these results were likely associated with the absence of appropriate levels of 350 nm and 580 nm that would normally be reflected from flower petals. These results indicate that future development of resistant germplasm should also address visual responses of pest insects.

Plants developed to reflect suboptimal levels of floral 350 nm and 580 nm (for *C. obstrictus*) may influence other anthophilous insects such as pollinators. Effects may be particularly apparent in scenarios where floral material with suboptimal reflective properties (for *C. obstrictus*) is presented to *C. obstrictus* populations in the presence of attractive genotypes in choice scenarios such as trap cropping with *B. rapa* or other similar plants. Pollinators such as the honeybee, *Apis mellifera* L. (Hymenoptera: Apidae), may differentiate among genotypes with different amounts or proportions of 350 nm and 580 nm foliar reflectance. Yoshioka et al. (2005) found differences in visitation rates by pollinators including honeybees and bumblebees, *Bombus* spp. (Hymenoptera:

Apidae), and hoverflies (Diptera: Syrphidae) among different plant genotypes with varying UV and visible reflectance properties.

Although pollen transfer in *B. napus* oilseed agroecosystems also occurs by wind, gravity and collisions among flowers, visitation by pollinators is an essential component of pollination and so seed production in crops grown in western Canada (Sabbahi et al. 2005). Delivery of pollen by bees is more than one hundred times more rapid than other pollination modes (Hayter and Cresswell 2006). Sabbahi et al. (2005) reported a 46 per cent increase in seed yield in test plots with three honeybee hives per hectare relative to those from which pollinators were excluded with cages. *Brassica napus* continues to produce new flowers until its 'maximum carrying capacity' is reached; flower production ceases when this threshold is reached though fertilization (Sabbahi et al. 2006). Unfertilized *B. napus* flowers live longer (Williams et al. 1987; Mesquida et al. 1988). Williams et al. (1987) also reported more advanced *B. napus* pod growth in bee-fertilized plots than plots without this type of pollen transfer. The introduction of mobile hives into commercial fields shortens the flowering period of canola (Sabbahi et al. 2006), and so likely promotes more synchronous maturation of the crop. An evaluation of the impact of wild bees including *Andrena* spp. and *Bombus* spp. (Hymenoptera: Apidae), and *Halictus* spp. (Hymenoptera: Halictidae) in northern Alberta, Canada on canola (*B. napus* and *B. rapa*) also found that seed production increased with wild bee abundance (Morandin and Winston 2005). Although the relative contributions of wild and domesticated bees to canola seed set are poorly understood, differential visitation by either

group due to visual attractiveness could influence productivity of novel *C. obstrictus*-resistant *S. alba* x *B. napus* genotypes and offset some yield gains made through resistance.

Near infrared (NIR) reflectance properties of these *S. alba* x *B. napus* genotypes were not examined in my study. Morandin and Winston (2005) reported reduced wild bee abundance in genetically modified, glyphosate-resistant (Roundup-Ready®) *B. napus* crops; they attributed mechanisms of reduced bee abundance in part to decreases in weed diversity (as per Haughton et al. 2003). However, because fibre structures change during the transgenic process, visible and NIR analysis can be used to screen transgenic crops (Xu et al. 2009). Hurburgh et al. (2000) distinguished transgenic soybean, *Glycine max* L., from a non-transgenic genotype using NIR reflectance with 84 % accuracy. Rui et al. (2005) distinguished transgenic and non-transgenic corn, *Zea mays* L., with 100 % accuracy. Xie et al. (2007) could differentiate tomatoes, *Lycopersicon esculentum* Miller, with an inserted antisense ethylene receptor gene (*LeETR1*) from parental genotypes using visible/NIR reflectance properties. Quantification of Brassicaceae foliar glucosinolate content is achieved through evaluation of NIR reflectance properties (Font et al. 2005). NIR reflectance properties, albeit of single seeds, can also be used to distinguish *B. napus* with both an inserted antisense gene for cruciferin and inserted yeast sn-2 acyltransferase gene from parental germplasm (Kohno-Murase 1995; Zou 1997). Rigorous comparisons of floral and foliar NIR reflectance properties of transgenic and parental *B. napus* genotypes have not, to my knowledge, been conducted but may account for some

differences in wild bee abundance detected by Morandin and Winston (2005) and may also influence *C. obstrictus* responses.

Differences were detected in the glucosinolate contents of immature seeds of *C. obstrictus*-resistant and -susceptible *S. alba* x *B. napus* genotypes (Shaw 2008). This compound was proposed to be 2-phenylethyl glucosinolate (Chapter 4). Immature seeds of genotypes susceptible to *C. obstrictus* expressed greater levels of this glucosinolate than nonsusceptible genotypes (Shaw 2008). Greater responses of *C. obstrictus* to susceptible genotypes in olfactometer tests were attributed in part to the attractive effects of hydrolysis products of this compound (Chapter 4). However, the effects of hydrolysis products of 2-phenylethyl glucosinolate on other prevalent pests of brassicaceous oilseeds in western Canada, the flea beetles *Phyllotreta cruciferae* (Goeze) and *Phyllotreta striolata* (Fabricius) (Coleoptera: Chrysomelidae), appear to be negligible although they are attracted to allyl isothiocyanate (Pivnick et al. 1983). Because no significant polymorphisms in the content of allyl glucosinolate were detected among the *S. alba* x *B. napus* genotypes examined by Shaw (2008), olfactory responses of *P. cruciferae* and *P. striolata* should not differ among the resistant and susceptible germplasm tested in this study. However, olfactory responses of these *Phyllotreta* spp. and those of other brassicaceous oilseed pests to these *S. alba* x *B. napus* genotypes should be assessed.

