University of Alberta

Integrated Pest Management of *Sitona lineatus* L. (Coleoptera: Curculionidae) in Crops of *Pisum sativum* L. (Fabales: Fabaceae) in Western Canada

by

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For my grandfathers,

Frank Miner (September 12, 1936 – June 8, 1988)

and

Ernie Vankosky (October 28, 1932 – June 8, 2010)

Abstract

Sitona lineatus L. (Coleoptera: Curculionidae) is a pest of *Pisum sativum* L. (Fabales: Fabaceae) and managing it is a challenge because of its fecundity, migratory behavior and concealed larval habitat. Potential components of an integrated pest management program for *S. lineatus* were investigated near Lethbridge and Vauxhall, Alberta over three years. Cage studies indicated that larval feeding is more damaging than adult feeding but that larval populations are not dependent on adult weevil density. In open plot experiments, thiamethoxam-treated plants experienced significantly less foliar feeding damage than plants receiving no insecticide treatment but no consistent effects on yield were observed for any plot treatment over six site-years. *Rhizobium* inoculation had a synergistic interaction with thiamethoxam. Laboratory trials showed that *Bembidion quadrimaculatum* L. (Coleoptera: Carabidae) consumed *S. lineatus* eggs. Seed treatment with thiamethoxam and *Rhizobium* inoculant, and egg predation should be included in an integrated pest management program for *S. lineatus*.

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1.1. Introduction

Current agricultural cropping practices, especially the mass production of cash crops grown in extensive monocultures over vast geographical areas and the rapid transport of goods on a global scale, have facilitated the introduction and movement of invasive insect pests in agroecosystems worldwide (Pimentel 2002; Pimentel *et al*. 2002). The pea leaf weevil, *Sitona lineatus* L. (Coleoptera: Curculionidae), is an invasive pest that has dramatically expanded its geographical range and abundance in association with these practices. *Sitona lineatus* has been responsible for enormous losses in yield and quality of some legume crops (Prescott and Reeher 1961; Doré and Meynard 1995; Williams *et al*. 1995; Corre-Hellou and Crozat 2005), and its pest status appears poised to increase in future years.

Field pea, *Pisum sativum* L. (Fabales: Fabaceae), one of two primary host plants of *S. lineatus* (Jackson 1920), has recently undergone considerable expansion in production and this has been accompanied by increases in pea leaf weevil crop damage. The crop is currently grown on over 25 million ha worldwide (Schatz and Endres *et al.* 2003), with approximately 1.5 million ha annually in Canada (Statistics Canada 2008), and 0.35 million ha in the U.S.A. (National Agricultural Statistics Service 2007). Approximately 3 million ha are committed to field pea production in Australia and Europe combined (McKay *et al.* 2003). In Europe, fertilizer inputs are greater relative to that in North America (Herzog *et al.* 2006), while the scale of field pea production tends to be reduced relative to that of North America, due to the smaller average size of farms in Europe and the production of monocultures over vast areas in North America (Marvier 2001). Other significant production regions include Russia and China (McKay *et al.* 2003). Field pea is an attractive rotational crop because its nitrogen-fixing capability benefits soil fertility (Corre-Hellou and Crozat 2005; Anonymous 2008) which reduces nitrogen-input costs for subsequent crops (Walley *et al.* 2007). In addition, its high protein content is ideal for animal and human consumption (Verkleij *et al.* 1992; Corre-Hellou and Crozat 2005).

Areas devoted to the production of legume crops worldwide are expected to continue to increase in future years, with concomitant increases in crop damage inflicted by insect pests including *S. lineatus*. The primary appeal to producers for increasing production of crops like field pea, especially in North America, is the opportunity to reduce fertilizer input costs (Walley *et al.* 2007). Escalating agricultural expenses associated with the impact of world fuel price increases on nitrogen fertilizer production, especially anhydrous ammonia (Agriculture and Agri-Food Canada 2008), are important incentives to increase production of crops that fix nitrogen and so help reduce dependence on inorganic fertilizers.

Climate change poses a considerable new threat to crop production due to invasive insect pests of agricultural crops. Climate change is predicted to cause a northward shift of suitable habitat for many insect species, and bioclimatic models of several agricultural crop pests predict that both the geographical ranges and abundances of these species will increase with global temperature increases between 1 and 7 °C (Olfert and Weiss 2006). In North America, the pea leaf weevil is currently well established in Alberta and Saskatchewan, Canada (Dow AgroSciences 2001; Saskatchewan Agriculture and Food 2007), and its continued northward range expansion, potentially exacerbated by climate change, poses a serious threat to sustainable pulse crop production in the Canadian prairies.

