

University of Alberta

The effects of soil and plant nutrients on the oviposition preference, larval performance and spatial dynamics of *Ceutorhynchus obstrictus* and its parasitoids

by

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Abstract

The effects of nitrogen and sulfur fertilization on the oviposition, feeding preferences, and larval performance of *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae) on *Brassica napus* L. were examined in a series of laboratory experiments. The associations between *C. obstrictus* adults, larvae and parasitoids, and environmental factors including plant vigor indicators and soil and plant nutrients were evaluated within two commercial fields of *B. napus* in southern Alberta. Nitrogen fertilization, and sulfur fertilization at low levels of nitrogen fertilization had positive effects on oviposition preference. Nitrogen had a positive effect on larval development times and no effect on larval weights. Within one field, gravid *C. obstrictus* females were dissociated with high levels of plant nutrients including nitrogen. The synthesis of the lab and field experiments seems to support the plant stress and the preference-performance hypotheses. Differences in olfactory and visual cues are identified as a possible mechanism for the observed differences.

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List of Abbreviations

EC	Electrical Conductivity
GSL	Glucosinolate
HSB	Hue Saturation and Brightness Color Space
K	Potassium
N	Nitrogen
OM	Organic Matter Content
P	Phosphorous
RGB	Red Green and Blue Color Space
S	Sulfur
SADIE	Spatial Analysis by Distance Indices
TEC	Total Extractable Cations
SEM	Structural Equation Model(ing)
UV	Ultraviolet
λ	Wavelength
v_i	SADIE's patch index
v_j	SADIE's gap index
X	SADIE's index of association

Chapter 1 Introduction

1.1 Canola or oilseed rape (*Brassica napus* L. and *Brassica rapa* L.)

Canola and other types of oilseed brassicaceous crops are important both in North America and Europe (Alford 2003), with value that encompasses uses in human and animal nutrition, and most recently in the biofuel industry (Canola Council of Canada 2008). Worldwide over 30 million hectares of oilseed rape are in production, including 6.5 million hectares in Canada (FAOSTAT 2008). Canola production contributes roughly 14 billion dollars annually to the Canadian economy (Canola Council of Canada 2008). Canola production in Canada is concentrated in the western provinces of Alberta, Saskatchewan and Manitoba, with minor production in British Columbia, Ontario and Québec (Statistics Canada 2010). The canola species grown in western Canada include *Brassica napus* L., *Brassica rapa* L., and *Brassica juncea* (L.) Czern. (Thomas 2003). *Brassica napus* is the dominant crop in most areas (Alford 2003) due to its higher yields compared with *B. rapa* (Holmes 1980).

The term 'canola' refers to varieties that have been selected for low seed glucosinolate (GSL) and erucic acid contents, which allow the oil to be used for human consumption and the meal for animal feed (Downey and Rimmer 1994). Winter-dormant, biennial or winter varieties are preferred over spring-seeded varieties in Europe because they have greater yield (Alford 2003). Winter varieties have insufficient frost hardiness to withstand Canadian climatic conditions (Holmes 1980), and as a consequence all canola grown in western

Canada comprises spring-planted annual or spring canola varieties. Herbicide-resistant transgenic varieties of canola have been widely adopted by western Canadian producers (Cárcamo and Blackshaw 2007). The resistance of these varieties to herbicides such as glyphosate or glufosinate-ammonium allow for more effective control of dicotyledonous weeds within the crop than is possible with non-transgenic varieties (Thomas 2003).

1.2 *Ceutorhynchus obstrictus* (Marsham), the cabbage seedpod weevil

1.2.1 Taxonomic status

The cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae), is a member of the subfamily Ceutorhynchinae Gistel, 1856 and the tribe Ceutorhynchini Gistel, 1848 (Colonnelli 2004). The genus *Ceutorhynchus* Germar 1824 is a large diverse genus with an Holarctic distribution, and its members are almost exclusively associated with cruciferous hosts.

The nomenclature of this species has been recently revised as a result of a historical misidentification. Consequently, *C. assimilis* (Paykull) was recognized as a junior synonym of *C. obstrictus* (Colonnelli 1990, 1993). The junior synonym *C. assimilis* was in wide use for over a hundred years and as a result much of the literature refers to the cabbage seedpod weevil as *C. assimilis* (Williams 2006). A petition to conserve the name of *C. assimilis* was recently reviewed by the International Commission of Zoological Nomenclature (Alford 2006), and the Commission ruled that the appropriate name is *C. obstrictus* (ICZN 2007).

1.2.2 European origin and North American invasion

The cabbage seedpod weevil occurs throughout Europe (Hill 1987) and is an introduced species in the Nearctic that was first recorded in North America in the vicinity of Vancouver, British Columbia in 1931 (McLeod 1953). It was subsequently reported in Washington (Baker 1936), California (Hagen 1946; Crowell 1952), Georgia (Buntin et al. 1995), and Tennessee (Boyd and Lentz 1994). It is now thought to occur throughout continental United States (Cárcamo et al. 2001). It has since been recorded in Alberta, Saskatchewan (Dosdall et al. 2002), Ontario (Mason et al. 2003) and Québec (Brodeur et al. 2001) and is predicted to eventually occupy all areas of canola production in western Canada (Dosdall et al. 2002). Molecular analysis of weevil populations has provided evidence of two separate introductions, with populations in Québec distinct from those in the remainder of the continent (Laffin et al. 2005).

1.2.3 Life history

The cabbage seedpod weevil is oligophagous on Brassicaceae (Dmoch 1965; Dosdall and Moisey 2004) and has been recognized as a serious pest of cultivated mustards in Europe since the beginning of the 19th century (Bonnemaison 1957). The weevil is univoltine and overwinters as an adult under the leaf litter layer in shelterbelts or wooded areas near canola fields (Dmoch 1965; Ulmer and Dosdall 2006a). Mortality during overwintering accounts for approximately 50% of generational mortality and is an important limiting factor

on population growth (Haye et al. 2010). Adults begin to emerge from these overwintering areas when soil temperatures warm to 9-12°C, and emergence peaks when temperatures reach 15°C (Dmoch 1965; Ulmer and Dossdall 2006a). Adults then disperse to feed on a variety of brassicaceous weeds, such as wild mustard (*Sinapis arvensis* L.), flixweed (*Descurainia sophia* (L.) Webb) and field pennycress (*Thlaspi arvense* L.) (Fox and Dossdall 2003; Dossdall and Moisey 2004). The host plants of *C. obstrictus* can be divided into two categories, food plants such as the species listed above, and true hosts usually from the genus *Brassica* that allow for both feeding and larval development (Dmoch 1965).

Ceutorhynchus obstrictus invades fields of canola in the bud to early flower stages (Dmoch 1965; Dossdall and Moisey 2004). The adults are drawn to these large concentrations of true hosts by several GSL-derived plant volatiles (Bartlet et al. 1993; Moyes and Raybould 2001). Females experience limited egg development at the time of dispersing to canola, but this increases rapidly after they begin to feed on their true hosts (Dmoch 1965; Ni et al. 1990; Fox and Dossdall 2003). Mating also begins at this time and occurs throughout flowering (Dossdall and Moisey 2004). Adults feed primarily on structures of the plant inflorescence, such as flower buds, flower parts, and developing pods, but they can also feed on young leaves and the upper stem (Dmoch 1965; Dossdall and Moisey 2004). During flowering the adults feed primarily on pollen (Dmoch 1965).

Following a period of feeding on true hosts, mated females begin oviposition on host plants (Dossdall and Moisey 2004). Most oviposition activity

occurs in mid flowering, when pods on lower racemes are approximately 40 to 60 mm long (Dosdall and Moisey 2004). Eggs are laid most frequently within canola pods that develop on the lower portions of racemes (Dosdall and Moisey 2004). Often only one egg is laid per pod because females mark pods after oviposition with an oviposition deterring pheromone (Kozlowski et al. 1983), but in outbreak conditions it is not uncommon for a single pod to be inhabited by two or three larvae (Cárcamo et al. 2001). The larva develops within the pod, feeding on the developing seeds and consumes an average of five seeds in the course of three instars (Dmoch 1965). When mature, the final-instar larva chews an exit hole in the pod and drops to the soil surface where it burrows down to pupate in soil (Dmoch 1965). This period of development from egg to pupa spans 21-31 days and correlates well with pod development (Dmoch 1965). Pupation occurs 3-8 cm below the soil surface in an earthen cell (Dmoch 1965). Pupation lasts 11-24 days; the new generation adults emerge and feed upon host plants until the temperature drops in the fall and they migrate to overwintering sites (Dmoch 1965; Dosdall and Moisey 2004). Development from egg to adult requires 31 to 58 days in spring canola in western Canada (Dosdall and Moisey 2004), and developmental rate is sufficiently flexible to allow *C. obstrictus* to exploit the shorter development window available with spring-planted canola (Dosdall and Moisey 2004).

1.2.4 Economic importance

Ceutorhynchus obstrictus cause damage to canola crops as larvae and adults. Feeding on developing buds when plants are in the bud or early flowering stage can destroy vascular tissue causing ‘bud-blasting’, which prevents flowering and reduces the number of developing pods (Dmoch 1965; Dossdall et al. 2001). Larval feeding commonly reduces yields by 20% (Buntin et al. 1999). Even though the larvae consume a relatively small number of the total seeds per pod, infested pods are more predisposed to shattering before harvest than non-infested pods (Dossdall et al. 2001). Larval exit holes can also provide an entry site for disease pathogens (Dossdall et al. 2001). In Europe these exit holes also allow for subsequent infestation by the pod midge, *Dasineura brassicae* (Winnertz) (Diptera: Cecidomyiidae), resulting in further yield loss (Bartlett 1996). Newly emerged adults can cause further direct loss in yield and quality by feeding on seeds through the pod walls (Buntin et al. 1995; Dossdall et al. 2001). Feeding can also cause a loss in total seed weight and oil content (Buntin et al. 1995). The financial impact of larval feeding by *C. obstrictus* has been estimated at five million Canadian dollars per annum in Alberta alone (Colautti et al. 2006). This analysis also does not take into account indirect economic impacts or other sources of yield loss due to *C. obstrictus*.

1.2.5 Control strategies

Chemical control - Several foliar insecticides from the pyrethroid family are used to control cabbage seedpod weevil (Cárcamo et al. 2001; Cárcamo et al.

2005). Buntin (1999) and Cárcamo et al. (2005) showed that pyrethroids are more effective than organophosphates or organochlorines for controlling *C. obstrictus*. In Alberta the two pyrethroids, deltamethrin (Decis[®]) and cyhalothrin-lambda (Matador[®] or Silencer[®]), are registered for control of cabbage seedpod weevil (Government of Alberta 2010). These insecticides are typically applied when 10% of the crop is in flower to prevent oviposition; this timing is effective because substantial weevil invasion to crops has already occurred (Dosdall and Moisey 2004), but pollinators and many parasitoids are believed to be present in low densities (Dosdall et al. 2001). At moderate commodity prices, insecticide applications have been recommended when densities of *C. obstrictus* adults reach 3 to 4 per 180° sweep net sample (Dosdall et al. 2001), but at higher commodity prices applications have been recommended at densities of 2 *C. obstrictus* adults per sweep net sample (Dosdall, personal communication). While these applications are effective for reducing infestation levels, recolonization of fields after application is common (L. Dosdall, unpublished data) and these insecticides can have a substantial negative impact on beneficial insects such as pollinators (Government of Alberta 2010).

Insecticidal seed treatments have also been evaluated as a potential control method for *C. obstrictus*. Cárcamo et al. (2005) found that infestation levels of plants that developed from seeds treated with either lindane (an organochloride) or imidacloprid (a neonicotinoid) were not significantly different from an untreated control. These results may have been confounded by differences in flowering time between the treated and untreated plots. In contrast, Dosdall

(2009) conducted a field cage study and found that lindane, imidacloprid and clothianidin (a neonicotinoid) had negative effects on the development of larval *C. obstrictus* with the neonicotinoid compounds having a greater effect than lindane. These results were consistent with those of Bragg and Burns (1999), who found an absence of larval exit holes in plots with an imidacloprid seed treatment as compared with a high percentage of exit holes in the untreated control.

Resistant cultivars - Research has been conducted to develop resistant cultivars of canola (Cárcamo et al. 2001). Previous work has shown that *Sinapis alba* L. is not utilized by *C. obstrictus* for reproduction (Doucette 1947; Kalischuk and Dossdall 2004). Resistance genes from *S. alba* have been successfully transferred to *B. napus* through hybridization and back crossing (Dossdall and Kott 2006). These *S. alba* x *B. napus* hybrids have been shown to exhibit both antixenosis and antibiosis (Dossdall and Kott 2006; Tansey 2009). Resistant hybrids present distinct visual and olfactory cues as compared with susceptible *B. napus* plants (Tansey 2010a, b). There has also been research to develop low GSL and erucic acid cultivars of *S. alba* more suitable for human consumption while maintaining resistance to *C. obstrictus* (McCaffrey et al. 2004).

Cultural control – Some investigation has been undertaken into the use of cultural control for the management of *C. obstrictus*. Both seeding rate and date affected the levels of *C. obstrictus* infestation (Dossdall et al. 2006b). Plants seeded below the typical rate of 3-5 kg/ha and seeded in mid May both exhibited lower infestation rates than plants seeded at a lower rate and earlier in the season. The use of crop rotation is already recommended for canola production in western

Canada (Thomas 2003), but is unlikely to reduce damage from *C. obstrictus* as the adults are known to disperse widely (Cárcamo et al. 2001; Tansey et al. 2010c).

Trap cropping - Stimulo-deterrent diversion (push-pull) or trap cropping strategies (Miller and Cowles 1990) have been investigated for control of *C. obstrictus*. Buntin (1998) investigated this strategy using small plots of a winter canola cultivar with a border or trap crop of a spring cultivar. Both cultivars were seeded in the fall and as a result the spring-seeded cultivar flowered several weeks before the winter cultivar. The trap crop was sprayed with an insecticide after the weevils invaded in the spring. Adults were more numerous in the trap crop, but implementation of the trap crop failed to prevent damage to the main crop. The results of trap cropping were more successful on the scale of commercial fields (Cárcamo et al. 2007). The design was similar, though in addition to utilizing different varieties, Cárcamo et al. (2007) used trap crops of *B. rapa*, which is more susceptible to infestation than *B. napus* (Kalischuk and Dossdall 2004) and flowers at least a week earlier than *B. napus*. Cárcamo et al. (2007) also varied planting times. The trap crops were successful for preventing weevil damage in large square fields but unsuccessful in smaller, less regularly shaped fields (Cárcamo et al. 2007). A *B. rapa* trap crop was not effective when no insecticide was applied to the trap crop (Cook et al. 2004). The above investigations have only examined the pull portion of stimulo-deterrent diversion (push-pull) strategies. Cook et al. (2006) investigated the use of the less attractive variety

Starlight as a main crop in addition to a *B. rapa* trap crop; however, unfortunately *C. obstrictus* numbers were too low for meaningful analysis.

Site-specific management – The use of site-specific management (SSM) or precision agriculture could potentially be incorporated into an integrated pest management strategy for *C. obstrictus*. Site-specific management involves management at a spatial scale below that of the entire field (Plant 2001). This strategy is heavily dependent upon global positioning systems (GPS), geographic information systems (GIS), yield monitors, and variable rate application technology. While this strategy is not currently used to control *C. obstrictus*, SSM technology is widely used by producers, government agencies, and agronomic consultants with its use predicted to grow in the future (Alberta Agriculture and Rural Development 2001). Precision spraying of insecticides could be used to minimize the area sprayed and the negative effects to beneficial insects such as predators and parasitoids (Cárcamo et al. 2001). These approaches are dependent upon knowledge of spatial and temporal dynamics of *C. obstrictus* and the environmental factors governing them.

There have been several investigations of the spatio-temporal dynamics of *C. obstrictus*. Free and Williams (1979) showed that cabbage seedpod weevils were found in greater numbers on crop edges than in the interior during their immigration to the crop. This trend was observed in all subsequent studies (Murchie et al. 1999; Ferguson et al. 2000; Dossdall et al. 2006a). Murchie et al. (1999) confirmed this relationship statistically and also showed a temporal separation between the invasion of *C. obstrictus* and its principal parasitoid in

Europe, *Trichomalus perfectus* (Walker) (Hym., Pteromalidae). Invasion of oilseed rape by *C. obstrictus* occurs before invasion of the parasitoid, so properly timed insecticide application can minimize negative effects on the parasitoid. This temporal separation was not observed in the North American parasitoid fauna (Dosdall et al. 2006a). Ferguson et al. (2000) used Spatial Analysis by Distance IndicEs (SADIE) to quantify the clumped spatial pattern of cabbage seedpod weevil adults and larvae and its parasitoid *T. perfectus*. The analysis also showed a spatial association between weevil larvae and *T. perfectus*. Again using SADIE, spatial clustering was found in *C. obstrictus* adults, larvae and parasitoid numbers in North America by Dosdall et al. (2006a). They also confirmed spatial associations between the larvae of *C. obstrictus* and its North American parasitoid complex. There has been limited success in correlating bowl trap catches of adult weevils with observed larval densities as bowl traps are a measure of flight activity and may not adequately reflect oviposition behavior (Ferguson et al. 2000; Dosdall et al. 2006a). While there have been some investigations of the environmental factors underlying the spatial distribution of *C. obstrictus* (Ferguson et al. 2003; Blake et al. 2010), this understanding remains incomplete and consequently limits the use of site-specific management for *C. obstrictus*.

1.2.6 Parasitoids

Numerous parasitoids of the cabbage seedpod weevil have been recorded from both North America and Europe (Cárcamo et al. 2001). The main parasitoids of *C. obstrictus* in Europe include *Microctonus melanopus* Ruthe (Hym.,

Braconidae), *T. perfectus*, *Mesopolobus morys* (Walker), and *Stenomalina muscarum* (L.) (Hym., Pteromalidae) (Williams 2003). *Microctonus melanopus* is a koinobiont endoparasitoid of adult *C. obstrictus* while the remaining three are larval idiobiont ectoparasitoids (Williams 2003). Parasitism rates are variable but can be as high as 90% and are thought to play a major role in controlling *C. obstrictus* populations (Williams 2003).

The parasitoid fauna of North America differs considerably from Europe, and in western Canada it comprises a diverse assemblage of Chalcidoidea with 12 species from four families (Doddall et al. 2009). The dominant parasitoids include *Trichomalus lucidus* (Walker), *Mesopolobus moryoides* Gibson, *Stenomalina gracilis* (Walker) (Hym., Pteromalidae), *Necremnus tidius* (Walker) (Hym., Eulophidae) (Gibson et al. 2005; Gillespie et al. 2006), and *M. melanopus* (Harmon and McCaffrey 1997; Fox et al. 2004). With the exception of *M. melanopus*, all of these species are larval idiobiont ectoparasitoids of the cabbage seedpod weevil (Gibson et al. 2005). With the exception of *M. moryoides*, all North American parasitoids are either European introductions or are Holarctic in distribution (Gibson et al. 2005; Harmon and McCaffrey 1997a). Doddall et al. (2009) gives an exhaustive treatment of North American parasitoids of *C. obstrictus*. Observed rates of parasitism in North America are typically below 15% (Doddall et al. 2009) and are generally much lower than those observed in Europe (Harmon and McCaffrey 1997; Buntin 1998). This may be because the parasitoids observed in North America are not primary parasitoids of *C. obstrictus* (Gibson et al. 2005) and lack a long history of co-evolution (Doddall et al. 2006a)

hence host-seeking behaviors would be poorly attuned to *C. obstrictus* as compared with its primary European parasitoids. In Alberta, the main ectoparasitoids recorded include *N. tidius*, *T. lucidus*, *Chlorocytus* sp., and *Pteromalus* sp. (Hym., Pteromalidae) (Dosdall et al. 2006a). Observed rates of parasitism have increased from 0% in 1998-2001 to 5% in 2004 and approximately 15% in 2005 (Dosdall et al. 2006a; Dosdall et al. 2007; Dosdall et al. 2009), although parasitism levels can sometimes approach 50% for some sites and parasitoid species (Dosdall et al. 2007).