Shaw (2008) also detected a polymorphism in the glucosinolate content of cauline leaves among resistant and susceptible *S. alba* x *B. napus* genotypes. The likely identity of this compound was 1-methoxy-3-indolylmethyl glucosinolate

(Chapter 4). Reduced feeding and oviposition by *C. obstrictus* were attributed to this compound and its hydrolysis products in an apparently dose-dependent manner (Chapter 5). Several other Brassicaceae oligophages are apparently influenced similarly by 1-methoxy-3-indolylmethyl glucosinolate content. Increased levels of this compound were associated with reduced *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae) feeding (Bartlet et al. 1999). An oviposition-deterrent effect of 1-methoxy-3-indolylmethyl isothiocyanate has also been found for *Hellula undalis* (Fabricius) (Lepidoptera: Pyralidae) (Mewis et al. 2002). Indolyl glucosinolates are derivatives of indole-3-acetaldoxime in *Arabidopsis*; the phytoalexin camalexin is also a derivative of this compound (Glawischnig et al. 2004). Camalexin synthesis is induced in response to *Brevicoryne brassicae* L. (Homoptera: Aphididae) feeding in *Arabidopsis thaliana* L. and reduces aphid fecundity (Kuśnierczyk et al. 2008). Constitutive and/or induced 1-methoxy-3-indolylmethyl glucosinolate, its hydrolysis products or phytoalexins associated with its synthesis, may also influence other Brassicaceae herbivores and present an opportunity for broad resistance to several insect pests. The inducibility of these weevil-resistant genotypes and responses of other herbivores to them require testing.

Antixenosis resistance to *P. cruciferae* and *P. striolata* has also been demonstrated for *S. alba* x *B. napus* genotypes and was attributed to variable levels of genetic material from *S. alba* (Gavloski et al. 2000). This suggests that *p*-hydroxybenzyl glucosinolate production may be conferred to hybrid lines; this glucosinolate is produced by *S. alba* but not *B. napus* (e.g. Bodnaryk 1991), and

has been suggested to be responsible for demonstrated antixenosis resistance of *S. alba* to *P. cruciferae* (Bodnaryk and Lamb 1991). However, Nielsen et al. (2001) found that incorporation of the *CYP79A1* gene from *Sorghum bicolour* L. to *A. thaliana* and subsequent production of *p*-hydroxybenzyl glucosinolate by modified plants did not influence *P. cruciferae* or *Phyllotreta nemorum* L. feeding. Ulmer and Dosdall (2006) found that a low *p*-hydroxybenzyl glucosinolate variety was not preferred for feeding or oviposition by *C. obstrictus* over a variety expressing high levels of this compound; both demonstrated antixenosis resistance (Ulmer and Dosdall 2006b). No detectable levels of *p*-hydroxybenzyl glucosinolate were associated with any of the genotypes tested in this study (Shaw 2008). Production of *p*-hydroxybenzyl glucosinolate was not conferred to resistant *S. alba* x *B. napus* germplasm in this study and likely not the germplasm tested by Gavloski et al. (2000).

Phyllotreta cruciferae feeding induced elevated levels of 1-methoxy-3-indolylmethyl glucosinolate in *B. napus* (Bodnaryk 1992). Production and inducibility of 1-methoxy-3-indolylmethyl glucosinolate is also associated with *S. alba* (Hopkins et al. 1998; Koritsas et al. 1991). A greater propensity for production and inducibility of 1-methoxy-3-indolylmethyl glucosinolate may have been conferred to *C. obstrictus*-resistant isolines tested in this study through introgression of *S. alba* x *B. napus*.

However, a more likely explanation for resistance that incorporates an association of defensive compounds and floral reflective properties may be a direct influence of genes contributing to flower colouration on production of

compounds associated with resistance. Variation in genes affecting floral colour may have pleiotropic effects on other characters that influence fitness (e.g. Charlesworth 1990). Other herbivores have been shown to respond differently to different colour morphs of the same plant species (Simms and Bucher 1996; Irwin et al. 2003), and their responses likely have adaptive value. For example, performance of several herbivores was greater on *Raphanus sativus* L. that were anthocyanin-recessive than anthocyanin-dominant colour morphs (Irwin et al. 2003). In *Raphanus* spp., two independently assorting loci determine petal colour; each locus is associated with two alleles. One locus controls carotenoid expression, the other controls anthocyanins (Panetsos 1964). Yellow petals are associated with the presence of carotenoids; this trait is recessive to white petals that indicate a lack of carotenoids. Anthocyanin production is associated with pink petals and this trait is dominant to white petals (lack of anthocyanins). Bronze colour morphs are associated with expression of both anthocyanin and carotenoids (Panetsos 1964). Inheritance of yellow petal colour in *B. napus* and *B. rapa* is suggested to be under monogenic control (Séguin-Swartz 1988; Rahman 2001).

Carotenoids are isoprenoids (a subclass of terpenoids) that are ubiquitous among plants and microorganisms; these compounds are components of photosystems and associated with floral colours ranging from yellow to red (Tanaka et al. 2008 and references therein). Anthocyanins (a class of flavonoids) are ultimately derived from phenylalanine and are localized in foliar and floral epidermal vacuoles; these compounds are associated with colours ranging from

yellow to violet (Tanaka et al. 2008 and references therein). Colourless flavonoids are also associated with floral petal epidermal vacuoles in a great diversity of plant species (Kay et al. 1981). These compounds are associated with UV absorption (Mol et al. 1998). UV-absorbing flavonoids may be confined to limited areas of the petal, resulting in patterned displays of UV reflection; these are thought to influence responses of anthophilous insects (Yoshioka et al. 2005; Pfündel et al. 2006). Colourless flavonoids can also act as co-pigments to anthocyanins and augment reflected colours (Harborne and Williams 2000).

Strauss et al. (2004) detected differences in levels of induced glucosinolates among *R. sativus* colour morphs tested by Irwin et al. (2003); indolyl glucosinolates made up 71 per cent of the total glucosinolates detected in these plants. Strauss et al. (2004) suggested that glucosinolate induction was likely associated with pleiotropic effects between petal colour and defence loci, or tight linkage between these loci. An *Arabidopsis* mutant with a defective gene encoding *CYP83A1* (a cytochrome P450 highly similar to *CYP83B1*: an enzyme associated with glucosinolate biosynthesis) was shown to have an altered glucosinolate profile with reduced foliar aliphatic glucosinolates and increased indolyl glucosinolates (Hemm et al. 2003). This mutant was also shown to have an altered profile of products associated with the phenylpropanoid pathway. Although floral constituents were not examined, altered phenylpropanoid pathway product profiles were detected in leaves, seeds and stems (Hemm et al. 2003). Anthocyanin and colourless flavonoid pigments are derived from phenylpropanoid metabolism (Schwinn and Davies 2004; Kitamura 2006).

Altering this single gene has the potential to influence not only expression of aliphatic and indolyl glucosinolates, but also expression of anthocyanin and colourless flavonoids pigments and thus alter floral reflective properties.

A mechanism similar to that illustrated by Hemm at al. (2003) may be influencing effects detected in the introgressed genotypes I tested. If this is the case, the apparent negative relationship of apparency and antibiosis is clearer as is the adaptive significance of differential responses of *C. obstrictus* to variably resistant *S. alba* x *B. napus* genotypes. Further, alteration or deletion of a locus as part of the *S. alba* x *B. napus* introgression process, with function similar to that of the *Arabidopsis REF2* locus (associated with gene encoding *CYP83A1*) may explain the relationships between apparency, and antibiosis and antixenosis resistance detected in my studies. Many phenotypic variations can result from the introgression of alien material including deletion of segments, production of novel segments and gene silencing (Tu et al. 2009).

Although mechanisms for antixenosis and antibiosis in these weevil-resistant *S. alba* x *B. napus* genotypes are likely associated with 1-methoxy-3-indolylmethyl glucosinolate, responses to this compound and its hydrolysis products are not consistent among Brassicaceae oligophages. Although *Delia floralis* (Fallén) (Diptera: Anthomyiidae) attack can result in a 17-fold increase in 1-methoxy-3-indolylmethyl glucosinolate content, larvae are not negatively affected by increased concentrations of this compound (Birch 1992). In fact, *B. napus* varieties with the highest levels of inducible 1-methoxy-3-indolylmethyl glucosinolate support the most robust *D. floralis* larvae (Birch 1992). This

compound also stimulates oviposition in *Delia radicum* L. (Roessingh et al. 1992) and *Pieris rapae* (L.) (Lepidoptera: Pieridae) (Renwick et al. 1992). Resistance of these *S. alba x B. napus* genotypes to *C. obstrictus* and potential resistance to several other pests may benefit *Delia* spp. and complicate their deployment.

Possible mechanisms for this interaction may include induced plant responses associated with microorganisms. Induction of indolyl glucosinolates is associated with inoculation of Brassicaceae with pathogens (e.g. Li et al. 1999). Li et al. (1999) reported a correlation of indolyl glucosinolate induction and resistance to *Sclerotinia sclerotiorum* (Lib.) de Bary in *B. napus*. *Erwinia carotovora* (Jones) also induces indolyl glucosinolate synthesis in *A. thaliana* (Brader et al. 2001). *Delia radicum* preferentially oviposits on host plants attacked by conspecifics (Baur 1996) and a relationship between infection by *Erwinia* sp. and *D. radicum* oviposition has been demonstrated (Doane and Chapman 1964). Ellis et al. (1982) reported four-fold increase in *D. radicum* oviposition around *Raphanus* spp. seedlings grown from control seeds than those with no or few microorganisms.

Baur et al. (1999) reported increased *D. radicum* oviposition on *Brassica oleracea* L. var. *botrytis* exposed to the crucifer-specific phytoalexins methoxybrassinin, cyclobrassinin, and brassitin. These compounds are induced in response to bacterial infection in several Brassicaceae (Pedras et al. 2004 and references therein). Baur et al. (1999) also reported that brassicanate A, a metabolic derivative of the Brassicaceae phytoalexin brassinin (Pedras et al. 2004) was highly stimulatory to *D. radicum* oviposition. Methoxybrassinin and brassinin

are derived from indole-3-acetaldoxime in *B. napus.* ssp. *rapifera* (rutabaga) (Pedras et al. 2004); this compound is also a precursor to indolyl glucosinolates (Glawischnig et al. 2004). Oviposition responses of *Delia* spp. are apparently associated with induction of indolyl glucosinolate that several other Brassicaceae herbivores find deleterious. Interestingly, some *S. alba* x *B. napus* lines that have demonstrated antibiosis resistance to *D. radicum* (Dosdall et al. 2000) are not resistant to *P. cruciferae* (Gavloski et al. 2000).

Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae) feeding and exogenous application of volicitin (*N*-(17-hydroxylinolenoyl)-L-glutamine) increased induction of volatile emissions in *Z. mays* (Schmelz et al. 2003). The *S. exigua* gut bacterium, *Microbacterium arborescens* (Frankland and Frankland), contributes to this interaction (Piel et al. 2009). Koritsas et al. (1989) reported that bacterial isolates from *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae) increased indole glucosinolate content associated with artificial wounding. In light of the contributions of microbial organisms to insect herbivory in plants, the influence of microbial interactions on responses of *C. obstrictus* to its host plants including these *S. alba* x *B. napus* genotypes should be examined.

Perception of attractive kairomones and factors contributing to antixenosis and antibiosis resistance may also be influenced by epicuticular waxes. Epicuticular wax layers can influence the exudation of polar compounds like glucosinolates and their hydrolysis products and influence both long-distance and intimate range responses. Reifenrath et al. (2005) detected no glucosinolates in waxy layers removed from the adaxial or abaxial surfaces of *B. napus* with gum

arabic. Feeding preferences of the Brassicaceae oligophage *Phaedon cochleariae* Fabricius (Coleoptera: Chrysomelidae) were influenced by wax; these beetles fed preferentially on leaves from which epicuticular wax had been removed. Reifenrath et al. (2005) suggested that feeding behaviour was influenced by the availability of stimulants, particularly glucosinolates. Wax acted as a barrier between glucosinolates, which are not normally present on the leaf surface, and the insect. Small quantities of glucosinolate hydrolysis products resulting from spontaneous degradation of glucosinolates may be adsorbed to wax, allowing wax to act as a slow release substrate (Renwick et al. 2006). Low levels of attractive compounds may be detectable by *C. obstrictus* in contact with pods and influence host feeding and oviposition choices. It is also possible that *C. obstrictus* oviposition behaviour is influenced by compounds liberated by damage to tissues and subsequent release of myrosinases. Attractive hydrolysis products of glucosinolates released by probing may stimulate further probing, feeding and oviposition.

All of the genotypes tested supported larval development in choice scenarios in the field (Shaw 2008) and in laboratory assessments (Chapter 5). Antibiosis resistance may reduce *C. obstrictus* populations but deployment in monocultures of these toxic cultivars is not evolutionarily sustainable (Bernal et al. 2004). For example, St. Augustinegrass, *Stenotaphrum secundatum* (Walter) Kuntze var. Floratam, was developed for resistance to the southern chinch bug, *Blissus insularis* Barber (Hemiptera: Blissidae), and commercialized in 1973 (Horn et al. 1973). The mode or resistance of this variety was determined to be

antibiosis (Reinert and Dudeck 1974). Resistance was overcome by a local population of chinch bugs within 12 years in Florida (Busey and Center 1987) and by 2001-2002 throughout Florida (Nagata and Cherry 2003).