Although *T. perfectus*, *M. morys*, and *S. gracilis* were released in southwestern British Columbia as part of a classical biological control program in 1949, only *S. gracilis* has since become established (McLeod 1953; Gibson et al. 2006). The higher rates of parasitism observed in Europe make *C. obstrictus* a potential candidate for classical biological control (Muller et al. 2007). Given this potential, *T. perfectus* and *M. morys* are under current evaluation for release as biological control agents in North America (Kuhlman et al. 2006). The competition present among the already established native parasitoid fauna may potentially prove to be a barrier to introduction of European species (Dosdall et al. 2009).

1.3 Plant nutrients and their effects on oilseed rape

The management of soil nutrients is a key production requirement for optimal growth of canola (Grant and Bailey 1993). Nitrogen (N) is the most important nutrient for canola and the crop has a high requirement for N (Holmes

1980). Western Canadian soils tend to be deficient in N, resulting in a strong yield response of canola to N fertilizer (Krogman and Hobbs 1975; Cowell and Doyle 1993; Karamanos et al. 2007). *Brassica* species also have high sulfur (S) (Bole and Pitman 1984) and phosphorus (P) (Holmes 1980) requirements, which can also be deficient in western Canadian soils (Bettany et al. 1974; Cowell and Doyle 1993; Malhi et al. 2005). A significant interaction is often observed between N and S; the optimal ratio between the two nutrients being 7:1 (Janzen and Bettany 1984). While canola in general has a high requirement for potassium (K) (Holmes 1980), little K is removed through the harvest of seeds (Grant and Bailey 1993), preventing a significant drain on soil reserves and limiting the need for K fertilizer additions (Holmes 1980). Deficiencies in other soil nutrients are generally rare and thought to only occasionally limit canola yields (Grant and Bailey 1993).

Nutrients have several effects on the morphology and chemical composition of oilseed rape, which may affect selection of the plant as a host for insect herbivores. Fertilization with N and P has the general effect of increasing plant size, plant height, and inflorescence branching (Allen and Morgan 1972; Holmes 1980). Phosphorous deficiency can delay crop maturity (Holmes 1980) and S deficiency has been shown to reduce the number of seeds per pod (Schnug and Haneklaus 2005). Nitrogen and S have also been shown to affect both the protein and oil contents of canola seeds (Henry and MacDonald 1978; Nuttall et al. 1987; Malhi et al. 2005). Sulfur increases both protein and oil content (Malhi

et al. 2005) while N increases protein content while decreasing oil content (Grant and Bailey 1993).

Nutrients also affect GSL content in oilseed rape. Glucosinolates are N- and S-containing secondary plant compounds characteristic of the Brassicaceae (Halkier and Gershenzon 2006). They are a class of compounds related to the cyanogenic glucosides (Rask et al. 2000), and consist of a glucose molecule bonded to a non-sugar group via a S-glycosidic bond (Halkier and Gershenzon 2006). Glucosinolates are characterized by their variable R groups, which are derived from amino acids. Based on the amino acid precursors, GSLs can be categorized as aliphatic, aromatic or indolyl. Myrosinases, a type of thioglucoside glucohydrolase, react with GSLs to form glucose, sulfate, and the biologically active volatile isothiocyanates (Rask et al. 2000). Myrosinases and GSLs are separated spatially within the plant and isothiocyanates are generally only released following tissue damage (Halkier and Gershenzon 2006). Analysis of the headspace volatiles of undamaged *B. napus* plants has identified the presence of 3-butenyl, 4-pentenyl, and 2-phenylethyl isothiocyanates (Blight et al. 1995).

Nitrogen and S have been shown to increase the GSL content of the seed (Bilsborrow et al. 1993; Zhao et al. 1993, 1994; Asare and Scarisbrick 1995) and plant (Kim et al. 2002). The application of N in the absence of S also resulted in reduced levels of GSLs (Zhao et al. 1993). This effect may be explained through increased demand for S during protein synthesis, which is increased with N supply (Grant and Bailey 1993). As a result of this increased demand, there is less S available for GSL synthesis (Zhao et al. 1993). High levels of N also suppress

the positive effects of S on GSLs (Kim et al. 2002). This may be a result of high N levels suppressing the uptake of S. These contrasting interactions may have resulted from sampling of different plant parts in each study. Aljmli (2007) also found that high N levels suppressed the effect of S but this effect was only observed for the GSL content of leaves and not for the content of seeds. In general S has been shown to increase both indolyl and aliphatic GSLs including 3-butenyl and 4-pentenyl GSL (Zhao et al. 1994; Kim et al. 2002). This increase in 3-butenyl and 4-pentenyl GSLs could be suppressed by high N levels (Kim et al. 2002). Nitrogen increased indolyl GSLs (Zhao et al. 1994; Kim et al. 2002) and decreased aliphatic GSLs at low S levels and increased them at higher S levels (Zhao et al. 1994). Nitrogen has also been shown to decrease the levels of 4-pentenyl GSL. In addition to the differences in GSL content, S deficiency significantly alters the composition of volatiles emitted by the flowers of oilseed rape (Brauer et al. 2007).

Deficiencies in most nutrients cause at least some change in leaf color (Holmes 1980; Grant and Bailey 1993). Deficiencies of N and S are both known to cause chlorosis on old and new leaves respectively (Grant and Bailey 1993; Schnug and Haneklaus 2005). Severe S deficiency may also manifest in a reddish purple leaf and stem color due to anthocyanin enrichment in leaf tissue (Schnug and Haneklaus 2005). Sulfur deficient plants have flowers that appear white as opposed to the typical yellow (Schnug and Haneklaus 2005), and have a lower reflectance in the near ultraviolet (UV) (350 nm) and yellow (560-590 nm) wavelengths (λ) (Brauer et al. 2007). This color change is due to the build up of

leuco-anthocyanins within petal cells as a result of incomplete protein synthesis (Schnug and Haneklaus 2005). Petals of S-deficient plants are also smaller and more oval in shape (Schnug and Haneklaus 2005; Brauer et al. 2007).

1.4 Host plant location and selection by *Ceutorhynchus obstrictus*

Phytophagous insects exhibit great selectivity among olfactory and visual cues during host location (Prokopy and Owens 1983). Host location by phytophagous insects is thought to occur in a stepwise fashion with insects first responding to volatile host plant odors through upwind anemotaxis, followed by response to visual cues at close ranges and finally responses to tactile and non-volatile chemical cues when physical contact is made with the host plant (Kennedy 1965; Finch and Collier 2000).

The volatile isothiocyanates, which are breakdown products of GSLs, play an important role in regulating the host-finding and host-acceptance behaviors of specialist herbivores of *Brassica* spp. (Bartlett 1996). 3-butenyl, 4-pentenyl and 2-phenylethyl isothiocyanates have been shown to be attractive to *C. obstrictus* adults (Evans and Allen-Williams 1992; Bartlett et al. 1993; Blight et al. 1995; Smart and Blight 1997). In addition, the 1-methoxy-3-indolylmethyl GSL and its breakdown products are repellent to *C. obstrictus* (Tansey et al. 2010b). This GSL is also associated with lower levels of infestation by *C. obstrictus*, decreased larval weights and increased larval development time (Tansey 2009). There is debate as to the attractive range of olfactory cues for phytophagous insects (Bartlett 1996). It is often found that host plant odors are attractive within the

range of several meters (Finch 1980), but in the case of *C. obstrictus*, adults can respond to host plant odors at distances of 20 m (Evans and Allen-Williams 1993). *Ceutorhynchus obstrictus* adults respond to host plants through odor-mediated upwind anemotaxis (Evans and Allen-Williams 1993).

Visual cues have perhaps the most important roles in host plant location for phytophagous insects (Prokopy and Owens 1983). *Ceutorhynchus obstrictus* most likely has a trichromatic visual system with response maxima at wavelengths of 350 (near UV), 450 (blue), and 550 nm (green) (Tansey 2009). *Ceutorhynchus obstrictus* also exhibits a supernormal response to yellow ($\lambda = 560\text{-}590$ nm) (Prokopy and Owens 1983; Tansey 2009). The maxima are very similar to those of a modeled system that was near optimal for distinguishing among flowers and between flowers and foliage (Chittka 1996). These UV, blue and green receptors are thought to be ancestral to Insecta (Chittka 1996). Adult *C. obstrictus* respond maximally to high levels of yellow reflectance and moderate UV reflectance with deviations from this maximum being associated with decreased attractiveness (Tansey 2009). Blue and green reflectance also affected *C. obstrictus* with blue generally decreasing response and green generally increasing response. Males in general had lower responses to visual cues than females. Differences in the reflectance properties have been associated with differences in rates of *C. obstrictus* infestation in several lines derived from *S. alba* x *B. napus* hybrids (Tansey 2010a).

Finch and Collier (2000) suggest that non-volatile chemical cues govern the final oviposition decision with less suitable plants providing a reduced

stimulus requiring additional appropriate landings when compared with a more suitable host. The assessment of host quality by *C. obstrictus* is associated with a stepwise behavioral procedure ending in oviposition (Kozłowski et al. 1983). The steps are slower in less preferred hosts (Ulmer and Dossdall 2006b), and these protracted behaviors may similarly be the result of a reduced stimulus from the plant. *Ceutorhynchus obstrictus* has already shown the ability to discriminate among differing host species (Kalischuk and Dossdall 2004; Ulmer and Dossdall 2006b) and genotypes derived from *S. alba* x *B. napus* hybrids (Dossdall and Kott 2006; Tansey 2009), based solely on the cues presented by excised pods.

1.5 Nutrients and their effects on cruciferous specialists

Several phytophagous insect pests of *Brassica* spp. respond to differences in soil and plant nutrients. Although fertilizer rate had no influence on the rate of oviposition, higher rates of fertilization increased both larval survival and damage by root maggots (*Delia* spp.) (Diptera: Anthomyiidae) (Dossdall et al. 2004). In contrast, the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), oviposits preferentially, develops more quickly, and achieves higher pupal weights on plants grown at an intermediate level of fertilization (Sarfranz et al. 2009). Diamondback moth also oviposits preferentially on leaves from or treated with extracts of plants grown under a high S regime (Gupta and Thorsteinson 1960; Marazzi et al. 2004).

The abundances of pollen beetles, *Meligethes* spp. (Coleoptera, Nitidulidae); the cabbage seedpod weevil; the brassica pod midge, *Dasineura*

brassicae (Winnertz) (Diptera: Cecidomyiidae); and the cabbage root fly, *Delia radicum* (L.) (Diptera, Anthomyiidae), have been increased in plots fertilized with S (Aljmlí 2007). The abundances of oilseed rape pests also generally increased with N fertilization, although the abundance of *C. obstrictus* adults was reduced with N fertilization in the absence of S fertilization.

Plant nutrient levels have also been shown to correspond with the spatial distribution of *P. xylostella* (Sarfráz et al. 2010) and *C. obstrictus* (Blake et al. 2010). The distributions of *C. obstrictus* adults and larvae were associated with areas of the crop with high levels of S and low levels of N, and dissociated with areas of the crop with low levels of S and high levels of N (Blake et al. 2010).

Although some investigation of the effects of soil and plant nutrient levels has been performed, there are still several gaps in our understanding of these effects. Chapter 2 examines the effects of N and S fertilization on oviposition preferences and larval development of *C. obstrictus*. While previous work has examined the spatial associations between plant nutrients and the distribution of *C. obstrictus*, unlike research described in Chapter 3, previous studies have not examined the system as a whole and have not investigated the relationships with plant vigor and soil nutrients. The mechanism for nutrient effects on *C. obstrictus* field distributions remains uncharacterized and is investigated in Chapter 3. The results of Chapters 2 and 3 should allow for further investigation of the plant vigor (Price 1991), plant stress (White 1984), and preference-performance hypotheses (Jaenike 1978). The plant vigor hypothesis predicts female insects will select rapidly growing plants or plant parts that reach a large size relative to the

mean size of the population as larval hosts. These plants or plant parts provide an improved environment for larval development due to an increased quantity of tissues and lower levels of defensive chemicals (Price 1991). In contrast, the plant stress hypothesis predicts ovipositing females will prefer stressed plants as these plants are more nutritious due to higher concentrations of free amino acids in tissues (White 1984) and compromised defense systems (Rhoades 1985). These hypotheses have been suggested as two ends of a continuum of insect response with the plant vigor hypothesis being most relevant for flush-feeders and the plant stress hypothesis being most relevant for senescence-feeders (White 2009). The preference-performance hypothesis is a more general hypothesis that females will exhibit an ovipositional preference for environments in which the fitness of their larvae is maximized (Jaenike 1978).

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Chapter 2 Bottom-up effects of *Brassica napus* nutrition on the oviposition preference and larval performance of *Ceutorhynchus obstrictus* (Coleoptera: Curculionidae)

Note: A version of this chapter has been accepted for publication.

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2.1 Introduction

Canola (*Brassica napus* L. and *Brassica rapa* L.) is an important agricultural crop in Europe, Asia, Australia, and North America (Smith and Jimmerson 2005). The cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (syn. *Ceutorhynchus assimilis* Paykull) (Coleoptera: Curculionidae), is an introduced invasive pest of canola in North America. First reported in British Columbia in 1931 (McLeod 1953), it has since spread across North America (Cárcamo et al. 2001) and it is predicted to eventually colonize all areas of canola production in Canada (Dossdall et al. 2002). *Ceutorhynchus obstrictus* was first recorded within Alberta in 1995 (Cárcamo et al. 2001).

The cabbage seedpod weevil is univoltine and oligophagous on members of the Brassicaceae (Dmoch 1965; Dossdall and Moisey 2004). In spring *C. obstrictus* adults emerge from overwintering sites in shelterbelts and wooded areas when soil temperatures reach approximately 15°C (Dmoch 1965; Ulmer and Dossdall 2006a). Peak oviposition occurs during mid flowering when both developing pods and flowers are present in the crop (Dossdall and Moisey 2004).

Gravid females select immature pods, excavate a cavity in the pod wall and deposit a single egg within (Dosdall and Moisey 2004). After oviposition the female marks pods with an oviposition deterring pheromone (Kozlowski et al. 1983). This pheromone acts as a deterrent for a period of 1-2 h (Ferguson and Williams 1991) and it is not uncommon for a single pod to be inhabited by two or three larvae (Cárcamo et al. 2001). Larvae complete three instars within the pod, each larva consuming five to six developing seeds (Dmoch 1965; Dosdall and Moisey 2004). Larval development times range between 17 - 44 d depending on temperature (Bonnemaïson 1957; Dmoch 1965; Dosdall and Moisey 2004). Mature larvae then excavate holes in the pod wall, and drop to the soil surface where they burrow into the soil to pupate (Dmoch 1965). New generation adults emerge after approximately 14 d (Dosdall and Moisey 2004) and feed on brassicaceous plants until temperatures decline in the fall when they migrate to overwintering sites (Dmoch 1965).

The feeding of overwintered adult *C. obstrictus* on developing flowers can result in ‘bud-blasting’ and a reduction in the number of pods (Dosdall et al. 2001). Direct yield loss also results from both larvae and newly emerged adults feeding upon developing seeds. Larval feeding commonly results in yield reductions of 20% (Buntin et al. 1999). In addition to feeding damage, infested pods are more susceptible to both premature shattering and fungal infection (Dosdall et al. 2001). In Europe, larval exit holes also allow for subsequent infestation by the pod midge, *Dasineura brassicae* (Winnertz) (Diptera: Cecidomyiidae), resulting in further yield loss (Bartlett 1996). While insecticides

are effective in controlling *C. obstrictus* (Cárcamo et al. 2001; Cárcamo et al. 2005), recolonization following treatment is common (L. Dossall, unpublished data) and these insecticides have negative effects on natural enemies and other beneficial insects (Cárcamo et al. 2001).

The supply of essential plant nutrients can have several effects on the physical and biochemical characteristics of canola. The most commonly limiting nutrients for canola production include nitrogen (N) and sulfur (S) (Holmes 1980; Cowell and Doyle 1993). The ratio of N and S in soil and plant tissue is also important, with the optimum being 7:1 (Janzen and Bettany 1984). Plant size, plant height, and inflorescence branching all increase with N fertilization (Allen and Morgan 1972; Holmes 1980). Nitrogen fertilization has also been shown to increase the protein content of the seed while decreasing oil content (Grant and Bailey 1993). Sulfur fertilization can increase both oil and protein contents (Malhi et al. 2005). There is generally a positive relationship between the concentration of glucosinolates (GSLs) within plant tissue and N and S supply (Bilsborrow et al. 1993; Zhao et al. 1993 1994; Asare and Scarisbrick 1995; Kim et al. 2002). Increases in S have been associated with increases in 3-butenyl and 4-pentenyl GSL (Zhao et al. 1994; Kim et al. 2002) whose breakdown products have been shown to be attractive to *C. obstrictus* (Evans and Allen-Williams 1992; Bartlet et al. 1993; Blight et al. 1995; Smart and Blight 1997). Increased N supply has been associated with decreases in these attractive GSL and increases in the relative composition of indolyl GSLs. 1-methoxy-3-indolylmethyl GSL is one such indolyl GSL and its has been shown to have a repellent effect on *C. obstrictus*

(Tansey et al. 2010). This GSL has also been correlated with lower larval weight and longer development times in *C. obstrictus*.

It is conceivable that these described changes in plant characteristics due to differing supplies of N and S may influence maternal or larval fitness, and as a consequence could influence female choice of oviposition sites. The two dominant hypotheses governing the effect of plant nutrients on the fitness of polyphagous insects are the plant vigor (Price 1991) and stress hypotheses (White 1984). In this case the plant vigor hypothesis would predict that female *C. obstrictus* will choose plants with greater levels of N and S during oviposition as these plants or plant parts will grow more rapidly and provide a more optimal environment for larval development (Price 1991). In contrast the plant stress hypothesis predicts that ovipositing females will prefer hosts with lower levels of N and S because these plants would have higher concentrations of free amino acids resulting from reduced protein synthesis (White 1984). In addition, both hypotheses predict lower concentrations of defensive chemical in preferred plants (Rhoades 1985; Price 1991).

While the relative supply of N and S has been recognized as important for *Brassica* specialists in the choice of oviposition sites and larval fitness (Doddall et al. 2004; Sarfraz et al. 2009; Sarfraz et al. 2010) their responses do not consistently support either the plant vigor or plant stress hypotheses. The spatial distributions of *C. obstrictus* adults and larvae in commercial fields have been associated with areas of high S and low N contents in plant tissue (Blake et al.

2010). Plot experiments have also shown increased abundances of *C. obstrictus* adults and larvae with N and S fertilization (Aljmlí 2007).