Partial resistance, as demonstrated for these *S. alba* x *B. napus* genotypes (Chapter 5) is preferable to total resistance due to reduced selective pressures (Palaniswamy 1996). Vacher et al. (2003) modeled tobacco budworm, *Heliothis virescens* L. (Lepidoptera: Noctuidae), mortality and gene flow in mixed resistant and susceptible genotype cotton agroecosystems. They reported that the currently adopted 'high dose refuge' strategy will delay pests overcoming resistance but will not prevent its emergence. Transgenic corn, *Z. mays*, and cotton, *Gossypium hirsutum* L., that express *Bacillus thuringiensis* Berliner (*Bt*) δ- endotoxins are sufficiently toxic to achieve complete mortality of pest populations (Vacher et al. 2003). Thus, deployment of transgenic plants with low toxicity interspersed with susceptible refugia occupying approximately 25 percent of the production area is suggested for greatest durability of resistance traits (Vacher et al. 2003). Resistant genotypes tested in this study are of low toxicity and should require relatively small refugia to preserve resistance traits (as per Vacher et al. 2003). However, potential issues arising from this low toxicity may include rapid evolution of search efficiency associated with the conditional suitability of these resistant genotypes to *C. obstrictus* (Parmesan 1991). This potential problem may be offset somewhat by demonstrated differences in the olfactory responses of *C. obstrictus* to susceptible and resistant germplasm and antifeedant and anti-oviposition characteristics (Chapters 4, 5).

Incorporation of susceptible refugia into cropping schemes has dealt primarily with preservation of novel resistance traits in commercial settings (e.g. Gould 1998). However, contributions of susceptible refugia to associational resistance have also been demonstrated (Chapter 6). Differences in *C. obstrictus* adult and larval spatial distribution and reduced levels of larval infestation in small plot tests of mixes of *C. obstrictus*-resistant and -susceptible germplasm were attributed to differences in apparency (Feeny 1976) and antixenosis (Chapter 5). According to Andow (1991), associational resistance refers to a reduction in herbivore attack when hosts plants are associated with genetically or taxonomically diverse cohabitants; a primary mechanism of this phenomenon is reduced apparency of susceptible plants. Results (Chapter 6) were consistent with the concept of associational resistance (as per Tahvanainen and Root 1972) and results of a meta-analysis and review by Barbosa et al. (2007). Barbosa et al. (2007) indicated that associational resistance in susceptible plants is inversely related to the palatability (antixenosis resistance) associated with neighbours.

Kennedy et al. (1987) indicated that simultaneous presentation of moderate levels of antixenosis, antibiosis, and tolerance to pest populations can be an effective strategy for controlling pest populations. In addition to selective pressures associated with antibiosis, antixenosis also introduces selective pressures. Thus, coupling antixenosis with antibiosis modes can influence the longevity of resistant lines. These resistance modes, presented simultaneously to pest populations, reduce the likelihood of overcoming either mode individually; this relationship requires that susceptible hosts are accessible to pests (Gould

1984). However, antixenosis and antibiosis are likely associated with the same compound(s) in resistant *S. alba* x *B. napus* genotypes tested in this study (Chapter 4); this relationship may mean that *C. obstrictus* overcomes these resistance traits at a rate comparable to either moderate antixenosis or antibiosis presented alone.

Crop resistance is highly compatible with integrated pest management (IPM) (Palaniswamy 1996). Reduced apparency and antixenosis (as they contribute to associational resistance) and antibiosis resistance might best be exploited in a manner consistent with stimulo-deterrent diversion (Miller and Cowles 1990). Stimulo-deterrent diversion or 'push-pull' that incorporates a trap cropping scheme may improve successes already demonstrated in *C. obstrictus* control associated with trap cropping alone (Cárcamo et al. 2007). The combined effects of the 'pull' of attractive *B. napus* or *B. rapa* genotypes as the trap crop and the 'push' of resistant *S. alba* x *B. napus* genotypes as the main crop may concentrate *C. obstrictus* populations more effectively than a trap crop of highly attractive *B. rapa* and main crop of attractive *B. napus* for localized insecticidal control. The stability of the IPM system would benefit from incorporation of resistant germplasm and reduce pesticide input (Palaniswamy 1996), and as susceptible and attractive host plants are easily accessible to *C. obstrictus*, reduce selective pressures associated with antibiosis and antixenosis resistance.

Cárcamo et al. (2007) suggested that augmenting the efficacy of trap crops in small or narrow fields and/or when populations reach outbreak levels is required. The effectiveness of cropping systems that integrate 'push-pull' with

susceptible and resistant germplasm under outbreak conditions, as occur in the Lethbridge, Alberta region and are predicted in southern Manitoba in the near future (Dosdall et al. 2002), need to be evaluated. Resistance associated solely with these *S. alba* x *B. napus* genotypes may be insufficient to control *C. obstrictus* populations below current nominal thresholds (Dosdall et al. 2001) under outbreak conditions. However, the novel *S. alba* x *B. napus* genotypes may still benefit from reduced apparency (Chapters 3, 4), antixenosis resistance and antibiosis resistance (Chapter 5).

Differences in the olfactory responses of *C. obstrictus* to resistant and susceptible genotypes also indicate difference in the compositions of headspace volatiles associated with these plants. In addition to influencing the responses of *C. obstrictus*, olfactory cues may also influence the behaviours of natural enemies of the weevil, recruited from native or naturalized populations or deliberately introduced as classical biological control agents (Gibson et al. 2005, 2006; Kuhlman et al. 2006; Dosdall et al. 2007, 2009). Examples of parasitoids responding to host plant olfactory cues are numerous. For example, *Phradis interstitialis* Thomson and *Tersilochus heterocerus* Thomson (Hymenoptera: Ichneumonidae) are parasitoids of pollen beetles (*Meligethes* spp.; Coleoptera: Nitiduliae) in northern Europe (Nilsson and Andreasson 1987; Billqvist and Ekbom 2001; Büchi 2002). Jönsson et al. (2005) found that *P. interstitialis* were attracted to odours of oilseed rape in the bud stage and *T. heterocerus* preferred odours of flowering rape; they also detected a synergistic interaction of host plant odour and yellow paper for both parasitoid species. The responses of predators

and parasitoids of *C. obstrictus* to olfactory and visual cues associated these *S. alba* x *B. napus* genotypes have not yet been evaluated, but represent an important direction for future research.

Antibiosis resistance demonstrated for some *S. alba* x *B. napus* genotypes may also influence natural enemies of *C. obstrictus*, particularly the larval ectoparasitoids *Trichomalus perfectus* (Walker) and *Mesopolobus morys* (Walker) (Hymenoptera: Pteromalidae). Larvae of these parasitoids occupy inner regions of canola siliques and so are exposed to the same conditions as *C. obstrictus* larvae. Consequently populations of these parasitoids may be affected by changes in host plant biochemistry. For instance, Soler et al. (2007) found that increases in indole glucosinolates occurred in the roots of *Brassica nigra* L. (Brassicaceae) plants that were subject to foliar feeding by *Pieris brassicae* L. (Lepidoptera: Pieridae). The growth of both *D. radicum* and its koinobiont parasitoid, *Trybliographa rapae* (Westwood) (Hymenoptera: Figitidae), were reduced by approximately 50 per cent as a result of this increase of root glucosinolate content (Soler et al. 2007).

A final concern is associated with the distribution of resistant *S. alba* x *B. napus* genetic material by pollen vectors. Honeybees are capable of distributing *B. napus* pollen up to 4 km (Ramsay et al. 1999). Wind is a less efficient vector; *B. napus* pollen density decreases by 90 per cent 20 m from the field border (McCartney and Lacey 1991). Distribution of genetic material from resistant to susceptible genotypes will occur and may influence regional durability of resistance traits. Also, because preservation of resistance traits will likely require

its incorporation into appropriate planting arrangements with susceptible refugia, control over the distribution of resistant germplasm may be required. The use or sale of seed saved from previous years by producers will likely need to be restricted to control deployment and thus exposure of *C. obstrictus* to selective pressures that will allow it to overcome antibiosis and antixenosis resistance and reduced apparency associated with these *S. alba* x *B. napus* genotypes.

References

Andow, D. A. 1990. Population dynamics of an insect herbivore in simple and diverse habitats. Ecology 71: 1006–1017.

Bartlet, E., G. Kiddle, I. Williams, and R. Wallsgrove. 1999. Wound induced increases in the glucosinolate content of oilseed rape and their effect on subsequent herbivory by a crucifer specialist. Entomologia Experimentalis et Applicata 91: 163–167.

Barbosa, P., I. Kaplan, Z. Szendrei, A. Szczepaniec, J. Hines, and H. Martinson. 2007. Associational resistance and susceptibility: Having right or wrong neighbours. Proceedings of the joint meetings of the Ecological Society of America and the Society for Ecological Restoration. 5-10 August, 2007. San Jose, California.

Baur, R., A.N.E. Birch, R.J. Hopkins, D.W. Griffiths, M.S.J. Simmonds, and E. Städler. 1996. Oviposition and chemosensory stimulation of the root flies *Delia radicum* and *D. floralis* in response to plants and leaf surface extracts from resistant and susceptible *Brassica* genotypes. Entomologia Experimentalis et Applicata 78: 61-75.

Baur, R., E. Städler, K. Monde, and M. Takasugi. 1999. Phytoalexins from *Brassica* (Cruciferae) as oviposition stimulants for the cabbage root fly, *Delia radicum.* Chemoecology 8: 163-168.

Bernal, J.S., J. Prsifka, M. Sétamou, and K.M. Heinz. 2004. Transgenic insecticidal cultivars in integrated pest management: Challenges and opportunities, pp. 123- 145. *In*: Koul, O., G.S. Dhaliwal, and G.W. Cuperus

(eds.). Integrated pest management: Potential, constraints and challenges. CABI Publishing, Oxon, United Kingdom.

Billqvist, A., and B. Ekbom. 2001. The influence of host plant species on parasitism of pollen beetles (*Meligethes* spp.) by *Phradis morionellus*. Entomologia Experimentalis et Applicata 98: 41-47.

Birch, A.N.E., D.W. Griffiths, R.J. Hopkins, W.H. Macfarlane Smith, and R.G. McKinlay. 1992. Glucosinolate responses of swede, kale, forage and oilseed rape to root damage by turnip root fly (*Delia floralis*) larvae. Journal of the Science of Food and Agriculture 60: 1-9.

Bodnaryk, R.P. 1991. Developmental profile of sinalbin (*p*-hydroxybenzyl glucosinolate) in mustard seedlings, *Sinapis alba* L., and its relationship to insect resistance. Journal of Chemical Ecology 17: 1543–1556.

Bodnaryk, R.P. 1992. Effects of wounding on glucosinolates in the cotyledons of oilseed rape and mustard. Phytochemistry 31: 2671–2677.

Bodnaryk, R.P., and R.J. Lamb 1991. Mechanisms of resistance to the flea beetle, *Phyllotreta cruciferae* (Goeze), in mustard seedlings, *Sinapis alba* L. The Canadian Journal of Plant Science 71: 13-20.

Brader, G., É. Tas, and E.T. Palva. 2001. Jasmonate-dependent induction of indole glucosinolates in *Arabidopsis* by culture filtrates of the nonspecific pathogen *Erwinia carotovora*. Plant Physiology 126: 849-860.

Büchi, R. 2002. Mortality of pollen beetle (*Meligethes* spp.) larvae due to predators and parasitoids in rape fields and the effects of conservation strips. Agriculture, Ecosystems and Environment 90: 255-263.

Busey, P., and B. J. Center. 1987. Southern chinch bug (Hemiptera: Heteroptera: Lygaeidae) overcomes resistance in St. Augustinegrass. Journal of Economic Entomology 80: 608-611.

Cárcamo, H.A., R. Dunn, L.M. Dosdall, and O. Olfert. 2007. Managing cabbage seedpod weevil in canola using a trap crop – a commercial field-scale study in western Canada. Crop Protection 26: 1325-1334.

Charlesworth, B. 1990. Optimization models, quantitative genetics, and mutation. Evolution 44: 520-538.

Doane, J.F., and R.K. Chapman. 1964. The relation of the cabbage maggot, *Hylemyia brassicae* (Bouché), to decay in some cruciferous crops. Entomologia Experimentalis et Applicata 7: 1-8.

Dosdall, L. M., and L.S. Kott. 2006. Introgression of resistance to cabbage seedpod weevil to canola from yellow mustard. Crop Science 46: 2437-2445.