The objective of this study was to examine the effect of N and S supply on the oviposition host choice and larval development of *C. obstrictus* under controlled laboratory conditions to investigate possible mechanisms for associations observed in field experiments. The study was designed to distinguish among the contrasting predictions of the plant vigor and plant stress hypotheses on ovipositional preference and larval performance of *C. obstrictus*. It is also possible that N and S may have competing effects on *C. obstrictus* similar to the effects observed in field experiments by Blake et al. (2010). This study was designed to develop improved understanding of the environmental factors driving oviposition choices and the resulting spatial structure of *C. obstrictus* populations within fields of *B. napus*., and so enable more effective strategies of control that require fewer inputs and reduce detrimental impacts on beneficial insects.

2.2 Methods

2.2.1 Pod choice bioassay

2.2.1.1 Nutrient treatments

Potted *B. napus* of the *C. obstrictus* susceptible cv. Q2 were grown at four levels of N and two levels of S fertilization in a fully factorial design to yield eight nutrient treatments. All chemicals used in the preparation of the nutrient treatments were of laboratory grade quality. Nitrogen was applied as ammonium bicarbonate at 0.0, 0.5, 1.0, and 2.0 times the base rate of 1.024 g/pot, which

corresponds to a fertilization rate of 100.0 kg/hectare. Sulfur was applied as sodium sulfate decahydrate in either a 5:1 or 10:1 ratio by weight with the ammonium bicarbonate for all treatments except the N-0 treatment. To the two S levels of the N-0.0 level we applied sodium sulfate at one-half the rate of the corresponding N-0.5 level. Consequently, S levels varied from 0.046 to 0.729 g/pot or 2.4 to 40.0 kg/ha. Monopotassium phosphate was also applied at a rate of 0.360 g/pot or 57.0 kg/ha. The levels of these fertilizers are summarized in Table 2.1. All chemicals were applied in an aqueous solution in various amounts to create eight distinct soil mixes. Before potting, each soil mix was folded using a plastic sheet to ensure uniform distribution of nutrients. To avoid phytotoxicity in the N-2.0 treatments, the application of N and S fertilizers was split with one-half applied to the original soil and one-half delivered to the plant via aqueous solution at the initiation of flowering.

2.2.1.2 Plant material

Five untreated *B. napus* seeds were planted in each 15-cm-diameter pot to ensure adequate germination. Pots were later thinned to two plants per pot to minimize resource competition. Sunshine mix LA4 (Sun Gro Horticulture Canada Ltd., Seba Beach, AB), a peat moss aggregate, was used as a soil medium. This medium contained an initial charge of major and minor nutrients as well as being amended with gypsum. The plants were watered daily according to need using water treated by reverse osmosis to better control the supply of nutrients to the plant. Plants were grown in a glasshouse (approximately 22°C and 16h L : 8h D),

for approximately six weeks or until they reached early flowering (growth stage 4.1 of Harper and Berkenkamp 1975). The plants were systematically arranged to minimize locational effects.

2.2.1.3 Choice arenas

The experimental design was based on the choice experiments performed by Harmon and McCaffrey (1997) and Ulmer and Dosdall (2006b). A set of closed arenas with a volume of 2166 cm³ (19 cm long × 19 cm wide × 6 cm high) were constructed to enclose eight rows and eight columns of treatment locations. Pods 40 to 60 mm in length were selected randomly from our eight N S treatments, excised and arranged in these arenas in an eight by eight Latin square for a total of 64 pods (Fig. 2.1). Adult *C. obstrictus* were collected with sweep nets after weevils had invaded fields of *B. napus* near Lethbridge, Alberta (49°41'N; 112°44'W). Before use in the experiment, adults were sexed by examining pygidia (Cook et al. 2006). Ovipositional status of each female was determined using a single pod of *B. napus* following the method of Harmon and McCaffrey (1997). Sixteen females were then introduced to the center of each arena and the females were allowed to feed and oviposit for a period of 24 h. This number of females yielded a ratio of four pods to one adult. We assumed the effect of oviposition deterring pheromone would be minimal given the duration of the exposure and the short period of bioactivity of the pheromone (Ferguson and Williams 1991). After 24 h, the pods were dissected and examined for feeding marks and egg deposition. This procedure was replicated five times.

2.2.1.4 Data analysis

The number of feeding marks and the number of eggs deposited per pod were analyzed as a mixed model ANOVA using PROC MIXED with N, S, and the N-S interaction included as fixed effects (SAS Institute Inc 2004). Replicate as well as row and column nested within replicate were included in the model as random factors. The data for both feeding marks and egg deposition exhibited a Poisson distribution and were transformed [$\sqrt{x+0.5}$] to normalize model residuals. When significant treatment effects were observed, the means were compared with a Tukey-Kramer test.

2.2.2 Larval growth and development

2.2.2.1 Nutrient treatments

To achieve the level of control over plant nutrient uptake necessary to create lower ranges of plant vigor, the method of nutrient delivery was changed from that used in the pod choice bioassay experiment. Instead of a single highly concentrated nutrient application to the soil medium, multiple low concentration applications were added to the water supply of the plant. An additional N level was also added to this experiment to test for response differences at high N levels similar to those observed by Sarfraz et al. (2009).

Brassica napus plants were grown at five N and two S levels again in a fully factorial design resulting in 10 distinct nutrient regimes. The distinct nutrient regimes were created by the differential application of a modified Hoagland's

solution (Hoagland and Arnon 1950) (Table 2.2). Sulfur was applied in a separate solution in either a 10:1 or 5:1 ratio by weight with N to create the S-low and S-high treatments. The full strength solutions used for the N-4.00 S-high treatment were diluted to enable the correct nutrient quantities to be added while still applying the same volume of solution. High purity, laboratory grade chemicals were used in the preparation of all solutions. Nutrients were applied at planting, two weeks after planting and every subsequent week for 15 weeks.

2.2.2.2 Plant material

Plants were grown in an equal mixture of perlite and vermiculite. Six untreated *B. napus* cv. Q2 seeds were planted in each 15-cm-diameter pot to ensure adequate germination. At the seedling stage, pots were thinned to three plants per pot. Six pots of each treatment were prepared for a total of 60 pots. These pots were arranged according to treatment into large plastic trays (Fig. 2.2). The positions of the trays were shifted weekly until the start of the experiment to avoid positional effects. Within these trays the pots were elevated 2.5 cm above the tray bottom by placing each pot within a second empty pot (Fig. 2.3). Pots were watered using reverse osmosis water using six approximately 30-cm acrylic wicks extending from the tray bottom into the soil medium. These wicks supplied a constant moisture level through capillary action. The nutrient treatments as well as any needed water were applied directly to each tray. Pots were grown in a growth chamber set at $22\pm 1^\circ\text{C}$, 16h L/ $18\pm 1^\circ\text{C}$, 8h D until they reached early to mid flower (growth stages 4.1 - 4.2 of Harper and Berkenkamp 1975). At this

time, plant vigor metrics of plant height and stem diameter were measured and recorded. Upon the completion of the experiment, pods from each plant were collected and dried for four weeks at room temperature. The samples were then oven dried at approximately 80°C for 24 h before being weighed.

2.2.2.3 Plant exposure and larval capture

The experimental procedure was adapted from that of Dossdall and Kott (2006) and Ulmer and Dossdall (2006b). Adult *C. obstrictus* were again collected from commercial canola fields near Lethbridge, AB. Their sex ratio was assumed to be approximately 1:1 (Free and Williams 1979). Pots were exposed individually to 100 adults for a period of 24 h in a series of six experimental blocks which occurred over a three week period. Adults were confined to individual potted plants using fine mesh bags tied at both ends. To emulate natural conditions, the exposure to adult *C. obstrictus* occurred in the presence of natural light with mean temperature and light regime being approximately 18°C and 16h L : 8h D.

After 24 h both the mesh bags and the adults were removed, the pods were counted and approximately 25% were systematically collected from each flowering raceme and used for nutrient analysis. The pods were dried, ground and analyzed for percent N and S contents. Total N and S contents were determined using the combustion method (AOAC – 990.03) (AOAC 2003). After pod collection the pots were returned to the growth chamber. Approximately 10 d later, the pods from each pot were bound together with string, and a stiff paper

cone (approximately 1 m in length and 30 cm in diameter) was placed on the plants in each pot so the cone enclosed all developing pods (Fig. 2.4). The cone funneled emerging larvae to a smaller collecting plate (10 cm in diameter with sides 4 cm high) secured to the stem below the level of the pods. Larvae were collected from these plates twice daily for 20 to 30 d following exposure to adult *C. obstrictus*. Larvae were immediately frozen and later freeze-dried for 48 h before being weighed. Development time from pod exposure to emergence of final-instar larvae was also recorded.

2.2.2.4 Data analysis

Due to low numbers of larvae collected in the later experimental blocks, most likely due to reduced oviposition from females field-collected later in the year, and a small number of escaped adults in the growth chamber, only four of the experimental blocks could be utilized in the analysis of larval weight. For similar reasons only three of the experimental blocks could be utilized in the analysis of development time. For each pot the mean development time and larval dry weight were calculated. These means were then analyzed with ANOVA using PROC MIXED (SAS Institute Inc 2004). The models both included N, S, and the N-S interaction as fixed effects, with experimental block included as a random effect. Similar models were used to analyze the plant vigor metrics including plant height, stem diameter, and final pod weight. The data for final pod dry weight and pod number were transformed $[\ln(x)]$ to reduce heteroscedasticity.

When significant treatment effects were observed, the means were compared with a Tukey-Kramer test.

2.3 Results

2.3.1 Pod choice bioassay

The results of the choice bioassays indicated no significant effect of N ($F = 2.13$; $df = 3, 237$; $P = 0.0966$), S ($F = 0.84$; $df = 1, 237$; $P = 0.3595$), or their interaction ($F = 1.02$; $df = 3, 237$; $P = 0.3829$) on the mean number of feeding marks per pod. A small non-significant decrease in feeding marks was noted for the N-0 treatments (Fig. 2.5).

The mean number of eggs deposited per pod increased significantly with increased N fertilization ($F = 3.33$; $df = 3, 237$; $P = 0.0202$). This effect was largely driven by an increase of more than 10% in the mean number of eggs deposited in the higher N treatments as compared to the N-0.0 treatments (Fig. 2.6). Although there was no significant S effect ($F = 0.94$; $df = 1, 237$; $P = 0.3321$), a significant N by S interaction was observed ($F = 2.84$; $df = 3, 237$; $P = 0.0387$). In the lower two N treatments the mean number of eggs deposited per pod in the high S treatment increased relative to the low S treatment (Fig. 2.6). This trend then seemed to reverse or disappear in the two higher N treatments.

2.3.2 Larval growth and development

I observed a large increase in plant vigor with increasing N supply (Fig. 2.7; Table 2.3), as indicated by plant height ($F = 29.07$; $df = 4, 45$; $P < 0.0001$),

stem diameter ($F = 115.08$; $df = 4, 45$; $P < 0.0001$), pod number ($F = 81.80$; $df = 4, 45$; $P < 0.0001$) and final pod dry weight ($F = 102.89$; $df = 4, 45$; $P < 0.0001$). I also observed a much smaller though significant increase in plant vigor between the low and high S treatments, again as indicated by plant height ($F = 4.47$; $df = 1, 45$; $P = 0.0401$), stem diameter ($F = 6.00$; $df = 1, 45$; $P = 0.0182$), and final pod dry weight ($F = 7.41$; $df = 1, 45$; $P = 0.0105$). I also observed a significant interaction between N and S on the final dry weight of pods ($F = 2.94$; $df = 4, 45$; $P = 0.0305$), with greater final pod weights from the high S treatment but only at the highest N level, but no other significant N S interactions were observed. The observed range in plant vigor was much greater than that observed in the pod choice bioassay with both higher and lower values of plant vigor observed.

Plant tissue analysis of the developing pods collected after exposure in this experiment was hampered by the limited number of pods we could remove without negatively impacting measurements of larval development time and weight. Consequently we were required to pool all pods from a given nutrient treatment, which precluded statistical analysis. The yields of pods from treatment levels N-0.25 S-Low, N-0.25 S-High, and N-0.50 S-Low were particularly low and there was insufficient material to perform the analysis. The quantity of N within pods ranged from 1.14 – 3.91% and in general increased with N supply. The S present within pods ranged from 0.34 - 0.65% and in most cases increased with S supply (Table 2.3). Under the N-2 and N-4 treatments, the levels of N and S approached the lower end of the sufficiency range for N and S of *B. napus* leaf tissue (4.00% and 0.65% for N and S respectively) (Haneklaus and Schnug 1991).

There was a strong N effect on the development time between egg deposition and the end of the third instar ($F = 50.45$; $df = 4, 17$; $P < 0.0001$). The increase was generally linear from the N-0.25 to the N-2 treatment, with a leveling off or decline from the N-2 to the N-4 treatment (Fig. 2.8). We found no evidence for a S effect ($F = 0.02$; $df = 1, 17$; $P = 0.8817$), or N by S interaction ($F = 1.92$; $df = 4, 17$; $P = 0.1542$).

No similar N effect was observed for the mean larval dry weight ($F = 0.76$; $df = 4, 24$; $P = 0.5585$, Fig. 2.9). We again found no evidence for a S effect ($F = 0.10$; $df = 1, 24$; $P = 0.7597$), or N by S interaction ($F = 0.69$; $df = 4, 24$; $P = 0.6080$).

2.4 Discussion

Contrary to both the plant stress and plant vigor hypotheses, we did not observe a positive relationship between ovipositional preference and larval performance. Results from the pod choice bioassay showed significantly more eggs deposited in pods from plants grown with a higher supply of N. In contrast, results of the larval development experiment showed a significant increase in development time in plants with greater access to N. These results conflict with the preference-performance hypothesis, which predicts that female phytophagous insects should select oviposition sites that provide an optimal environment for larval development so as to maximize their fitness (Jaenike1978). Unfortunately this contrast between preference and performance could not be confirmed statistically because differences in methodology between the experiments resulted

in a greater range of plant vigor in the larval growth and development experiments. Despite these differences, the positive effect of N on both oviposition and development time refutes the preference-performance hypothesis. These results are in contrast to previous laboratory studies with *C. obstrictus* which have shown at least a weak positive relationship between larval performance and adult ovipositional preference on host plants of different species and differing resistant and susceptible lines but equivalent nutrient contents (Dosdall and Kott 2006, Ulmer and Dosdall 2006b, Tansey et al. 2010).

Several factors have been hypothesized to explain a lack of association between ovipositional preference and larval feeding (Thompson 1988; Scheirs et al. 2000; Mayhew 2001; Scheirs and De Bruyn 2002; Agosta 2006; Marshall and Uller 2007). It is possible that female *C. obstrictus* may maximize their own reproductive fitness by trading offspring quality for offspring quantity (Marshall and Uller 2007). *Brassica napus* plants with a greater nutrient supply produce more pods (Holmes 1980), and hence offer the female more opportunities for oviposition. The selection of plants with a high nutrient supply may be adaptive for ovipositing females if the benefits of a higher frequency of oviposition outweigh any reductions in larval fitness.

Females may also have avoided selecting pods from plants with a low nutrient supply in the choice bioassay, as these plants are generally rare in canola cropping systems under conventional fertilizer management regimes. This rarity may prevent a strong directional selection for the utilization of these plants (Thompson 1988).

Additionally, these experiments did not investigate the effects of host plant nutritional quality on natural enemies of *C. obstrictus* such as its larval parasitoids. The lower number of pods per plant in a low N fertility regime may result in a higher rate of larval infestation per pod creating a more concentrated and more apparent resource for larval parasitoids. However, this increase in apparency may be offset by a shorter development time and a smaller window in which larvae are at risk of parasitism. It may be adaptive for females to select plants with a greater number of pods so as to avoid parasitism of their larvae (Thompson 1988), but this hypothesis needs to be tested under field conditions. Future research may also want to replicate the pod choice bioassay and examine the effect of N and S levels on the preference of the larval parasitoids of *C. obstrictus*.

Another factor to consider is the interaction of optimal foraging on optimal oviposition (Scheirs and De Bruyn 2002). When due to temporal or spatial separation of optimal larval and adult resources, females may be unable to optimize both oviposition and adult feeding. In this case it may be adaptive for females to optimize adult fitness, through adult feeding, at the expense of larval development. If so, one would expect females to oviposit in the vicinity of adult feeding resources. Such a situation has been observed in the grass miner *Chromatomyia nigra* (Meigen) (Diptera: Agromyzidae) where a strong positive relationship occurs between feeding and oviposition preference (Scheirs et al. 2000). A similar relationship was found between the number of feeding marks and the number of eggs deposited per pod which suggests a similar conflict

between adult and larval fitness. The relationship is consistent with past studies examining larval performance and adult preference of *C. obstrictus* (Ulmer and Dossdall 2006b; Dossdall and Kott 2006; Tansey et al. 2010). However, the number of feeding marks per pod may or may not be a reliable indicator of adult feeding preference as adults feed on a variety of plant tissues within the inflorescence in addition to developing pods (Dmoch 1965; Dossdall and Moisey 2004). When examining several genotypes developed by introgression of *Sinapis alba* L. x *B. napus*, a strong positive relationship was observed between the number of feeding marks per pod and the rate of ovarian development within *C. obstrictus* adults confined to an individual plant (Tansey et al. 2010). While in this particular example the number of feeding marks was positively related to potential adult fitness, future research should examine the effect of canola nutrition on egg development in *C. obstrictus* adults directly.

The observed differences in development time by themselves do not seem to imply a direct fitness cost to the larvae. The fact that increased development time is not associated with a decline in larval weight suggests that while these larvae may have developed more slowly, their lifetime fitness may be unaffected. Larval survival was not determined in this study and it is possible that a fitness cost was manifested through differential survival on the different nutrient treatments. It is also possible that increased development time would lower larval survival when exposed to larval parasitoids. Future research should examine the effects of canola nutrition on larval survival within a more natural setting.

Results of this experiment are similar to those of Dossdall and Kott (2006) in that the effects on larval performance were limited to increases in development time. Other results show both an increase in development time with an accompanying decline in larval weight (Ulmer and Dossdall 2006b; Tansey et al. 2010). Future research should investigate the fitness effects of canola nutrition on developing *C. obstrictus* larvae by observing the total adult reproductive output of those larvae.

Several possible factors may explain the positive relationship between the N supply to the host plant and larval development time of *C. obstrictus* under laboratory conditions. There were fewer pods present at the lower nutrient levels; larvae developing within these pods probably experienced greater competition for resources which may have resulted in shorter development times and lower larval weights.

Nutritional deficiencies in either N or S were unlikely a direct cause of the increased development time as the concentrations of these elements in pod tissue generally increased with increased supply of these elements. However, future research should attempt to test these increases statistically using greater replication as well as different plant tissues such as leaves. Differences in N and S supply can affect the composition of GSLs (Zhao et al. 1994; Kim et al. 2002), whose breakdown products have been shown to be attractive to *C. obstrictus* (Evans and Allen-Williams 1992; Bartlet et al. 1993; Blight et al. 1995; Smart and Blight 1997). Tansey (2009) showed a strong negative correlation between the concentration of 1-methoxy-3-indolylmethyl GSL and the larval performance and

adult preference of *C. obstrictus*. Given the positive effect of N on indolyl glucosinolates (Zhao et al. 1994; Kim et al. 2002), increases in 1-methoxy-3-indolylmethyl GSL seems a likely mechanism for the observed increases in development time with N fertilization. The positive effect of S on the attractive 3-butenyl and 4-pentenyl GSLs (Zhao et al. 1994; Kim et al. 2002) may also explain the observed increase in ovipositional preference with S fertilization at lower levels of N fertilization.

Differences in the protein or oil contents of pods could also affect oviposition preference and larval performance. Increased N fertilization can increase protein content while decreasing oil content (Grant and Bailey 1993) and this decreased oil content may be related to the relationship between N supply and larval development time. To test among these proposed causal factors, future research should directly measure GSL composition, oil content, protein content and level of larval competition within all host plants.

In conclusion, N and S supply of the canola plant can influence both the larval performance and the oviposition preference of *C. obstrictus* adults. The N supply of the plant is positively related to both the number of eggs laid per pod and the development time of the larvae, while S seems to affect only ovipositional preference through an interaction with N. Given these results, no changes to current fertilizer management practices can be suggested for canola production in regions infested annually with high population densities of *C. obstrictus*. Similar to the results of Dossdall et al. (2004), our results suggest that while greater nutrient supply results in increased oviposition and most likely increased crop

damage, these losses from *C. obstrictus* infestation would be small compared with the increased yields produced with increased levels of nutrient application.

Table 2.1. Quantities of nutrients added per plant pot for each of the fertilizer treatments used in the pod choice bioassay.

N Rate	S Rate	(NH ₄) ₂ CO ₃ (g)	Na ₂ SO ₄ (g)	KH ₂ PO ₄ (g)
0.0	low	0.000	0.046	0.360
0.0	high	0.000	0.091	0.360
0.5	low	0.512	0.091	0.360
0.5	high	0.512	0.182	0.360
1.0	low	1.024	0.182	0.360
1.0	high	1.024	0.365	0.360
2.0	low	2.048	0.365	0.360
2.0	high	2.048	0.729	0.360

Table 2.2. The quantities of nutrients added weekly per plant pot for the different treatments via dilution of the full strength solutions used for the N-4 S-high treatment.

N Rate	S Rate	KNO ₃ (mg)	Ca(NO ₃) ₂ (mg)	KH ₂ PO ₄ (mg)	K ₂ HPO ₄ (mg)	MgCl ₂ (mg)	Micros ^a (mg)	Na ₂ SO ₄ (mg)
0.25	low	10.43	34.09	5.29	0.42	8.39	0.30	5.52
0.25	high	10.43	34.09	5.29	0.42	8.39	0.30	11.03
0.50	low	20.85	68.19	10.58	0.83	16.77	0.60	11.03
0.50	high	20.85	68.19	10.58	0.83	16.77	0.60	22.06
1.00	low	41.71	136.38	21.15	1.67	33.55	1.20	22.06
1.00	high	41.71	136.38	21.15	1.67	33.55	1.20	44.13
2.00	low	83.42	272.75	42.30	3.34	67.09	2.40	44.13
2.00	high	83.42	272.75	42.30	3.34	67.09	2.40	88.25
4.00	low	166.83	545.51	84.60	6.68	134.18	4.80	88.25
4.00	high	166.83	545.51	84.60	6.68	134.18	4.80	176.50

^a Plant-Prod® chelated micronutrient mix (Plant Products Co. Ltd., Brampton, ON)

Table 2.3. Means \pm SE of plant vigor metrics, and the nutrient contents of developing pods from the larval growth and development experiment.

N Rate	S Rate	Plant Height (cm)	Stem Diameter (mm)	Pod Number	Final Pod Dry Weight (g)	% N	% S
0.25	low	47.7 \pm 3.7 a	1.90 \pm 0.16 a	14.8 \pm 2.5 a	0.6 \pm 0.2 a	— ^a	— ^a
0.25	high	54.2 \pm 5.5 ab	2.19 \pm 0.11 a	19.0 \pm 2.3 ab	1.1 \pm 0.2 b	— ^a	— ^a
0.50	low	68.2 \pm 6.2 abc	2.66 \pm 0.29 ab	33.0 \pm 7.5 b	2.4 \pm 0.6 bc	— ^a	— ^a
0.50	high	81.1 \pm 5.0 bcd	2.99 \pm 0.19 ab	37.0 \pm 4.8 bc	2.8 \pm 0.4 cd	1.14	0.39
1.00	low	97.4 \pm 6.6 de	3.91 \pm 0.11 bc	69.3 \pm 3.2 cd	5.9 \pm 0.3 e	1.66	0.34
1.00	high	106.4 \pm 5.9 de	4.33 \pm 0.25 c	67.7 \pm 12.8 cd	6.0 \pm 0.8 de	2.01	0.47
2.00	low	100.8 \pm 6.7 de	4.90 \pm 0.33 c	106.8 \pm 13.6 de	11.1 \pm 0.9 ef	2.44	0.42
2.00	high	94.9 \pm 2.6 cde	4.94 \pm 0.19 c	125.2 \pm 18.6 de	9.3 \pm 0.7 ef	3.20	0.65
4.00	low	92.7 \pm 6.6 cde	6.91 \pm 0.57 d	199.2 \pm 27.7 e	10.7 \pm 1.5 ef	3.91	0.58
4.00	high	109.2 \pm 7.6 e	7.99 \pm 0.39 d	236.2 \pm 64.0 e	17.3 \pm 2.3 f	2.96	0.55

Differing letters within each column indicate a significant difference as indicated by the Tukey-Kramer test ($P \leq 0.05$)

^a Insufficient sample for tissue analysis



Figure 2.1. The arrangement of pods within the choice arena used in the pod choice bioassay.

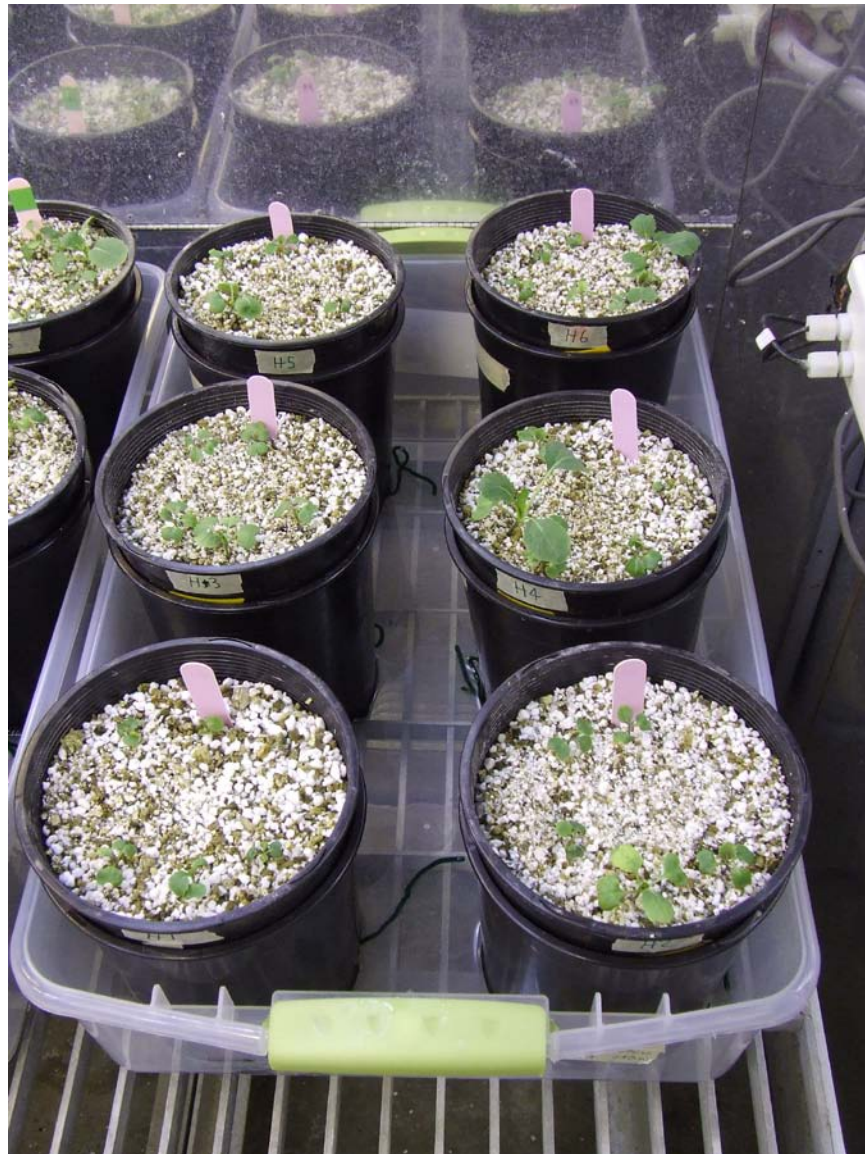


Figure 2.2. The arrangement of pots within the trays used to deliver water and nutrient solutions in the larval growth and development experiment.

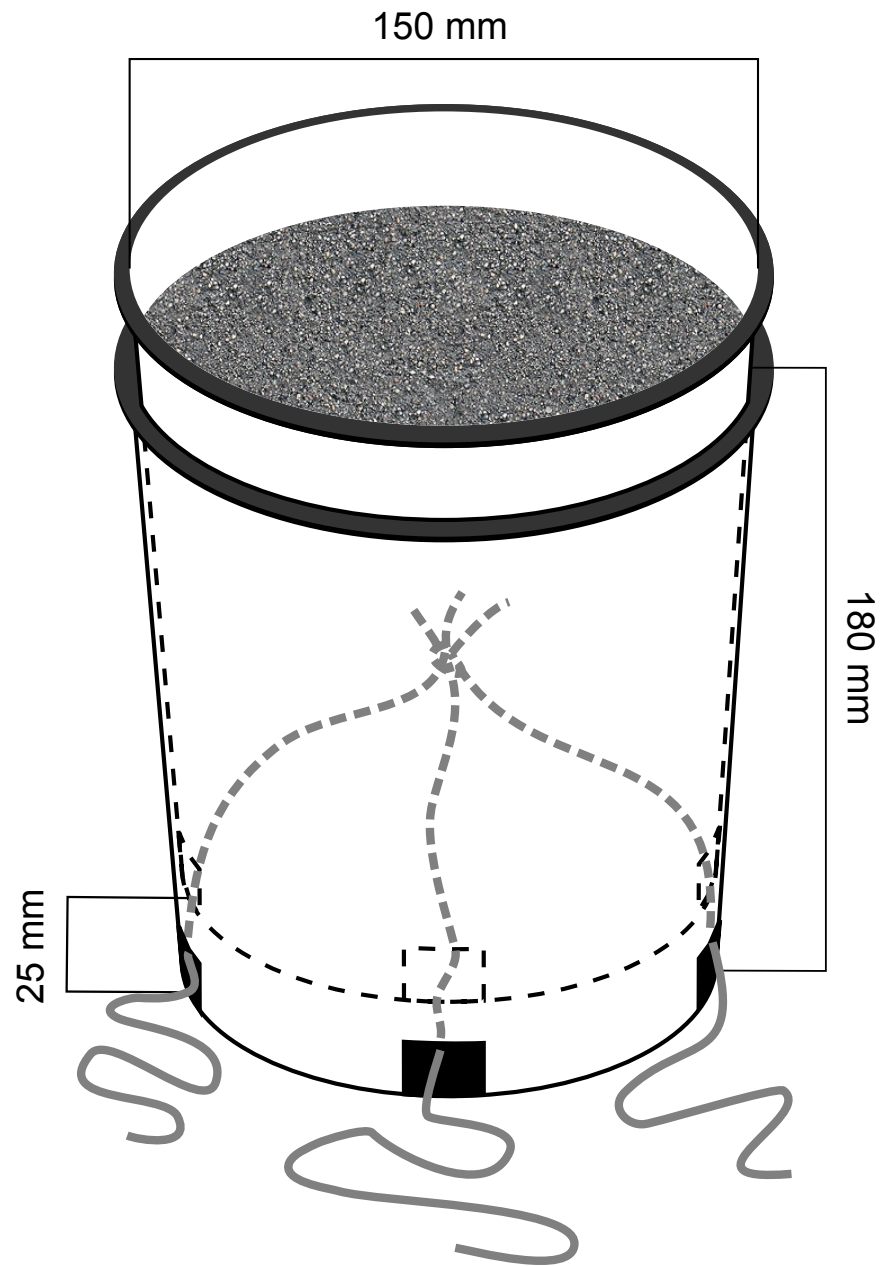


Figure 2.3. The arrangement of pots and wicks used to supply water and nutrient solution in the larval growth and development experiment.



Figure 2.4. The cone and collecting plates used to capture emerging final instar larvae in the larval growth and development experiment.

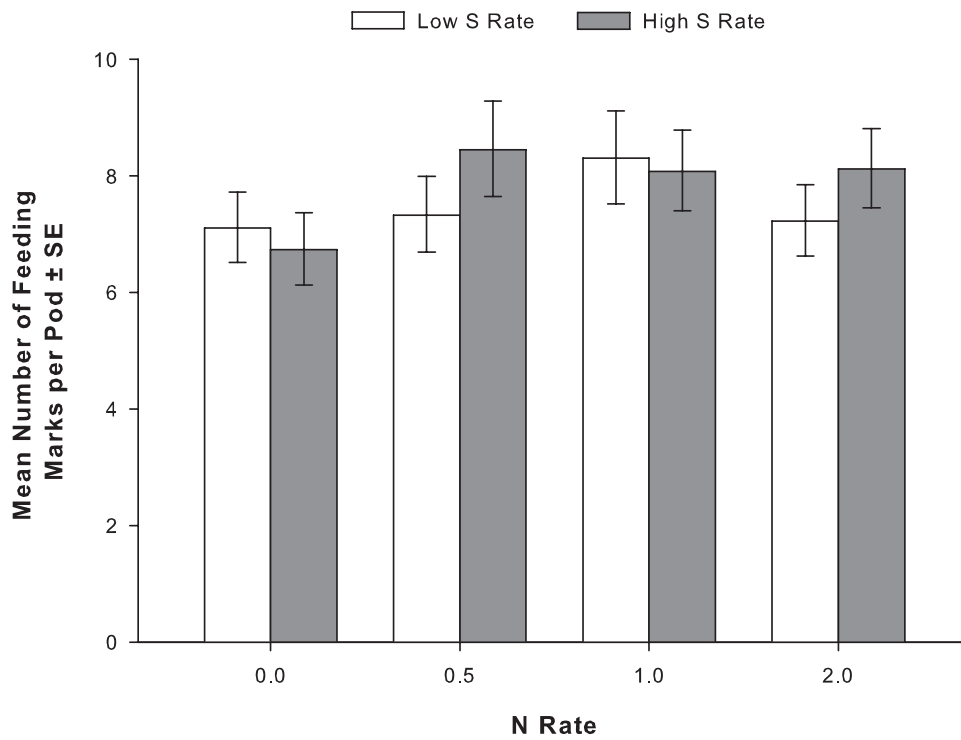


Figure 2.5. The mean numbers of *Ceutorhynchus obstrictus* feeding marks per pod after a 24 h exposure to 16 gravid *C. obstrictus* females. Pods were obtained from *Brassica napus* plants grown under four N and two S levels during the pod choice bioassay experiment.

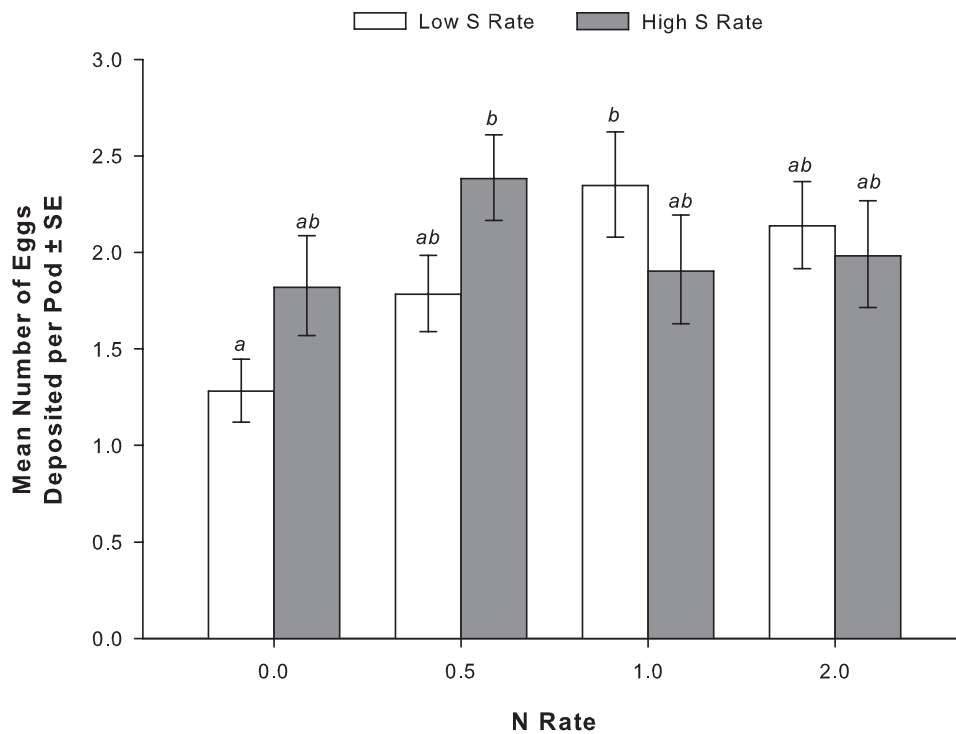


Figure 2.6. The mean numbers of *Ceutorhynchus obstrictus* eggs per pod after a 24 h exposure to 16 gravid *C. obstrictus* females. Pods were obtained from *Brassica napus* plants grown under four N and two S levels during the pod choice bioassay experiment. Differing letters within a nutrient treatment rate indicate a significant difference as indicated by the Tukey-Kramer test ($P \leq 0.05$).



Figure 2.7. Photographs showing the relative size and color of *Brassica napus* plants from the (a) N-0.25, (b) N-0.50, (c) N-1.00, (d) N-2.00 and (e) N-4.00 treatment levels.