Dosdall, L.M., A. Good, B.A. Keddie, U. Ekuere, and G. Stringam. 2000. Identification and evaluation of root maggot (*Delia* spp.) (Diptera: Anthomyiidae) resistance within Brassicaceae. Crop Protection 19: 247-253.

Dosdall, L. M., D. Moisey, H. Cárcamo, and R. Dunn. 2001. Cabbage seedpod weevil fact sheet. Alberta Agriculture, Food and Rural Development, Edmonton, AB, Canada.

Dosdall, L.M., R.M. Weiss, O. Olfert, and H.A. Cárcamo. 2002. Temporal and geographical distribution patterns of cabbage seedpod weevil (Coleoptera: Curculionidae) in canola. The Canadian Entomologist 134: 403-418.

Dosdall, L.M., G.A.P. Gibson, O. Olfert, B.A. Keddie, and B.J. Ulmer. 2007. Contributions to the life history, host range, and distribution of *Necremnus tidius* (Walker) (Hymenoptera: Eulophidae). Annals of the Entomological Society of America 100: 861–868.

Dosdall, L.M., G.A.P. Gibson, O.O. Olfert, and P.G. Mason. 2009. Responses of Chalcidoidea (Hymenoptera) parasitoids to invasions of the cabbage seedpod weevil (Coleoptera: Curculionidae) in western Canada. Biological Invasions 11: 109–125.

Ellis, P.R., J.D. Taylor, and I.H. Littlejohn. 1982. The role of microorganisms in the attraction of cabbage root fly, pp. 131–137. *In*: Visser, J.H., and A.K. Minks (eds.), Proceedings of the Fifth International Symposium on Insect-Plant Relationships. Pudoc**,** Wageningen**.**

Feeny, P. P. 1976. Plant apparency and chemical defence. Recent Advances in Phytochemistry 10: 1-40.

Font, R., R.M. Rio-Celestino, E. Cartea, and A. Haro-Bailon. 2005. Quantification of glucosinolates in leaves of leaf rape (*Brassica napus* spp. *pabularia*) by near-infrared spectroscopy. Phytochemistry 66: 175-185.

Frearson, D.J.T., A.W. Ferguson, N. Laugier, and I.H. Williams. 2006. The influence of petals on numbers of pests and their parasitoids in oilseed rape. *In* Proceedings of the International Symposium on Integrated Pest

Management in Oilseed Rape. 2-5 April 2006. Paulinerkirche, Göttingen, Germany.

Gavloski, J.E., U. Ekuere, A. Keddie, L. Dosdall, and L. Kott. 2000. Identification and evaluation of flea beetle (*Phyllotreta cruciferae*) resistance within Brassicaceae. The Canadian Journal of Plant Science 80: 881-887.

Gibson, G.A.P., H. Baur, B. Ulmer, L. Dosdall, and F. J. Muller. 2005. On the misidentification of the chalcid (Hymenoptera: Chalcidoidea) parasitoids of the cabbage seedpod weevil (Coleoptera: Curculionidae) in North America. The Canadian Entomologist 137: 381-403.

Gibson, G.A.P., D.R. Gillespie, and L.M. Dosdall. 2006. The species of Chalcidoidea (Hymenoptera) introduced to North America for biological control of the cabbage seedpod weevil, and the first recovery of *Stenomalina gracilis* (Chalcidoidea: Pteromalidae). The Canadian Entomologist 138: 285-291.

Glawischnig, E., B.G. Hansen, C. E. Olsen, and B.A. Halkier. 2004. Camalexin is synthesized from indole-3-acetaldoxime, a key branching point between primary and secondary metabolism in *Arabidopsis*. Proceedings of the National Academy of Sciences 101: 8245-8250.

Gould, F. 1984. Role of behavior in the evolution of insect adaptation to insecticides and resistant host plants. Bulletin of the Entomological Society of America 30: 34-41.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: Integrating pest genetics and ecology. Annual Review of Entomology 47: 701–722.

Harborne, J.B., and C.A. Williams. 2000. Advances in flavonoid research since 1992. Phytochemistry 55: 481-504.

Haughton, A.J., G.T. Champion, C. Hawes, M.S. Heard, D.R. Brooks, D.A. Bohan, S.J. Clark, A.M. Dewar, L.G. Firbank, J.L. Osborne, J.N. Perry, P. Rothery, D.B. Roy, R.J. Scott, I.P. Woiwod, C. Birchall, M.P. Skellern, J.H. Walker, P. Baker, E.L. Browne, A.J.G. Dewar, B.H. Garner, L.A. Haylock, S.L. Horne, N.S. Mason, R.J.N Sands, and M.J. Walker. 2003. Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. II. Within-field epigeal and aerial arthropods. Philosophical Transactions of the Royal Society of London, Series B 358: 1863-1877.

Hayter, K., and J. E. Cresswell. 2006. The influence of pollinator abundance on the dynamics and efficiency of pollination in agricultural *Brassica napus*: Implications for landscape-scale gene dispersal. Journal of Applied Ecology 43: 1196-1202.

Hemm, M.R., M.O. Ruegger, and C. Chapple. 2003. The *Arabidopsis* ref2 mutant is defective in the gene encoding CYP83A1 and shows both phenylpropanoid and glucosinolate phenotypes. Plant Cell 15: 179–194.

Hopkins, R.J., B. Ekbom, and L. Henkow. 1998. Glucosinolate content and susceptibility for insect attack of three populations of *Sinapis alba*. Journal of Chemical Ecology 24: 1203–1216.

Horn, G.C., A.E. Dudeck, and R.W. Toler. 1973. 'Floratam' St. Augustinegrass: a fast growing new variety for ornamental turf resistant to decline and chinch bugs. Florida Agricultural Experimental Station Circular S-224.

Hurburgh, C.R., G.R. Rippke, C. Heithoff, S.A. Roussel, C.L. and Hardy. 2000. Detection of genetically modified grains by near-infrared spectroscopy. Proceedings PITTCON 2000-Science for the 21st Century, New Orleans.

Irwin, R.E., S.Y. Strauss, S. Storz, A. Emerson, and G. Guibert. 2003. The role of herbivores in the maintenance of a flower-colour polymorphism in wild radish. Ecology 84: 1733–1743.

Jönsson, M., A. Lindkvist, and P. Anderson. 2005. Behavioural responses in three ichneumonid pollen beetle parasitoids to volatiles emitted from different phenological stages of oilseed rape. Entomologia Experimentalis et Applicata 115: 363-369.