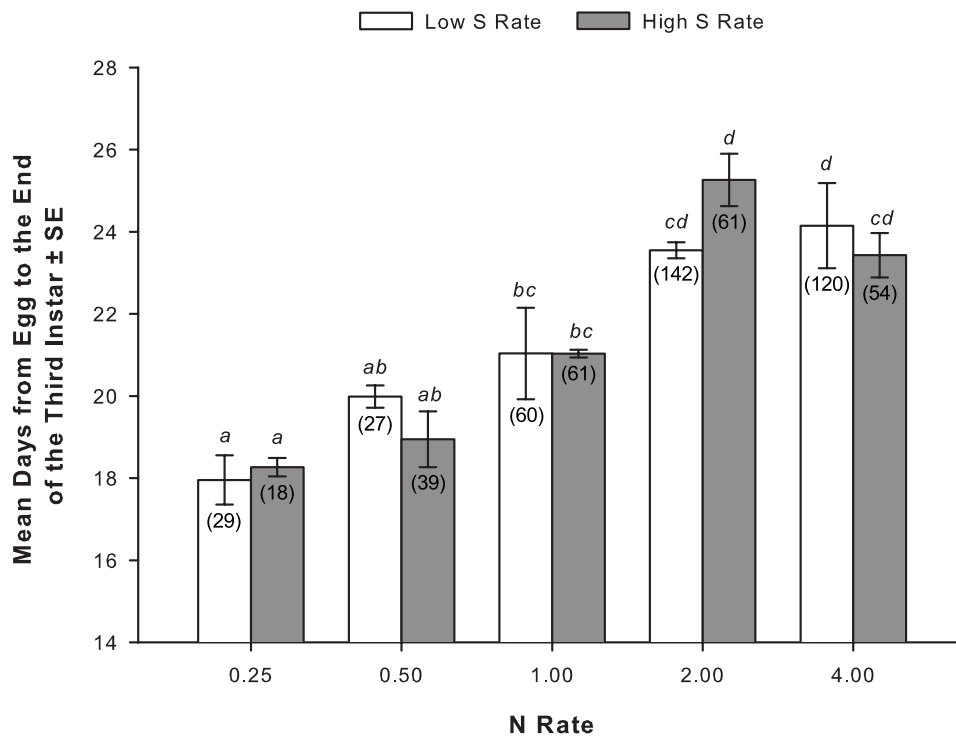


Figure 2.8. Mean numbers of days required for *Ceutorhynchus obstrictus* larvae to develop from egg to the end of the third instar on *Brassica napus* plants grown under five N and two S levels during the larval growth and development experiment. The numbers in parentheses indicate the number of larvae contributing to each mean. Differing letters indicate a significant difference as indicated by the Tukey-Kramer test ($P \leq 0.05$).

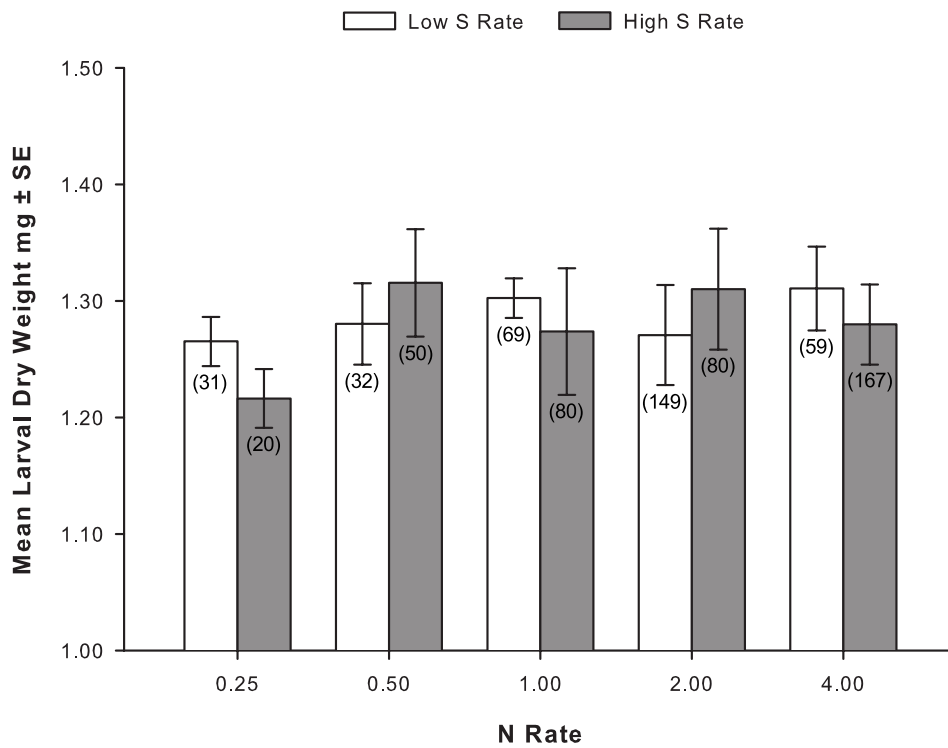


Figure 2.9. Mean dry weights of *Ceutorhynchus obstrictus* larvae developing on *Brassica napus* plants grown under five N and two S levels during the larval growth and development experiment. The numbers in parentheses indicate the number of larvae contributing to each mean.

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Chapter 3 The systemic spatial relationships among soil and plant characteristics and the distribution of *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae) and its parasitoids

3.1 Introduction

The cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (syn. *Ceutorhynchus assimilis* Paykull) (Coleoptera: Curculionidae), is a serious pest of canola or oilseed rape (*Brassica napus* L.) both in North America and Europe (Bonnemaison 1957; Dossdall et al. 2001). It is an invasive species in the Nearctic that has spread widely across North America (Cárcamo et al. 2001) and is predicted to eventually occupy all canola production regions in Canada (Dossdall et al. 2002). *Ceutorhynchus obstrictus* was first observed in Alberta in 1995 (Cárcamo et al. 2001) and its populations have since increased to outbreak densities in southern Alberta and Saskatchewan (Dossdall et al. 2009).

Ceutorhynchus obstrictus adults emerge from below-ground overwintering sites in the spring when soil temperatures reach approximately 15°C (Dmoch 1965; Ulmer and Dossdall 2006). Adults feed initially on brassicaceous weeds (Fox and Dossdall 2003; Dossdall and Moisey 2004), before invading fields of canola during the bud to early flower stage and immediately begin feeding on structures within or in the vicinity of the inflorescence (Dmoch 1965; Dossdall and Moisey 2004). Following ovarian maturation, gravid females deposit eggs within the developing pods of canola, with peak ovipositional activity occurring in mid-flowering (Dossdall and Moisey 2004). Larvae complete their development within

Pods, each consuming an average of five immature seeds (Dmoch 1965) before boring a hole in the pod wall and dropping to the ground to pupate below the soil surface. Development of *C. obstrictus* from egg to adult within spring-seeded canola in western Canada typically requires 31-58 days (Dosdall and Moisey 2004). The newly emerged adults resume feeding on available canola or other brassicaceous plants until temperatures decline in autumn (Dmoch 1965; Dosdall and Moisey 2004). Adults then migrate to nearby wooded areas or undisturbed soil where they overwinter under leaf litter.

Ceutorhynchus obstrictus larvae are parasitized by several larval idiobiont ectoparasitoids in both North America and Europe (Williams 2003; Dosdall et al. 2009). These parasitoids can play important roles in the control of *C. obstrictus* populations (Cárcamo et al. 2001), although parasitism rates are typically lower in North America than Europe (Harmon and McCaffrey 1997; Buntin 1998; Dosdall et al. 2007; Dosdall et al. 2009) as the primary parasitoids of *C. obstrictus* are absent in North America (Gibson et al. 2005). *Necremnus tidius* (Walker) (Hymenoptera: Eulophidae), *Trichomalus lucidus* (Walker), *Chlorocyclus* sp., and *Pteromalus* sp. (Hymenoptera: Pteromalidae) are the main ectoparasitoids recorded in western Canada (Dosdall et al. 2006, 2009).

Current management practices for *C. obstrictus* involve applications of broad spectrum insecticides to entire fields when populations reach economic threshold densities (Dosdall et al. 2001; Cárcamo et al. 2005). The use of precision spraying or site-specific management of *C. obstrictus* could reduce the amount of insecticide applied to fields, both reducing input costs and conserving

natural enemies (Ferguson et al. 2000; Dossdall et al. 2006). This management strategy would require that significant variability occurs in the abundance *C. obstructus*, that the causes of this variability are both identified and measurable, and that this information can be used to effectively modify management practices to improve yield or reduce environmental impact (Plant 2001). Numerous studies have identified significant spatial variability in the distribution of *C. obstructus* larvae and adults within fields (Murchie et al. 1999; Ferguson et al. 2000; Dossdall et al. 2006; Blake et al. 2010), and with the use of variable rate application technology, it is possible for producers to employ site-specific interventions (Bramley 2009; Plant 2001) especially within irrigated fields (King et al. 2009). There is currently insufficient understanding of the causal factors governing the spatial distribution of *C. obstructus* to allow for the prediction and site-specific management of areas of *C. obstructus* abundance (Ferguson et al. 2003; Blake et al. 2010). Previous studies have examined the relationships of *C. obstructus* with plant vigor and yield metrics (Ferguson et al. 2003) and plant nutrients (Blake et al. 2010), but these variables have never been examined concurrently or using a method that could examine the causal network among all of the variables (Grace 2006).

To improve understanding of the relationships among *C. obstructus* larvae and adults, their parasitoids, and the underlying soil and plant variables, a conceptual model (Figure 3.1) was evaluated using structural equation modeling (SEM). The concept of plant vigor is defined as an increased growth rate and larger size relative to other members within the population (Price 1991). As plant

vigor can be limited by several soil characters (Loomis and Connor 1992; Thomas 2003), it was hypothesized that plant vigor would be dependent upon soil moisture, soil properties and soil nutrients (Figure 3.1 a-c). Plant vigor can also be negatively affected by plant density (Figure 3.1 d; Rood and Major 1984; Seetseng 2008). Irrigation of the study area limited moisture stress, and for this reason and other logistical limitations, soil moisture was not investigated within the current study. Plant nutrient availability, and consequently plant tissue nutrient content, is also dependent upon soil characters (Figure 3.1 e, f; Baldwin 1975; Thomas 2003). The content of any one nutrient is ultimately dependent upon its availability within the soil (Figure 3.1 g), but the plant's requirement and uptake of nutrients is also mediated by limiting factors such as nitrogen (N) (Jackson 2000). Consequently the supply on one nutrient may influence the demand and uptake of other nutrients (Figure 3.1 g) ..

In this study, it was also hypothesized that the effect of plant nutrient composition on host plant choice of gravid females would be mediated by olfactory, visual, and chemical and tactile cues (Figure 3.1 h-j), as these are the three primary cues used in host location by phytophagous insects (Finch and Collier 2000). The nutritional status of plants can affect the composition of glucosinolates within plant tissues (Bilsborrow et al. 1993; Schnug 1993; Zhao et al. 1994; Asare and Scarisbrick 1995), affecting both the chemical and olfactory cues associated with these compounds (Bloem et al. 2010). Nutrition can also affect the thickness of epicuticular waxes, presumably modifying the tactile cues associated with the plant (Sarfranz et al. 2006). Foliar color is affected by both N

and sulfur (S) (Holmes 1980; Grant and Bailey 1993) while floral color and ultraviolet (UV) reflectance has been shown to be affected by S (Brauer et al. 2007). There is also strong evidence that *C. obstrictus* responds to olfactory (Tansey et al. 2010b) and visual cues (Tansey 2010a). *Ceutorhynchus obstrictus* also responds to chemical and tactile cues as adults can discriminate among pods grown under different fertility regimes (Chapter 2). This study focused on visual cues as a possible mechanism because vision seemed likely to operate on a spatial scale most relevant to the aggregation observed (Finch and Collier 2000). Additionally, it was not logistically possible to collect data associated with olfactory, chemical and tactile cues as part of this study.

It was hypothesized that the distribution of *C. obstrictus* males would be dependent upon the distribution of gravid *C. obstrictus* females as males are attracted to the odor of overwintered females (Figure 3.1 p; Evans and Bergeron, 1994). It was also hypothesized that while *C. obstrictus* males would respond to host plant cues when locating feeding sites, these responses would be less important than their responses to females. This assertion is supported by observations that *C. obstrictus* males tend to have similar though less discriminating responses to visual cues than females (Tansey et al. 2010a), and they also have a similar response to olfactory cues (Bartlet et al. 1993; Tansey et al. 2010b). Given these responses, direct paths from the three cues should have limited explanatory power for *C. obstrictus* males, and as a consequence they were omitted from the model. It was also hypothesized that the distribution of *C. obstrictus* larvae would be dependent upon that of *C. obstrictus* females (Figure

3.1 s), and the distribution of larval parasitoids would be dependent upon *C. obstrictus* larvae (Figure 3.1 d) as these associations have been observed previously (Ferguson et al. 2000; Dossdall et al. 2006). Larval infestation is measured as a proportion of pods collected; consequently, a negative relationship was predicted with plant density and plant vigor as both of these factors would increase pod density (Figure 3.1 q, r). Pods also serve as a potential food source for *C. obstrictus* adults. It was hypothesized that *C. obstrictus* males would respond differentially to these food sources, compared with females, as males respond more strongly to leaf volatiles (Evans and Allen-Williams 1992; Bartlett et al. 1993; Tansey et al. 2010b). As a consequence of this differential response, *C. obstrictus* males should have positive relationships with plant vigor and density (Figure 3.1 n, o).

The objective of this study was to evaluate the above conceptual model, and so investigate the effects of soil and plant nutrients on the spatial distribution of *C. obstrictus* and to assess visual cues as a possible mechanism to explain these effects. The conceptual model makes several testable predictions relevant to these objectives. It was predicted that soil nutrients would influence plant nutrient levels and those in turn would have a strong relationship on the distribution of gravid females, with N having a negative effect and sulfur having a positive effect. These effects would be mediated by the visual cues with nutrients having several effects on the visual appearance of *B. napus* flowers and foliage and these effects on appearance influencing the distributions on gravid females. It also follows that males, larvae and parasitoids would have associations similar to those

of gravid females with visual cues and plant nutrients due to their positive relationships with gravid females. To evaluate these predictions *in situ* an observational grid study was conducted over two site-years in southern Alberta. Soil nutrients were also manipulated to create nutrient ‘hot spots’ both to investigate a greater range of nutrients levels than would normally be present and to evaluate the effects of additional fertilization on the components of the conceptual model in a controlled field experiment.

3.2 Methods

3.2.1 2008 and 2009 Grid studies

3.2.1.1 Study area

The study was conducted in two adjacent commercial fields of *B. napus* located near Lethbridge, Alberta (49°41’N; 112°45’W) as this region has historically experienced high levels of *C. obstrictus* infestation. The fields were under pivot irrigation and as a consequence they comprised two halves of a circle that was approximately 380 m in diameter (Figure 3.2). Following a typical crop rotation, the fields were planted to canola in different years, the eastern field in 2008 and the western field in 2009. In 2008, the topography in conjunction with a large precipitation event during early flower (growth stages 4.1 of Harper and Berkenkamp 1975) resulted in flooding and crop mortality in approximately one-fifth of the grid (Figure 3.2). Due to plant mortality the samples of *C. obstrictus* adults, larvae, and parasitoids taken in these areas were no longer comparable

with the remainder of the grid. Consequently in all analyses of these variables, the flooded areas were excluded.

3.2.1.2 Grid layout

The sample grid was limited to the southwest quadrant in 2008 and the northeast quadrant in 2009 (Figure 3.2). The radius of the sampling quadrant was constrained to 180 m to avoid possible crop edge effects.

Within the quadrant in 2008, 25 points were placed on a 20 m grid in a checkerboard pattern (Figure 3.2). Four additional points were also placed randomly around each of these central points so that each of these random points was at least 4 m from all other points and no more than 10 m from the central point. A raised yellow bowl trap sampler was then placed at each of these points (Doddall et al. 2006). A clustered sampling design was chosen as this design better estimates autocorrelation in the first of several distance classes (Legendre et al. 2002).

Examination of the spatial structure of the data from 2008 determined that minimal differences occurred between traps within sampling clusters. Consequently, in 2009 the number of traps in each cluster was reduced to three and the number of clusters was expanded to 26 (Figure 3.2). In addition, the grid spacing was increased from 20 to 25 m and clusters were not placed in a checkerboard pattern but at every point in the grid. The N-S hotspot experiment was located along periphery of the grid as shown in Figure 3.2. In addition to the

analysis mentioned below, data from these hotspots were included in all analyses of the 2009 grid.

3.2.1.3 Measurement of soil characteristics

Before crop emergence in 2008, three 30-cm soil cores with a diameter of 2.54 cm were taken around each bowl trap and analyzed for soil nutrient composition (Figure 3.3). Soil cores were collected at each of the central points in each cluster and the three cores from each point were analyzed as a composite sample. In an effort to understand the spatial variation in soil nutrients, a hierarchical sampling regime was used at four of the sampling clusters. At these clusters all five points were sampled, to yield a total of 41 individual soil samples. Due to logistical constraints and poor correspondence of soil and pod nutrient levels, the soil sampling was not replicated in 2009.

Kelowna extraction was used to extract N in the form of nitrate, phosphorus (P), and potassium (K) (Carter 1993, Method 8.5). The concentrations of nitrate, P and K were determined by the cadmium reduction procedure (Carter and Gregorich 2008, Method 6.3), the stannous chloride method (Eaton et al. 2005, APHA 4500-P D), and the automated flame photometry method (AEC 1996, Method 19103 565), respectively. Sulfur in the form of sulfate was extracted using a dilute calcium chloride solution (McKeague 1978, Method 4.47) and its concentration was determined using the inductively coupled plasma spectroscopic method (Eaton et al. 2005, APHA 3120B). The pH, electrical conductivity (EC), and organic matter content (OM) of the soil were respectively

determined by using a pH meter within a 1:2 mixture of soil and water (McKeague 1978, Method 4.12), by measuring the resistance of a soil-water solution, and by the loss on ignition method (McKeague 1978, Method 3.8). The total number of extractable cations (TEC), which approximates the cation exchange capacity of the soil, was determined from the concentrations of K as well as calcium, magnesium and sodium. The additional cations were extracted using an ammonium acetate solution (McKeague 1978, Method 4.51), and their concentrations were measured using the inductively coupled plasma spectroscopic method (Eaton et al. 2005, APHA 3120B).

3.2.1.4 Adult *Ceutorhynchus obstrictus* sampling

Adult *C. obstrictus* captured in raised yellow bowl traps were used as an indicator of the adult abundance utilizing a method similar to that of Dossdall et al. (2006) and Blake et al. (2010). The traps were half filled with a 1:1 solution of propylene glycol and water, and set out while *B. napus* was still in the rosette stage (growth stages 2.1-2.4 of Harper and Berkenkamp 1975). The traps were sampled weekly and adult *C. obstrictus* were collected, counted, and their sex and reproductive status were determined by dissection. When the number of adults captured in a weekly trap sample was greater than 25, a subset of 25 was dissected to determine sex ratio, and this ratio was used to estimate the total numbers of males, females and gravid females from that trap sample. The sampling period lasted for an 11 to 12-week period spanning stem elongation, flowering and pod

development. To maintain apparency of the traps, their height was adjusted weekly so they remained level with the top of the crop canopy.

3.2.1.5 Measurements of the visual appearance of *Brassica napus*

Crop development and its visual appearance were monitored using a series of digital photographs taken at sampling locations throughout the grid. In 2008, photographs were limited to the central points of the grid, while in 2009 all grid points were photographed. Photographs were taken weekly during flowering. At each location photographed, two photographs were taken, one approximately 5 m south of the bowl trap facing north, and one approximately 5 m north facing south. The photographs were composed with the horizon at or slightly above the top of the photograph and the bowl trap centered horizontally within the photo.

The image analysis software, ImageJ (Ferreira and Rasband 2010), was used to process and measure certain aspects of the photographs. All work done in ImageJ utilized the HSB color space in which each pixel of the image is described by a hue, saturation and brightness value ranging from 0-255. Hue describes the dominant wavelength of light of each pixel, while saturation describes the intensity of the dominant wavelength relative to all other wavelengths and brightness can be described as the intensity of all wavelengths of light. The HSB color space was chosen as it is not dependent upon specific response maxima while the RGB color space is based on response maxima of human vision (Joblove and Greenberg 1978), which differ from those of *C. obstrictus* (Tansey 2009). A series of threshold filters was used to isolate either flowers or foliage

pixels from the photograph. ImageJ then calculated the percentage of pixels occupied by either flowers or foliage, henceforth referred to as percent cover. The mean hue, saturation, and brightness values of these isolated flowers and foliage pixels were also calculated. These values were averaged between the two photographs to produce final values for each sample location. In an effort to minimize the number of variables examined, statistical analysis was limited to the sampling week with the maximum capture of gravid females, representing the peak period of oviposition.

3.2.1.6 Measurements of plant vigor and density

Plant density was assessed for each point within the grid during the rosette stage (growth stage 2.1-3.1 of Harper and Berkenkamp 1975) using three equally spaced measurements with a 1 m² quadrat (Figure 3.3), averaged to give an overall estimate of plant density for that point. During early to mid flowering (growth stages 4.1-4.2 of Harper and Berkenkamp 1975), plant height was measured for five randomly selected plants arranged around each bowl trap (Figure 3.3). The mean height of those five plants was used as an indicator of plant vigor. Plant height was chosen as an indicator as this metric has been shown to be

–Jeromela et al. 2008).

3.2.1.7 Measurements of nutrient levels within pod tissue

During early to mid flowering, approximately 100 immature pods were collected from the vicinity of each bowl trap. Pods were dried, ground and

analyzed for percent N, P, K, and S contents. The total N and S contents were determined through the combustion method (AOAC 2003, Method 990.03), while the inductively coupled plasma spectroscopic method was used to determine P and K contents (AOAC 2003, Method 985.01).

3.2.1.8 *Ceutorhynchus obstrictus* larval and larval parasitoid sampling

In 2008, after the majority of *C. obstrictus* larvae had completed development (growth stages 5.2-5.4 of Harper and Berkenkamp 1975), all pods were removed from the plants used for plant vigor measurements. These pods were then placed in rearing boxes for six weeks and the adult parasitoids were then collected, preserved in ethanol, and later identified according to methods of Dossall et al. (2006). Larval and parasitoid exit holes in the pods were also examined and counted. Exit hole densities were used as indicators of the distributions of developing larvae and parasitoids.

3.2.1.9 Data analyses

The spatial structure in the abundance of *C. obstrictus* males, gravid females, larvae and parasitoids; the visual appearance of *B. napus*; measurements of plant density and vigor; and the nutrient content of developing pods were analyzed using Spatial Analysis by Distance IndicEs (SADIE) according to the method described in Perry et al. (1999). SADIE examines data for the structure of patches (multiple high values within an area) and gaps (multiple low values within an area). The indices \bar{v}_i , which is by convention positive, and \bar{v}_j , which is

by convention negative, indicate the overall spatial structure in terms of patches and gaps respectively. Expected values for these indices are generated through randomization and allow for a statistical test of significance.

In order to have comparable structural equation models between years, observed variables that were not collected in both years or at the same level of replication in both years were excluded from the structural equation analysis (Table 3.1). For this reason the relationships between the variables used in SEM and the soil variables as well as the variables describing the appearance of *B. napus* were analyzed using SADIE's overall index of association, X (Perry and Dixon 2002). X is comparable to a simple correlation coefficient between the two datasets but is calculated using the individual clustering indexes (v_i or v_j) of the two sets of data. It also adjusts for autocorrelation using Dutilleul's (1993) adjustment. The significance of the X is assessed through randomization; probability values <0.025 indicate significant association while values >0.975 indicate significant dissociation at an α of 0.05.

Structural equation modeling with observed variables was used to evaluate the conceptual model (Figure 3.1). The correspondence between observed variables and variables in the conceptual models is shown in Table 3.1. SEM is typically used in either a confirmatory or exploratory method (Grace 2006). When used in a confirmatory method, an initial model is first developed from existing theory, the covariance structure implied by the model is then compared with the covariance structure of the data using a χ^2 test. Unlike most statistical methods where priority is given to the null model, in SEM priority is given to the theory-

based initial model. A P value > 0.05 for the χ^2 test provides strong theoretical support for the initial model. SEM can also be used in an exploratory manner in which the results from model testing are used to modify the model to improve model fit. SEM results are usually displayed graphically where single-headed arrows represent a causal relationship in the direction of the arrow, while double-headed arrows represent an unexplained correlation between variables.

M-plus 4.1 (Muthén and Muthén 2006) was used to analyze the SEM for the data from 2008 and 2009. Structural equation modeling was initially used in an exploratory manner to fit the data from 2008 to develop an initial model, with the 2009 data reserved as a confirmatory test. The initial model did not fit the 2009 data well, and even when using an exploratory approach with both years, no model fit both the 2008 and 2009 data adequately. Consequently SEM was used in an exploratory fashion to fit individual models for the 2008 and 2009 grids. The bootstrap option in M-plus (Muthén and Muthén 2006) was used to generate standard errors to account for potential non-normality in the data and to avoid the assumption of large sample sizes required by maximum likelihood estimation (Grace 2006). Significance of path coefficients was assessed by dividing the path coefficients by their standard errors generated through bootstrapping. At large sample sizes these values approximate z-scores allowing for the calculation of P -values. When generating the individual models, paths without significant coefficients were removed from the model except in cases where the P value was marginal and there was strong theoretical support for that path. Each directed path between variables was examined via bivariate plot for potential nonlinear

relationships and influential points. The residuals generated from the model for individual observation for each endogenous variable were analyzed for autocorrelation with the program PASSaGE 2 using a resampling test of Moran's I values with a Bonferroni corrected experiment-wide α of 0.05 (Rosenberg and Anderson 2009).

3.2.2 Nitrogen-Sulfur hot spot experiment

The hot spot experiment was located as shown in Figure 3.2. Additional N and S fertilizer were applied to a circle 4 m in radius surround each bowl trap in the design shown in Figure 3.4. The arrangement was replicated in eight experimental blocks. The additional fertilizer was applied using a lawn spreader just after crop emergence. Additional N was applied in the form of granular urea at a rate equal to the 224 kg/ha baseline rate of the whole field, while S was applied as potassium sulfate at a ratio of 5:1 with N resulting in a rate of 45 kg/ha. These bowl traps were then sampled using the procedures described above.

The nutrient content of pods; the visual appearance of *B. napus* plants; and the abundance of *C. obstrictus* adults, larvae and parasitoids were analyzed as mixed model ANOVAs using PROC MIXED (SAS Institute Inc. 2004). The models included N fertilization, S fertilization, and the N-S interaction as fixed effects, with experimental block included as a random effect. The data for *C. obstrictus* males and gravid females were transformed [$\sqrt{x+0.5}$] to normalize the model residuals.

3.3 Results

3.3.1 2008 and 2009 Grid studies

3.3.1.1 Relationships with soil characteristics

With the exception of the association between soil S and the quantity of S in pod tissue ($X = 0.357$, $P = 0.0182$), no significant relationships were observed between nutrient levels in the soil and the nutrient levels within developing pod tissues. The nutrient contents in plant tissues were also not significantly related to other soil characteristic such as pH, OM, EC, or TEC. These characteristics did influence other variables within the structural equation model. Plant height was significantly dissociated with TEC ($X = -0.4293$, $P = 0.9947$) while gravid *C. obstrictus* females were associated with OM ($X = 0.4298$, $P = 0.0050$).

3.3.1.2 Relationships with the visual appearance of *Brassica napus*

In 2008, high levels of nutrients in pod tissues were significantly dissociated with plants showing foliage that was more blue and less green and significantly associated with plants showing flowers that were more yellow and less green (Table 3.2). A significant association between S levels and flower saturation, a significant dissociation between K levels and flower brightness, and a significant dissociation between P levels and the % cover of flowers were also observed. Similar relationships were not observed in 2009. In 2009 flower brightness was significantly dissociated with N and significantly associated with P. No similar association with P was observed in 2008, but a non-significant dissociation with N was observed in 2008. Plant density was significantly

associated with foliage brightness in 2008, while in 2009 it showed a dissociation with foliage brightness. Plant height showed a single association with percent cover of foliage in 2008. These relationships were again inconsistent between years.

Inconsistency was also observed in the relationships between the visual appearance of plants and the abundance of *C. obstructus* gravid females, males and larvae. In 2008 *C. obstructus* gravid females, males and larvae were associated with plants whose foliage appeared more blue and less green, plants whose flowers appeared less saturated and areas with reduced flower cover (Table 3.2). In 2009 *C. obstructus* males and gravid females showed an opposite response to these variables while larvae showed no relationships with these variables.

Significant differences were observed in the visual appearance of *B. napus* in the 2009 grid as compared with the 2008 grid (Table 3.3). In 2009 the foliage of plants appeared significantly less bright, more saturated, more blue and less green. The percent cover of flower was significantly increased and these flowers also appeared more saturated. Significantly less spatial structure was observed in the saturation values of foliage.

3.3.1.3 Structural equation models

No single model explained the observed covariance structure among the variables in the 2008 and 2009 grids. The individual models for both the 2008 ($\chi^2 = 21.649$, $P = 0.420$; Table 3.4) and 2009 ($\chi^2 = 26.847$, $P = 0.580$; Table 3.5) adequately fit the data (Figure 3.5). Significant spatial autocorrelation was only

observed in the residuals for gravid *C. obstrictus* females from 2008 and *C. obstrictus* males from 2009. While this autocorrelation would be expected to moderately inflate the strength of the relationships with these variables, this inflation is unlikely to affect the significance of the relationships as the *P* values of these paths were well below the α of 0.05 (Tables 3.4, 3.5).

While the two models differed considerably, several paths were similar between the two models (Figure 3.5). In both models larval parasitoids were positively related to *C. obstrictus* larvae, *C. obstrictus* males were positively related to gravid *C. obstrictus* females, and strong correlations occurred among nutrient levels in pod tissue. When both the direct and indirect effects through other variables are considered, the total effects of plant height and plant density on *C. obstrictus* larvae were also negative in both grids.

Several differences were observed in the structure of the model of the 2009 grid as compared with the model for the 2008 grid (Figure 3.5). The most important of these differences was the absence of any paths directed from pod nutrients to gravid *C. obstrictus* females. There are also no paths directed from gravid *C. obstrictus* females to *C. obstrictus* larvae, from plant density to *C. obstrictus* larvae, or from plant height to *C. obstrictus* males. There is also the addition of a path from plant density to *C. obstrictus* males, and the additional covariance between P, K and plant density. A reversal of the sign of the path coefficients was also observed between plant height and pod nutrient levels.

Some notable differences in mean, variability, and spatial structure were noted between the grids for many of the variables examined in the SEM (Table

3.3). Significantly higher levels of N, P and S occurred in pod tissue in the 2009 grid. Plants heights were also significantly greater in 2009. Numbers of *C. obstructus* males, gravid females, and larvae from the 2009 grid were approximately one-tenth of those observed in 2008. There was an approximate decline of one-half in the variability of the pod nutrients, plant height, and plant density from 2008 to 2009 as represented by their coefficients of variation. The variability of counts of *C. obstructus* males, gravid females, and larvae from the 2009 grid were increased relative to the 2008 grid. In 2009 there was significantly less spatial structure observed in the amounts of N and S in pod tissue, as well as the number of *C. obstructus* males, gravid females, and larval parasitoids, while significantly greater spatial structure was observed in the number of *C. obstructus* larvae per pod.

3.3.1.4 Larval parasitoids

In 2008 91 parasitoids were collected giving an overall parasitism rate of 0.33% with parasitoids emerging from 0.12% of collected pods. The parasitoid species composition was 47.25% *N. tidius*, 43.96% *T. lucidus*, 3.30% *Euderus albitarsis*, 2.20% *Chlorocytus* sp., 1.10% *Pteromalus* sp. and 2.20% *Necremnus* spp. In 2009 27 parasitoid exit holes were observed giving an overall parasitism rate of 1.93% with parasitoids emerging from 0.07% of collected pods. The parasitoids collected in 2009 consisted solely of *T. lucidus*.

3.3.2 Nitrogen-Sulfur hot spot experiment

Sulfur fertilization had a significant positive effect on the levels of S ($F = 24.11$; $df = 1, 21$; $P < 0.0001$) and K ($F = 6.10$; $df = 1, 21$; $P = 0.0222$) within developing pod tissue. Nitrogen fertilization had no significant effect on the levels of N or other nutrients within pod tissue. Neither N nor S fertilization had significant effects on plant height or plant density. Fertilization did, however, have significant effects on the visual appearance of the plants. The color of plant foliage fertilized with N appeared significantly less saturated ($F = 6.80$; $df = 1, 21$; $P = 0.0168$), more blue and less green ($F = 14.35$; $df = 1, 21$; $P = 0.0012$). The flowers of plants fertilized with N appeared more yellow and less green but only when the plants had no additional S fertilization ($F = 5.73$; $df = 1, 20$; $P = 0.0265$). Significantly fewer *C. obstrictus* males were observed in traps from areas fertilized with N ($F = 6.28$; $df = 1, 21$; $P = 0.0205$). Fertilization had no other significant effects on the numbers of *C. obstrictus* females, gravid females, larvae or larval parasitoids.

3.4 Discussion

Little evidence was found to support the hypothesis that soil variables affect plant vigor and pod nutrient content (Figure 3.1, b, c, f, g). With the exception of TEC, no soil properties had a significant relationship with plant vigor or plant nutrients. While these properties have been shown to limit the growth of *B. napus* in certain situations, the observed soil properties seem to fall within the known tolerances of *B. napus* and consequently no relationships would

be expected (Thomas 2003). Given the flooding that was present in 2008, it seems likely that topography, soil texture or soil moisture may have been more limiting than the measured soil properties, which would then suppress the relationships between these properties and plant vigor. The predicted relationships were also not observed between soil nutrients and plant vigor, and between soil nutrients and plant nutrients with S being a notable exception. In contrast with the results, numerous studies have shown reduced plant vigor with deficiencies in various plant nutrients (Grant and Bailey 1993; Thomas 2003). Previous studies have also shown a link between plant nutrient content and fertilization rate (Bullock and Sawyer 1991; Jackson 2000). These studies have generally compared fertilized and unfertilized areas and have not attempted to link these variables in an observational manner. Similar to the situation described above, an unmeasured factor may also have limited plant growth and plant nutrient uptake preventing soil nutrients from becoming limiting, thereby obscuring the relationship. In addition, soil tests are limited in their ability to predict nutrient uptake as many soil nutrients are highly mobile and have small pools of available nutrients (Barker and Bryson 2007; Haneklaus et al. 2007). These limitations may reduce the strength of the relationships between soil and plant nutrients. Future studies might utilize ion exchange membranes to better capture nutrient uptake (Qian and Schoenau 2002).

The results from the two structural equation models provide mixed support for the hypothesis that plant nutrient composition has an indirect effect on the distribution of *C. obstrictus* adults and larvae. In 2008 significant relationships

were observed between plant nutrient levels and the distribution of gravid *C. obstrictus* with an overall association between gravid females and areas of lower plant nutrients. Due to the correlation among plant nutrients, it is difficult to determine if a single nutrient or a combination of nutrients is driving the response. Despite this difficulty, N, S or a combination of these two nutrients seems the most likely explanation as these nutrients had plausible mechanisms and larger path coefficients. Given the previously reported positive effect of S described in the literature (Aljmlí 2007; Blake et al. 2010) and the decreased abundance of *C. obstrictus* males with N fertilization in the hotspot experiment, it seems likely that N may be responsible for the observed dissociation with high nutrient areas. Contrary to previous studies (Aljmlí 2007; Blake et al. 2010), no interaction between N and S was observed. This difference may result from the observational approach used, which did not include an unfertilized control, or from the absence of areas low in N and high in S at our study sites. Unlike 2008, no significant relationships were observed between plant nutrients and the distribution of gravid *C. obstrictus* females in 2009. There are several possible explanations for the differential response between years. It is possible that a threshold exists in the response of gravid females in which the higher, less variable and more uniformly distributed levels of plant nutrients may not have resulted in the same dissociation with nutrients seen in 2008. This threshold interpretation is consistent with previous findings which show the development time of *C. obstrictus* larvae increasing with N fertilization but at a decreasing rate (Chapter 2). A stronger response to plant nutrients may have been observed as a result of the greater

populations of *C. obstrictus* observed in 2008. The flooding in 2008 may also have influenced the response of *C. obstrictus*.

The evidence to support the hypothesis that plant nutrients influence visual cues was conflicting (Figure 3.1, i). Results from the 2008 grid showed an association between areas high in N and other nutrients and areas with foliage that appeared more green and less blue and flowers that appeared less yellow and more green while the results of the hotspot experiment showed N having the opposite effect on the visual appearance of *B. napus*. Results of the hot spot experiment are consistent with color differences typically seen between N-deficient and sufficient plants (Barker and Bryson 2007). The anomalous results from 2008 may be the result of other stresses, most likely from excess soil moisture, affecting the visual appearance of the plants. In addition to the effects of N, in 2008 it was found that plants with a greater supply of S appeared more saturated, which is consistent with the paler floral colorations observed in *B. napus* under S deficiency. No similar relationships were observed between plant nutrients and visual appearance in the 2009 grid, most likely due to higher and less variable levels of plant nutrients recorded in 2009.

Conflicting evidence was observed for the response of adult *C. obstrictus* to the visual appearance of their host plants (Figure 3.1, i). The responses of male and gravid female *C. obstrictus* from 2009 were consistent with the current model of visual attraction in *C. obstrictus* (Tansey 2009). The inconsistent responses observed in 2008 could be the product of the anomalous visual appearance of high nutrient areas in that year. It is possible that adults may have been responding

more to the olfactory and non-volatile chemical cues, which may have been a more accurate representation of the nutritional status of the plants. The stronger response of males to visual cues contrast with the findings of Tansey et al. (2010a) who found that males had a less discriminatory response to visual cues than males. The differing responses of larval and adult *C. obstrictus* in 2009 could be explained by the absence of a relationship between larval and gravid female *C. obstrictus*. Additional laboratory experiments need to be performed to confirm the effect of plant nutrient levels on the visual appearance of *B. napus* and to confirm the behavioral response of *C. obstrictus* to these changes.

The results of SEM supported the interpretation that plant height and plant density affect the distribution of *C. obstrictus* males and larvae through effects on pod density (Figure 3.1, n, o, q, r). Differences in the variability of plant density and the average value of plant height between site-years may explain the differences in the pathways between the two models. Ferguson et al. (2003) found some correlation between plant height, plant density and the distribution of *C. obstrictus* adults but contrary to this study they found no relationships between plant height, plant density and larval populations of *C. obstrictus*.

The 2008 model provided strong support for the relationships among *C. obstrictus* males, females, larvae, and parasitoids (Figure 3.1, p, s, t). The strength of these relationships declined in 2009, mainly as a result of the large reduction in *C. obstrictus* populations between years. This decline could have been the result of poor overwintering survival due to low winter temperatures (Cárcamo et al. 2009). This decline is likely responsible for the increased levels of parasitism seen

in 2009. The weak relationships observed between gravid female *C. obstrictus* and their larvae may be a result of the yellow bowl traps used to sample adult *C. obstrictus*. Yellow bowl trap captures are indicative of flight activity and may not reflect the oviposition behavior of *C. obstrictus* females (Ferguson et al. 2000; Dossdall et al. 2006). The relationships observed among *C. obstrictus* males, females, larvae, and parasitoids are consistent with previous studies which showed consistent association between *C. obstrictus* females and males and between *C. obstrictus* larvae and parasitoids, and a less consistent association between *C. obstrictus* females and larvae (Murchie et al. 1999; Ferguson et al. 2000; Dossdall et al. 2006). The observed level of parasitism and species composition were also consistent with previous observations of *C. obstrictus* larval parasitoids (Dossdall et al. 2006, 2009).