Kay, Q.O.N., H.S. Daoud, and C.H. Stirton. 1981. Pigment distribution, light reflection and cell structure in petals. Botanical Journal of the Linnaean Society 83: 57-84.

Kennedy, G.G., F. Gould, O.M. DePonti, and R.E. Stinner. 1987. Ecological, agricultural, genetic and commercial considerations in the development of insect resistant germplasm. Environmental Entomology 16: 327-338.

Kitamura, S. 2006. Transport of flavonoids: from cytosolic synthesis to vacuolar accumulation, pp. 123-146. *In*: Grotewold, E. (ed.). Science of flavonoids. Springer, Berlin.

Kohno-Murase, J., M. Murase, H. Ichikawa, and J. Imamura. 1995. Improvement in the quality of seed storage protein by transformation of *Brassica napus* with an antisense for cruciferin. Theoretical and Applied Genetics 91: 627-631.

Koritsas, V.M., J.A. Lewis, and G.R. Fenwick. 1989. Accumulation of indole glucosinolates in *Psylliodes chrysocephala* L. infested-, or damaged-tissues of oilseed rape (*Brassica napus* L). Experientia 45: 493-495.

Koritsas, V.M, J.A. Lewis, and G.R. Fenwick. 1991. Glucosinolate responses of oilseed rape, mustard and kale to mechanical wounding and infestation by cabbage stem flea beetle (*Psylliodes chrysocephala*). Annals of Applied Biology 118: 209-221.

Kott, L.S., and L.M. Dosdall. 2004. Introgression of root maggot resistance (*Delia* spp.) derived from *Sinapis alba* L. into *Brassica napus* L. Brassica 6: 55–62.

Kuhlmann, U., P.G. Mason, H.L. Hinz, B. Blossey, R.A. De Clerck-Floate, L.M. Dosdall, J.P. McCaffrey, M. Schwarzlaender, O. Olfert, J. Brodeur, A. Gassmann, A.S. McClay, and R.N. Wiedenmann. 2006. Avoiding conflicts between insect and weed biological control: Selection of non-target species to assess host specificity of cabbage seedpod weevil parasitoids. Journal of Applied Entomology 130: 129–141.

Kuśnierczyk, A., P. Winge, T.S. Jørstad, J. Troczynska, J.T. Rossiter, and A.M. Bones. 2008. Towards global understanding of plant defence against aphids – timing and dynamics of early *Arabidopsis* defence responses to cabbage

aphid (*Brevicoryne brassicae*) attack. Plant, Cell and Environment 31: 1097–1115.

Li, Y., G. Kiddle, R.N. Bennett**,** and R.M. Wallsgrove**.** 1999**.** Local and systemic changes in glucosinolates in Chinese and European cultivars of oilseed rape *(Brassica napus* L.) after inoculation with *Sclerotinia sclerotiorum* (stem rot). Annals of Applied Biology 134: 45-58

McCartney, H.A., and M.E. Lacey. 1991 Wind dispersal of pollen from crops of oilseed rape (*Brassica napus* L.). Journal of Agricultural Science 107: 299-305.

Mesquida, J., M. Renard, and J.S. Pierre. 1988. Rapeseed (*Brassica napus* L.) productivity: The effect of honey bees (*Apis mellifera* L.) and different pollination conditions in cage and field tests. Apidologie 19: 51-72.

Mewis, I., C. Ulrichs, W.H. Schnitzler. 2002. Possible role of glucosinolates and their hydrolysis products in oviposition and host-plant finding by cabbage webworm, *Hellula undalis*. Entomologia Experimentalis et Applicata 105: 129–139.

Miller, J.R., and R.S. Cowles. 1990. Stimulo-deterrent diversion: A concept and its possible application to onion maggot control. Journal of Chemical Ecology 16: 3197–3212.

Mol, J., E. Grotewold, and R. Koes. 1998. How genes paint flowers and seeds. Trends in Plant Science 3: 212-217.

Morandin, L.A., and M.L. Winston. 2005. Wild bee abundance and seed production in conventional, organic, and genetically modified canola. Ecological Applications 15: 871-881.

Nagata, R.T., and R.H. Cherry. 2003. New source of chinch bug (Hemiptera: Lygaeidae) resistance in a diploid selection of St. Augustinegrass. Journal of Entomological Science 38: 654-659.

Nielsen, J.K., M.L. Hansen, N. Agerbirk, B.L. Petersen, and B.A. Halkier. 2001. Responses of the flea beetles *Phyllotreta nemorum* and *P. cruciferae* to metabolically engineered *Arabidopsis thaliana* with an altered glucosinolate profile. Chemoecology 11: 75-83.

Nilsson, C., and B. Andreasson. 1987. Parasitoids and predators attacking pollen beetles (*Meligetes aeneus* F.) in spring and winter rape in southern Sweden. IOBC/WPRS Bulletin 10: 64-73.

Palaniswamy, P. 1996. Host plant resistance to insect pests of cruciferous crops with special reference to flea beetles feeding in canola – a review. Acta Horticulture (ISHS) 407: 469-482.

Panetsos, C.P. 1964. Sources of variation in wild populations of *Raphanus* (Cruciferae). Ph.D. thesis, University of California, Berkeley, California.

Parmesan, C. 1991. Evidence against plant "apparency" as a constraint on evolution of insect search efficiency (Lepidoptera: Nymphalidae). Journal of Insect Behavior 4: 417-430.

Pedras, M.S.C., S. Montaut, and M. Suchy. 2004. Phytoalexins from the crucifer rutabaga: Structures, syntheses, biosyntheses, and antifungal activity. Journal of Organic Chemistry 69: 4471-4476.

Pfündel E.E., G. Agati, and Z.G. Cerovic. 2006. Optical properties of plant surfaces, pp. 216-249. *In*: Riederer, M., and C. Müller (eds.). Biology of the Plant Cuticle. Annual Plant Reviews 23. Blackwell Publishing, Oxford.

Piel, J. 2009. Metabolites from symbiotic bacteria. Natural Products Reports 26: 338-362.

Pivnick, K.A., R.J. Lamb., and D. Reed. 1992. Response of flea beetles, *Phyllotreta* spp., to mustard oils and nitriles in field trapping experiments. Journal of Chemical Ecology 18: 863-873.

Rahman, M.H. 2001. Inheritance of petal colour and its independent segregation from seed colour in *Brassica rapa*. Plant Breeding 120: 197-200.

Ramsay G., C.E. Thompson, S. Neilson, and G.R. Mackay. 1999. Honeybees as vectors of GM oilseed rape pollen, pp. 209-221. *In*: Gene Flow and Agriculture: Relevance for Transgenic Crops. BCPC Symposium Proceedings No. 72. Keele, UK.

Reifenrath, K., M. Riederer, and C. Muller. 2005. Leaf surface wax layers of Brassicaceae lack feeding stimulants for *Phaedon cochleariae*. Entomologia Experimentalis et Applicata 115: 41-50.

Reinert, J.A., and A.E. Dudeck. 1974. Southern chinch bug resistance in St. Augustinegrass. Journal of Economic Entomology 67: 275-277.

Renwick, J.A.A., C.D. Radke, K. Schdev-Gupta, and E. Städler. 1992. Leaf surface chemicals stimulating oviposition by *Pieris rapae* on cabbage. Chemoecology 3: 33-38.

Renwick, J.A.A., M. Haribal, S. Gouinguené, and E. Städler. 2006. Isothiocyanates stimulating oviposition by the diamondback moth, *Plutella xylostella.* Journal of Chemical Ecology 32: 755-766.

Roessingh, P., E. Städler, G.R. Fenwick, J.A. Lewis, J.K. Nielsen, J. Hurter, and T. Ramp. 1992. Oviposition and tarsal chemoreceptors of the cabbage root fly are stimulated by glucosinolates and host plant extracts. Entomologia Experimentalis et Applicata 65: 267-282.

Rui, Y.K., Y.B. Luo, K.L. Huang, W.M. Wang, and L.D. Zhang. 2005. Application of near-infrared diffuse reflectance spectroscopy to the detection and identification of transgenic corn. Spectroscopy and Spectral Analysis 25: 1581-1583.

Sabbahi, R., D. de Oliveira, and J. Marceau. 2005. Influence of honey bee (Hymenoptera: Apidae) density on the production of canola (Crucifera: Brassicacae [sic!]). Journal of Economic Entomology 98: 367-372.

Sabbahi, R., D. de Oliveira, and J. Marceau. 2006. Does the honeybee (Hymenoptera: Apidae) reduce the blooming period of canola? Agronomy and Crop Science 192: 233-237.

Schmelz, E.A., H.T. Alborn**,** and J.H. Tumlinson**.** 2003**.** Synergistic interactions between volicitin, jasmonic acid and ethylene mediate insect-induced volatile emission in *Zea mays*. Physiologia Plantarum 117: 403-412.

Schwinn, K.E., and K.M. Davies. 2004. Flavonoids, pp. 92-149. *In*: Davies, K.M. (ed.). Plant pigments and their manipulation. Annual Plant Reviews 14. CRC Press, Blackwell Publishing, Boca Raton.

Séguin-Swartz, G. 1988. Inheritance of a pale yellow petal mutant of summer rape. EUCARPIA Cruciferae Newsletter 13: 24-25.

Shaw, E. 2008. The detection of biochemical markers for cabbage seedpod weevil (*Ceutorhynchus obstrictus*) resistance in *Brassica napus* L. x *Sinapis alba* L. germplasm. M.Sc. Thesis, University of Guelph, Guelph, ON. 155 pp.

Simms, E.L., and M.A. Bucher. 1996. Pleiotropic effects of flower-colour intensity on herbivore performance on *Ipomoea purpurea*. Evolution 50: 957–963.

Soler, T., M. Bezemer, A. M. Cortesero, W.H. Van der Putten, L.E.M. Vet, and J.A. Harvey. 2007. Impact of foliar herbivory on the development of a root-feeding insect and its parasitoid. Oecologia 152: 257-264.

Strauss, S.Y., R.E. Irwin, and V.M. Lambrix. 2004. Optimal defence theory and flower petal colour predict variation in the secondary chemistry of wild radish. Journal of Ecology 92: 132–141.

Tahvanainen, J. O., and R. B. Root. 1972. The influence of vegetational diversity on the population ecology of a specialized herbivore, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). Oecologia 10: 321–346.

Tanaka, Y., N. Sasaki, and A. Ohmiya. 2008. Biosynthesis of plant pigments: Anthocyanins, betalains and carotenoids. The Plant Journal 54: 733-749.

Tu, Y., J. Sun, X. Ge, and Z. Li. 2009. Chromosome elimination, addition and introgression in intertribal partial hybrids between *Brassica rapa* and *Isatis indigotica.* Annals of Botany doi:10.1093/aob/mcp045.

Ulmer, B.J., and L.M. Dosdall. 2006. Glucosinolate profile and oviposition behaviour in relation to the susceptibilities of different Brassicaceae to the cabbage seedpod weevil. Entomologia Experimentalis et Applicata 121: 203-213.

Vacher, C., D. Bourguet, F. Rousset, C. Chevillon, and M.E. Hochberg. 2003. Modelling the spatial configuration of refuges for a sustainable control of pests: A case study of Bt cotton. Journal of Evolutionary Biology 16: 378–387.

Williams, I.H., A.P. Martin, and R.P. White. 1987. The effect of insect pollination on plant development and seed production in winter oil-seed rape (*Brassica napus* L.). Journal of Agricultural Science (Cambridge) 109: 135-139.

Xie, L., Y. Ying, T. Ying, H. Yu, and X. Fu. 2007. Discrimination of transgenic tomatoes based on visible/near-infrared spectra. Analytica Chimica Acta 584: 379-384.

Xu, W.T., W.B. Bai, Y.B. Luo, and Y.F. Yuan. 2009. Research progress in techniques for detecting genetically modified organisms. Chinese Journal of Agricultural Biotechnology 6: 1–9.

Yoshioka, H., A. Horisaki, K. Kobayashi, Syafaruddin, S. Niikura, S. Ninomiya, and R. Ohsawa. 2005. Intraspecific variation in the ultraviolet colour proportion of flowers in *Brassica rapa* L. Plant Breeding 124: 551-556.

Zou, J., V. Katavic, E.M. Giblin, D.L. Barton, S.L. MacKenzie, W.A. Keller, X. Hu, and D.C. Taylor. 1997. Modification of seed oil content and acyl composition in the Brassicaceae by expression of a yeast sn-2 acyltransferase gene. Plant Cell 9: 909-923.