The use of SEM allowed for an evaluation of the current understanding of environmental factors and their effects upon the spatial distribution of *C. obstrictus* and its parasitoids. Application of SEM, in comparison with the use of SADIE (Blake 2010) or regression (Ferguson 2003), allowed for the untangling of the effects of plant nutrient levels from plant vigor. The effective use of SEM was hampered by large variations in plant vigor, plant nutrient levels, and populations of *C. obstrictus* between site-years. These variations in conjunction with the theory that plant growth is controlled by the most limiting factor complicates prediction, as the effect of the addition of one nutrient will be dependent upon the availability of others (Loomis and Connor 1992). These complications may explain the inability to find one model to describe both site-years. Additional field

testing and refinement of our SEM will be necessary before this model can be used in a management context. With that qualification, future research should investigate the economic and logistical feasibility of using site-specific management zones. The utility of management zones for *C. obstrictus* should be investigated as it is unlikely that adult distributions are dependent solely upon environmental factors, as *C. obstrictus* adults are attracted to conspecifics (Evans and Bergeron 1994). This endogenous factor would lessen the spatial dependence between environmental factors and *C. obstrictus* making their precise distributions more difficult to predict (Wagner and Fortin 2005). The current model suggests that management zones with low plant density, plant height and N content will be at higher risk of infestation with *C. obstrictus*. Management of these zones through differential application of insecticide, fertilizer or seed may serve to lower the risk of *C. obstrictus* infestation while minimizing inputs and conserving natural enemies (Plant 2001). It is also possible that with further research, knowledge of the visual responses of *C. obstrictus* in combination with aerial or ground-based imagery could be used within a growing season to enhance the management of *C. obstrictus*.

Table 3.1. The correspondence between observed variables and variables within the conceptual model. Conceptual variables are shown in bold. Unmeasured conceptual variables and conceptual variables omitted from SEM analysis are also identified

Soil Moisture (unmeasured)	Olfactory Cues (unmeasured)
Soil Properties (omitted from model)	Visual Cues (omitted from model)
pH	Hue Value of Foliage
Electrical Conductivity	Saturation Value of Foliage
% Organic Matter	Brightness Value of Foliage
Total Extractable Cations	% Cover of Foliage
Soil Nutrients (omitted from model)	Hue Value of Flowers
Soil N	Saturation Value of Flowers
Soil P	Brightness Value of Flowers
Soil K	% Cover of Flowers
Soil S	Chemical & Tactile Cues (unmeasured)
Plant Vigor	<i>C. obstrictus</i> Gravid Females
Plant Height	<i>C. obstrictus</i> Gravid Females per Trap
Plant Density	<i>C. obstrictus</i> Males
Plant Density per m ²	<i>C. obstrictus</i> Males per Trap
Plant Nutrients	<i>C. obstrictus</i> Larvae
Pod N	<i>C. obstrictus</i> Larvae per Pod
Pod P	Larval Parasitoids
Pod K	Larval Parasitoids per Pod
Pod S	

Table 3.2. The spatial relationships, as represented by SADIE's index of overall association X, between the variables describing the visual appearance of *Brassica napus* plants and the variables included in the structural equation models of the 2008 and 2009 grids.

	Hue Value of Foliage		Saturation Value of Foliage		Brightness Value of Foliage		% Cover of Foliage		Hue Value of Flowers		Saturation Value of Flowers		Brightness Value of Flowers		% Cover of Flowers	
	Grid 2008	Grid 2009	Grid 2008	Grid 2009	Grid 2008	Grid 2009	Grid 2008	Grid 2009	Grid 2008	Grid 2009	Grid 2008	Grid 2009	Grid 2008	Grid 2009	Grid 2008	Grid 2009
N	-0.51	-0.19	0.10	0.12	0.20	-0.11	-0.21	-0.10	0.45	-0.15	0.29	0.14	-0.30	-0.22	0.26	0.14
P	-0.57	0.05	0.20	0.10	0.12	-0.05	-0.20	-0.13	0.59	-0.14	0.37	-0.05	-0.40	0.22	-0.45	0.00
K	-0.49	-0.10	-0.04	0.15	0.13	-0.09	-0.49	-0.16	0.53	-0.08	0.16	0.06	-0.52	0.02	-0.03	0.11
S	-0.59	-0.09	0.33	0.19	0.14	-0.16	-0.14	-0.19	0.47	-0.06	0.47	0.10	-0.40	0.01	0.40	0.14
Plant Density	-0.31	-0.18	0.15	0.13	0.47	-0.13	0.28	-0.05	-0.02	0.18	0.07	0.20	0.23	-0.21	0.26	0.19
Plant Height	0.31	0.00	0.22	0.17	-0.15	-0.08	0.43	-0.07	0.24	0.00	0.20	0.13	0.19	0.09	0.24	0.22
<i>C. obstrictus</i> Gravid Females	0.30	-0.19	-0.11	0.14	-0.40	-0.11	-0.18	-0.08	-0.06	0.00	-0.08	0.19	-0.13	-0.13	-0.34	0.22
<i>C. obstrictus</i> Males	0.62	-0.23	-0.39	0.21	0.07	-0.20	0.19	-0.15	-0.18	0.01	-0.42	0.28	0.29	-0.26	-0.34	0.30
<i>C. obstrictus</i> Larvae	0.23	-0.05	-0.21	-0.06	-0.41	0.07	-0.41	-0.06	-0.06	0.08	-0.11	-0.06	-0.33	0.01	-0.45	-0.03
Larval Parasitoids	-0.04	0.05	0.22	-0.12	-0.25	0.07	-0.06	-0.12	0.23	0.00	0.27	-0.13	-0.06	0.14	0.26	-0.02

Bold values indicate a significant association or dissociation based on SADIE's index of association X

Table 3.3. The means \pm SD, coefficients of variation (CV), minimum and maximum values, and the \bar{v}_i and \bar{v}_j values of variables describing the visual appearance of *Brassica napus* plants as well as all of the variables included in the structural equation models of the 2008 and 2009 grids.

	Mean \pm SD		CV		\bar{v}_i		\bar{v}_j	
	Grid 2008	Grid 2009	Grid 2008	Grid 2009	Grid 2008	Grid 2009	Grid 2008	Grid 2009
Hue Value of Foliage	78.65 \pm 1.76	81.49 \pm 3.73	0.02	0.05	1.92	1.30	-1.76	-1.24
Saturation Value of Foliage	110.05 \pm 4.66	122.83 \pm 10.78	0.04	0.09	1.75	0.95	-1.54	-1.00
Brightness Value of Foliage	134.08 \pm 4.36	122.25 \pm 8.37	0.03	0.07	1.07	1.01	-1.04	-1.08
% Cover of Foliage	56.34 \pm 6.20	62.41 \pm 6.69	0.11	0.11	-1.22	1.00	1.18	-1.16
Hue Value of Flowers	45.92 \pm 0.21	45.99 \pm 0.34	0.00	0.01	1.40	1.34	-1.14	-1.20
Saturation Value of Flowers	144.93 \pm 7.41	164.74 \pm 12.86	0.05	0.08	1.81	1.54	-1.73	-1.58
Brightness Value of Flowers	226.34 \pm 4.18	224.12 \pm 6.84	0.02	0.03	1.05	1.13	-1.16	-1.03
% Cover of Flowers	4.25 \pm 1.50	10.61 \pm 3.12	0.35	0.29	1.39	1.22	-1.57	-1.34
% of N in Pod Tissue	4.25 \pm 0.60	4.73 \pm 0.23	0.14	0.05	2.97	1.24	-3.13	-1.48
% of P in Pod Tissue	0.49 \pm 0.09	0.74 \pm 0.07	0.18	0.09	2.85	3.37	-3.09	-3.54
% of K in Pod Tissue	1.84 \pm 0.19	1.82 \pm 0.10	0.10	0.05	2.07	2.39	-1.98	-2.55
% of S in Pod Tissue	0.57 \pm 0.09	0.74 \pm 0.06	0.16	0.08	2.79	1.76	-2.87	-2.02
Plant Density per m ²	48.99 \pm 25.25	44.04 \pm 9.62	0.52	0.22	1.03	1.02	-1.03	-1.12
Plant Height (cm)	70.08 \pm 6.93	103.04 \pm 6.62	0.10	0.06	2.42	2.10	-2.43	-1.99
<i>C. obstrictus</i> Gravid Females per Trap	24.91 \pm 10.58	2.53 \pm 1.84	0.42	0.73	2.55	1.15	-2.66	-1.19
<i>C. obstrictus</i> Males per Trap	26.62 \pm 15.53	3.81 \pm 2.79	0.58	0.73	3.51	1.76	-3.17	-1.58
<i>C. obstrictus</i> Larvae per Pod	0.3465 \pm 0.1087	0.0328 \pm 0.0169	0.31	0.52	1.10	1.58	-0.92	-1.52
Larval Parasitoids per Pod	0.0011 \pm 0.0020	0.0006 \pm 0.0011	1.81	1.97	1.30	1.03	-1.15	-0.94

Bold value indicate a significant difference in the means between grids according to a t-test assuming unequal variance with an $\alpha = 0.01$

Table 3.4. Unstandardized and standardized coefficients, standard errors of the unstandardized coefficients, and the z -values and associated P -values for the structural equation model for the 2008 grid. The paths described below, with the exception of the covariances, were directed from the unbolded variables below to the variable in bold above.

	Unstandardized Coefficients	SE	z -value	P -value	Standardized Coefficients
Covariance with Plant Height					
N	-1.357	0.359	-3.776	0.0002	-0.341
K	-0.371	0.112	-3.309	0.0009	-0.304
S	-0.107	0.041	-2.583	0.0098	-0.200
Covariance with N					
P	0.048	0.007	7.435	< 0.0001	0.854
K	0.083	0.013	6.587	< 0.0001	0.683
S	0.042	0.006	7.498	< 0.0001	0.794
Covariance with P					
K	0.012	0.002	6.839	< 0.0001	0.715
S	0.006	0.001	6.943	< 0.0001	0.790
Covariance with K					
S	0.011	0.002	7.048	< 0.0001	0.669
C. obstrictus Gravid Females					
N	9.015	3.137	2.874	0.0041	0.547
P	-49.184	22.517	-2.184	0.0290	-0.426
K	18.283	7.821	2.338	0.0194	0.340
S	-80.912	21.432	-3.775	0.0002	-0.661
C. obstrictus Males					
C. obstrictus Gravid Females	1.024	0.143	7.186	< 0.0001	0.653
Plant Height	0.465	0.201	2.319	0.0204	0.180
C. obstrictus Larvae					
C. obstrictus Gravid Females	0.0023	0.0012	1.831	0.0671	0.216
Plant Density	-0.0012	0.0004	-2.992	0.0028	-0.252
Larval Parasitoids					
C. obstrictus Larvae	0.0024	0.0014	1.689	0.0912	0.147

Table 3.5. Unstandardized and standardized coefficients, standard errors of the unstandardized coefficients, and the z -values and associated P -values for the structural equation model for the 2009 grid. The paths described below, with the exception of the covariances, were directed from the unbolded variables below to the variable in bold above.

	Unstandardized Coefficients	SE	z-value	P-value	Standardized Coefficients
Covariance with Plant Height					
P	0.057	0.029	1.986	0.0470	0.134
S	0.104	0.030	3.455	0.0006	0.270
Covariance with Plant Density					
P	-0.138	0.045	-3.078	0.0021	-0.224
K	-0.223	0.085	-2.636	0.0084	-0.243
Covariance with N					
P	0.005	0.002	2.867	0.0041	0.352
K	0.006	0.002	2.620	0.0088	0.286
S	0.007	0.002	4.395	< 0.0001	0.516
Covariance with P					
K	0.004	0.001	5.864	< 0.0001	0.694
S	0.002	0.000	5.726	< 0.0001	0.574
Covariance with K					
S	0.002	0.001	3.883	0.0001	0.369
Plant Height					
Plant Density	0.136	0.052	2.631	0.0085	0.196
<i>C. obstrictus</i> Males					
<i>C. obstrictus</i> Gravid Females	0.382	0.162	2.352	0.0187	0.250
Plant Density	0.056	0.023	2.433	0.0150	0.189
<i>C. obstrictus</i> Larvae					
Plant Height	-0.0007	0.0003	-2.811	0.0049	-0.283
Larval Parasitoids					
<i>C. obstrictus</i> Larvae	0.0300	0.0071	4.255	< 0.0001	0.450

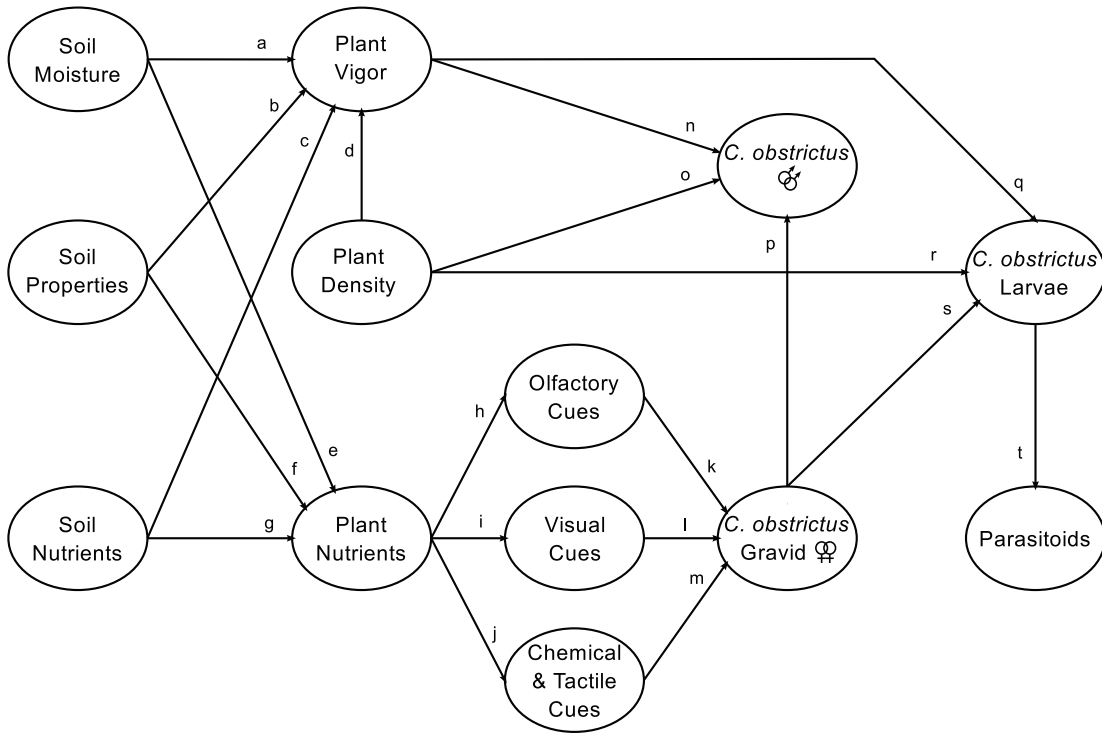


Figure 3.1. The hypothesized conceptual model showing the relationships between the soil, plant, *Ceutorhynchus obstrictus* and parasitoid variables. The paths are identified with lowercase letters and are discussed within the text of the paper.

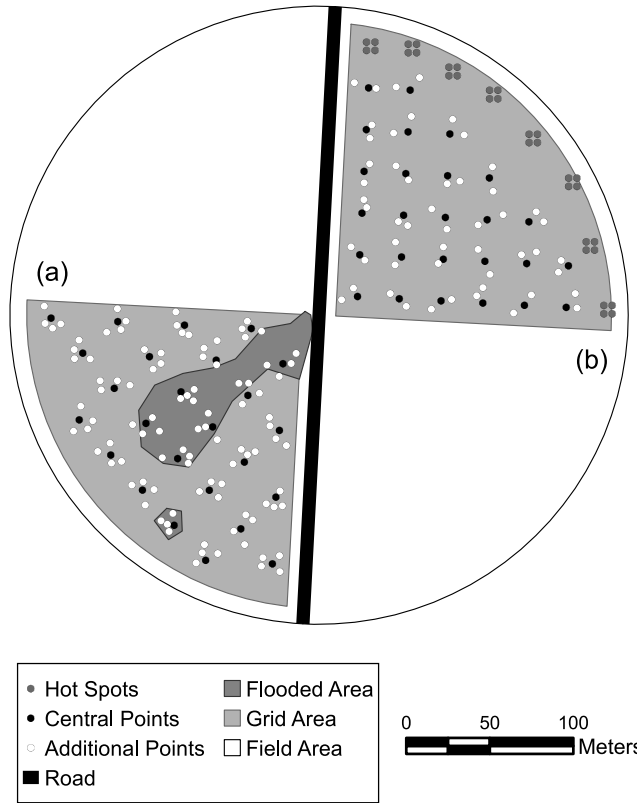


Figure 3.2. An overview of the study area showing the shape and arrangement of the (a) 2008 and (b) 2009 grids, the location of the flooded area in the 2008 grid, and the location of the sampling points within both grids.

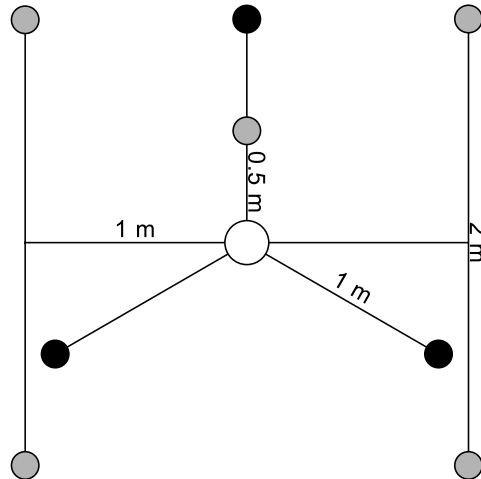


Figure 3.3. The arrangement of sampling locations around each bowl trap. The central white point represents the bowl trap while the surrounding gray points show the locations for taking plant vigor measurements and larval collection sites in 2008. The black points show the locations of soil sampling in 2008 and the locations of the plant density quadrats.

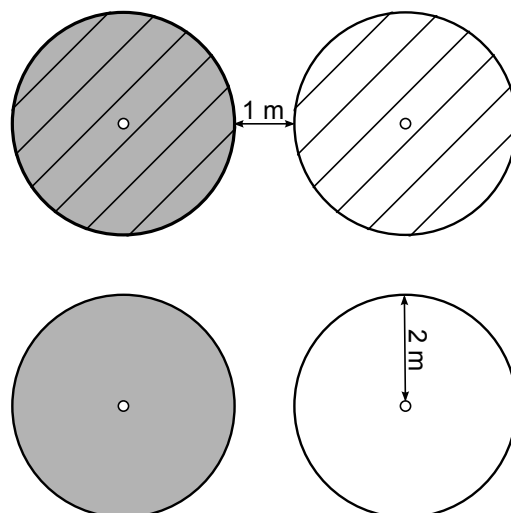


Figure 3.4. The arrangement and spacing of the treatments for the nitrogen-sulfur hot spot experiment. The bowl traps are represented as white points; the grey areas indicate additional nitrogen fertilization, while the hatched areas indicate additional sulfur fertilization.

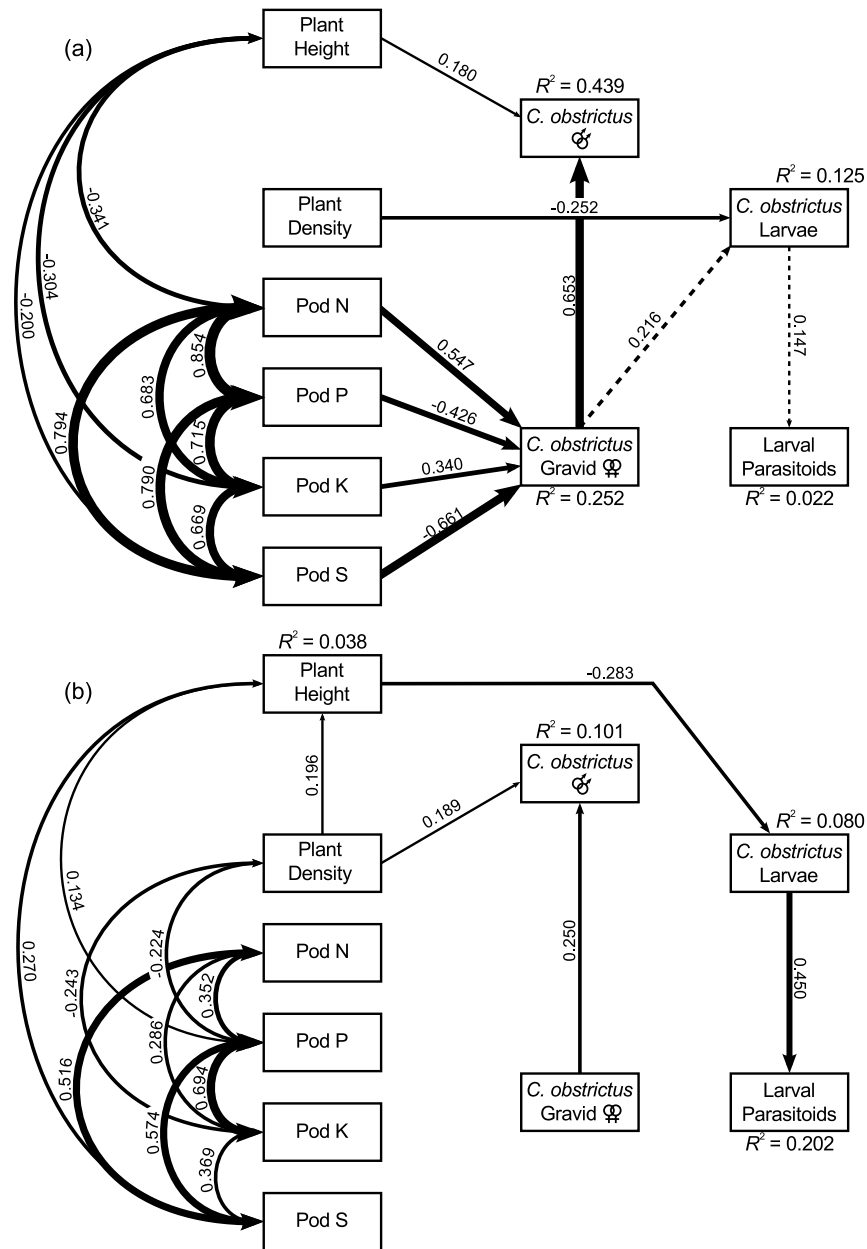


Figure 3.5. The structural equation models for the 2008 (a) and 2009 (b) grids showing the relationships between plant height (cm), plant density per m^2 , the percentage of nutrients in pod tissue, the number of *Ceutorhynchus obstructus* males and gravid females per trap, and the number of larval *C. obstructus* and larval parasitoids per pod. The two non-significant paths are shown with dotted lines. Standardized coefficients are shown along each path and the thicknesses of these paths are proportional to the magnitude of these coefficients.

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Chapter 4 Conclusion

In Chapter 2 I investigated the effects of nitrogen (N) and sulfur (S) supply on feeding preferences, oviposition preferences, and larval development of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham). N and S affected oviposition preference with S increasing the attractiveness of pods at lower N levels and N increasing attractiveness but only relative to the lowest level of N fertilization. Similar though non-significant differences in feeding preferences were observed among the nutrient treatments. These results did not correspond to either the plant stress (White 1984) or plant vigor hypothesis (Price 1991) as in either case one would expect the extreme fertilization treatments to be preferred. The plant vigor and plant stress hypotheses likely represent two extremes on a continuum of response, and as a consequence, responses intermediate between the two hypotheses should be expected. Responses intermediate between the plant stress and plant vigor hypotheses have also been observed for the crucifer specialist *Plutella xylostella* (L.) (Sarfraz et al. 2009).

In contrast, some of the results of the larval development experiment are consistent with the plant stress hypothesis. All measured plant vigor metrics as well as the developmental time of larval *C. obstrictus* increased with N fertilization. These results support the plant stress hypothesis, although no increase in developmental time was observed between the two highest N treatments. Also inconsistent with the plant vigor hypothesis is the lack of a S effect on larval weight and developmental time and the lack of a N effect on

larval weight. Under a simple interpretation of the plant stress hypothesis, one would expect larvae to develop more slowly and weigh less when developing on plants with higher levels of both N and S.

The contrast between adult preference for, and larval performance on, plants fertilized under differing N and S regimes refutes the preference-performance hypothesis (Jaenike 1978). Results of my study differ from previous investigations of adult preference and larval performance of *C. obstrictus* on different *Brassica* species and on several susceptible and resistant lines of *B. napus* derived from hybridization between *Sinapis alba* L. x *Brassica napus* L. where at least a weak association was observed between preference and performance (Ulmer and Dossdall 2006; Dossdall and Kott 2006; Tansey et al. 2010b). Two differing fertilizer regimes were used in the determination of adult preference and larval performance, and it is possible that this difference in methodology may have contributed to the differences in response to N and S between larvae and adults. It is also possible that different cues are used when recognizing species and when assessing host quality within a species. However, previous investigations of the grass leaf miner *Chromatomyia nigra* (Meigen) (Diptera: Agromyzidae) have shown a similar discrepancy between adult preference and larval performance when comparing host species and when manipulating nutrient supply to one host species (Scheirs et al. 2000; Scheirs and De Bruyn 2004).

There are several possible factors which might contribute to the observed differences between adult preference and larval performance. These include

natural enemies (Thompson 1988), the rarity of low nutrient levels in canola cropping systems, optimal foraging theory (Scheirs and De Bruyn 2002) and conflicts between maternal and larval fitness (Marshall and Uller 2007). The similarities between feeding and oviposition preferences of *C. obstrictus* give support to the hypothesis that optimal foraging theory may affect the oviposition preferences of *C. obstrictus*. The large increase in pod number observed with N fertilization is also consistent with the hypothesis that *C. obstrictus* females maximize their own fitness by selecting plants with large numbers of pods, which offer more sites for oviposition, at the cost of the fitness of their larvae, which develop more slowly on these plants.

In Chapter 3, the effects of soil and plant nutrients on the spatial distributions of *C. obstrictus* and its parasitoids were investigated in commercial fields of *B. napus* in southern Alberta. In general no significant relationships were found between soil characters and the levels of plant nutrients. There were also no significant relationships between soil nutrients and the distributions of *C. obstrictus* and its parasitoids. The strength of observed relationships with soil properties might be improved through examinations of soil texture or topography, or the use of ion exchange membranes (Qian and Schoenau 2002).

Strong negative relationships were observed between plant nutrient levels in 2008 and the distribution of gravid *C. obstrictus* females. In contrast, the relationships observed between plant nutrient levels in 2009 and the distribution of gravid females were not significant. This difference could easily be explained by the less variable and high average levels of plant nutrients seen in 2009. A

large degree of multicollinearity was observed among plant nutrients making it difficult to determine which nutrients were driving the response of gravid *C. obstrictus* females. Nitrogen was viewed as the element most responsible for affecting responses of gravid weevil females, given the known effects of N on visual and olfactory cues of *B. napus* (Bilsborrow et al. 1993; Grant and Bailey 1993; Zhao et al. 1993, 1994; Asare and Scarisbrick 1995; Kim et al. 2002), and the previously reported positive effect of S on *C. obstrictus* (Aljmli 2007; Blake et al. 2010). This conclusion is supported by the negative effect of N on the trap captures of *C. obstrictus* males observed in the hot spot experiment.

The results of Chapter 3 were mostly consistent with the plant stress hypothesis. When plants with low levels of nutrients were available, these plants were preferred by gravid *C. obstrictus* females. Diminished numbers of *C. obstrictus* males were observed on plants fertilized with additional N. Finally, a negative relationship was observed between the plant vigor indicator plant height and the numbers of *C. obstrictus* larvae. The lack of a relationship between soil nutrients and *C. obstrictus*, and the lack of an effect of additional N levels upon gravid *C. obstrictus* females and larvae, refute this hypothesis although this absence of relationship could be explained by other factors. In addition, given the reported effects of S on the olfactory and visual cues emitted by *B. napus* (Zhao et al. 1993, 1994; Asare and Scarisbrick 1995; Kim et al. 2002; Schnug and Haneklaus 2005; Brauer et al. 2007) and on the abundance of *C. obstrictus* (Aljmli 2007; Blake et al. 2010), it seems likely that S levels, acting in addition to or interacting within the negative effects of N, increase the abundance of *C.*

obstrictus adults and larvae. A positive effect of S was not observed but as N and S levels were associated, no areas with low N levels and high S levels were observed.

When considering the results of both laboratory (Chapter 2) and field experiments (Chapter 3) the overall results of the two studies seem to support a plant stress interpretation. Larvae of *C. obstrictus* develop more quickly on plants with lower levels of N (Chapter 2), while adult *C. obstrictus* are associated with areas of the field with lower levels of N (Chapter 3). Combined results of the two experiments also seem to support the preference-performance hypothesis as adult females are associated with optimal host plants. In contrast, the results of the pod choice experiment are in conflict with both of these hypotheses (Chapter 2).

Gravid *C. obstrictus* females are associated with areas of fields that are low in N, but they prefer pods from plants grown at moderate to high levels of N. These differing results may be a consequence of the relative importance of different host plant cues at different spatial scales. Olfactory cues are thought to be most important at longer ranges, visual cues are most important at medium ranges, while nonvolatile chemical and tactile cues are most important at relatively close range (Kennedy 1965; Finch and Collier 2000). As a consequence, different cues may be important at the scale of fields compared to the scale of pods. Differences in olfactory and visual cues are the most plausible possible mechanism to explain the distribution of *C. obstrictus* adults and larvae in fields, while non-volatile cues are most likely driving oviposition and feeding preferences among pods. The diminished attractiveness of pods from the lowest N

treatments may have resulted from differences in non-volatile plant compounds such as sugars, proteins or amino acids (Bartlett et al. 1996).

Differences in the visual appearance of the foliage and flowers of *B. napus* were investigated as a possible mechanism to explain the observed relationships between plant nutrient levels and the distribution of gravid *C. obstrictus* females (Chapter 3). Deficiencies in N result in foliage chlorosis while the foliage of N-sufficient plants appears greenish-blue (Barker and Bryson 2007). Deficiencies in S are also associated with foliar chlorosis, and in extreme cases with accumulations of purplish anthocyanins within leaf tissues. Sulfur deficiency also results in smaller, more pale flowers (Schnug and Haneklaus 2005; Brauer et al. 2007). Current models of *C. obstrictus* vision predict increased blue reflectance having a negative effect and increased yellow reflectance having a positive effect on attractiveness (Tansey 2009).

Based on this theory, N would be predicted to have a negative relationship with the distribution of *C. obstrictus* adults based on the predicted increase in the reflectance of blue light from plants high in N. Additional N fertilization in the hot spot experiment resulted in foliage that appeared more blue in color. These areas were associated with greater numbers of *C. obstrictus* males but not gravid females. In 2008 a contrasting response to N was observed with plants with a high N level appearing less blue. This response could result from other sources of plant stress that affect foliar color. Also in 2008, in contrast with my expectations *C. obstrictus* adults showed an association with areas with foliage appearing more blue. This association may be the result not of visual but of olfactory and non-

volatile chemical cues. The responses of adults in 2009 were consistent with theory-based predictions with *C. obstrictus* adults associated with foliage appearing less blue.

The above model would predict S to have a positive effect on the abundance of *C. obstrictus* adults as S is associated with larger, more saturated flowers which would be expected to increase the reflectance of yellow. It is also possible that these changes in flower saturation may affect ultraviolet (UV) reflectance as decreases in both yellow and UV reflectance have been observed concurrently with S deficiency (Brauer et al. 2007). These potential changes in UV reflectance would also serve to increase their attraction to *C. obstrictus* adults (Tansey 2009). As predicted, an association was observed between S levels in the plant and the percent cover and saturation levels of *B. napus* flowers in 2008. No association was observed between S levels and the distribution of *C. obstrictus* adults in 2008, but the positive effect of S could have been masked by the effect of N which was associated with S in 2008. No effect of S on floral appearance or on *C. obstrictus* adults was observed in 2009. The higher S levels observed in 2009 may have prevented observable deficiency symptoms. Although we did not observe a positive effect of S on the abundance of *C. obstrictus*, this positive effect of S has been shown previously (Aljmlil 2007; Blake et al. 2010).

Unexpectedly, within the hotspot experiment N was also shown to affect flower color with flowers fertilized with additional N and no additional S appearing more yellow and less green. Based on the above model this should increase attractiveness (Tansey 2009). In contrast to this expectation significantly

fewer *C. obstrictus* males were observed. The observed change in floral color may shift the reflected wavelengths away from the supernormal response maxima of 590 nm contributing the reduced attractiveness. As stated above, N fertilization was associated with increased blue reflectance and it is possible that the negative effect of increased blue reflectance may have overpowered any positive effect of the more yellow flowers.

Based on these findings it is plausible that differences in N and S supply directly affect the spatial distribution of *C. obstrictus* adults and indirectly affect their larvae, through changes in foliar and floral reflectance. As stated above it is likely that these effects function at or beyond the scale of a single plant. Consequently differences in visual appearance seem unlikely to explain differences in oviposition or feeding preferences among pods. It also seems likely that volatile and non-volatile olfactory cues are also important determinants of the spatial distribution of *C. obstrictus*.

While differences in the glucosinolate (GSL) profile of *B. napus* were not determined, the observed relationships between plant nutrients and the distribution of adult *C. obstrictus* are consistent with the expected changes in GSL composition with N or S fertilization. Nitrogen levels within plants have been associated with increases in indolyl GSLs and decreases in 4-pentenyl GSL (Zhao et al. 1994, Kim et al. 2002). 4-pentenyl GSL has been shown to be attractive to *C. obstrictus* adults (Evans and Allen-Williams 1992; Bartlet et al. 1993; Blight et al. 1995; Smart et al. 1997) while the indolyl GSL 1-methoxy-3-indolylmethyl has been shown to be repellent (Tansey et al. 2010a). Consequently N was

predicted and observed to have a negative effect on the distribution of *C. obstrictus* adults.

The effects of N and S on the distribution of *C. obstrictus* adults have been previously shown to interact (Aljmlil 2007; Blake et al. 2010). Plants with high levels of N and low levels of S were less attractive than plants with low levels of N and high levels of S. The effects of N and S on the composition of GSLs were also shown to interact (Zhao et al. 1994; Kim et al. 2002). Sulfur increases levels of the attractive 3-butenyl and 4-pentenyl GSL but this effect is suppressed at higher N levels (Kim et al. 2002). Based on these findings we would expect plants with high levels of S and low levels of N to be the most attractive. No direct evidence was found for this attraction, but due to the observed association between N and S, no areas with high S levels were observed that were independent of high N levels.

Given the current understanding of the effects of plant nutrition on GSL composition, it seems likely that differences in olfactory cues are at least partially responsible for the observed effects of plant nutrients on the distributions of *C. obstrictus* adults and larvae. Differences in the relative amounts of 3-butenyl and 4-pentenyl GSL seem the most likely mechanism for large scale spatial structure observed as the breakdown products of 1-methoxy-3-indolylmethyl GSL are less volatile and unlikely to play a large role in long distance host location. The effect of plant nutrition on floral volatiles such as α -farnesene may also be important (Bartlett 1996; Brauer et al. 2007).

Differences in GSL content may also provide a plausible mechanism for the negative effect of N fertilization on the development of larvae observed in Chapter 2. As mentioned above, N increases the relative concentration of indolyl GSLs within *B. napus* (Zhao et al. 1994; Kim et al. 2002). These GSLs include 1-methoxy-3-indolylmethyl GSL, which has been associated with longer development times and lower larval weights in *C. obstrictus* (Tansey 2010b). The results of the pod choice experiment are in conflict with this potential mechanism as increased oviposition was observed in pods from plants grown under higher N levels. If 1-methoxy-3-indolylmethyl GSL concentrations increase with N fertilization, feeding and oviposition should decrease as 1-methoxy-3-indolylmethyl GSL has been shown to be a feeding and oviposition deterrent (Tansey 2010b). It is possible that differences in the concentrations of other feeding stimulants or deterrents masked this effect. For example, sucrose is a more effective feeding stimulus than the attractive GSL, sinigrin (2-propenyl GSL), for several crucifer specialists (Bartlett 1996).

In addition to the evaluation of ecological hypotheses and the investigations of possible mechanisms for the observed effects, results of these studies yield several recommendations for canola producers. One such recommendation involves the use of plant nutrition in addition to resistant cultivars and attractive *Brassica* spp. within a stimulo-deterrent diversion (push-pull) strategy (Miller and Cowles 1990) to achieve a more attractive trap crop. Reducing N and increasing S fertilization should create a trap crop more attractive

to *C. obstrictus* adults, which may better prevent invasion and subsequent damage of the main crop by *C. obstrictus*.

The results of this study also suggest that site-specific management techniques may be useful for the management of *C. obstrictus*. Site-specific management is already used to manage the application of fertilizers (Plant 2001). The use of such management strategies could minimize areas of the field with a nutrient profile that is attractive to *C. obstrictus* adults. This type of management attempts to maximize yield across the field and as such should already create vigorous, high density plant stands that are subjected to lower levels of infestation by *C. obstrictus* larvae. If future research is better able to link variations in soil nutrients and other properties to the distributions of *C. obstrictus* adults, it may then be possible to develop models predicting the relative risk of *C. obstrictus* attack within a field. This information could then be used to inform site-specific insect monitoring and insecticide application. Site-specific insecticide applications have the potential to reduce application costs (Plant 2001) and minimize the negative effects of applications on natural enemies and other beneficial insects (Cárcamo et al. 2001).

Although several technical hurdles remain, improved understanding of the visual cues driving the distribution of *C. obstrictus* may one day allow for continuous real time monitoring of plant characteristics contributing to the attractiveness of these plants as hosts for *C. obstrictus*. Commercial systems using imaging sensors to control the variable application rate of herbicide for the site-specific management of weeds are already commercially available (Brown and

Noble 2005), and models of *C. obstrictus* response to spectral reflectance have been developed (Tansey 2009). While a great deal of research would be required before this technology could be used in the site-specific applications of insecticide for the control of *C. obstrictus*, it remains an intriguing avenue of inquiry.

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