Potential changes in geographic ranges and relative abundances of insect pests like *S. lineatus*, wrought by modern production practices, crop expansion and climate change emphasize the need to design sustainable pest management programs based on a thorough understanding of the pest. The objective of this review is to describe the current distribution of the pea leaf weevil, its life history, current management strategies, and to identify new research directions needed to improve its sustainable management.

1.2. Pea Leaf Weevil Geographical Expansion

The pea leaf weevil had been reported throughout continental Europe, the United Kingdom (Jackson 1920) and northern Africa (Hoebeke and Wheeler 1985). The genus *Sitona* harbours well known legume pests, with at least one species associated with every species of legume (Jackson 1920). In North America, *S. lineatus* was first found in 1936 at Royal Oak, near Victoria, British Columbia, Canada (Downes 1938). It spread southward and established in Washington, Oregon, northern California, northwestern Idaho and other northwestern regions of the U.S.A in the following years (Hoebeke and Wheeler 1985). In 1984, the pea leaf weevil was recorded in northeastern U.S.A for the first time (Hoebeke and Wheeler 1985). In 2002, Bloem *et al.* reported that *S. lineatus* had been trapped in Jefferson County, Florida along with seven other non-native Curculionidae species.

Sitona lineatus is a significant pest throughout Europe, Africa, Asia and North America (Nilsson 1968; Hoebeke and Wheeler 1985; Bloem *et al.* 2002). A number of *Sitona* species have invaded Australia, including *Sitona humeralis* Stephens (now *Sitona discoideus* Gyllenhal (Aeschlimann 1980). The potential for expansion of the pea leaf weevil into Western Australia has raised concerns regarding pea crop damage and yield loss (Botha *et al.* 2004). In 1997, *S. lineatus* was reported for the first time in southern Alberta, and no geographical barriers remain to prevent its spread throughout Canada’s primary region of pulse crop production (Soroka and Cárcamo 2006; Coles *et al.* 2008). Ongoing surveys in southern Canada indicate northern and eastern spread of the weevil, as *S. lineatus* damage was reported in southern Saskatchewan, Canada for the first time in 2007 (Saskatchewan Agriculture and Food 2007).

Intercontinental dispersal of *S. lineatus* is most likely facilitated by import and export industries. Between 1940 and 1963, for example, *S. lineatus* specimens were intercepted along the eastern coast of North America, most commonly on cut flowers from Europe and Bermuda (Hobeke and Wheeler 1985). After reaching a new continent, range expansion is likely facilitated by flight. Although little research has focused on long range flight patterns of the pea leaf weevil, local migratory flight has been observed, and Hamon *et al.* (1987) found that most pea leaf weevil adults were trapped at heights of seven to 10 m above the ground. Flight at such heights suggests that this species has potential to disperse over substantial distances.

The recent expansion of the pea leaf weevil range into western Canada likely resulted from an increase in the area devoted to legume production, and climatic conditions conducive to the establishment and reproduction of the weevil. In 1991 in Canada, 198 000 ha were planted to field pea; by 2005, 1.32 million ha of pea were sown (Agriculture and Agri-Food Canada 2007). A similar increase in field pea production area occurred in France between 1980 and 1992, which was also associated with increased populations of legume pests (Doré and Meynard 1995).

1.3. *Sitona lineatus* Morphology, Life History and Host Plants

Morphological characters of the pea leaf weevil were described by Jackson (1920). Adults range from 3.6 to 5.4 mm in length and are distinguished by brown and grey ochreous scales arranged on the elytra in longitudinal stripes. Eggs are yellow-white when first deposited and become melanized in two to three days. Although some variation in egg morphology has been observed, eggs are commonly oblong and oval-shaped. Larvae have cylindrical bodies that taper at the extremities; the abdominal segments are creamy-white with reddish brown bristles on each segment. Larvae have reduced antennae, easily distinguishable jaws and no eyes. Pupae are creamy white with dorsal bristles. Adults and pupae can be sexed based on differences in the size and shape of the pygidium (eighth abdominal tergite).

Adult *S. lineatus* overwinter in shelter belts (Jackson 1920) or in the vicinity of perennial legumes including clover and vetch species (Schotzko and O'Keeffe 1988; Murray and Clements 1992). During the non-reproductive stage, adults are oligophagous, consuming a variety of leguminous species before and during overwintering (Landon *et al.* 1995), as feeding occurs when winter temperatures are mild (Murray and Clements 1992). Depending on the region, the pea leaf weevil emerges from overwintering sites in March and April (Jackson 1920), and adults then migrate to annual legume crops (Landon *et al.* 1995). Spring migration to annual legume hosts is limited by a lower temperature threshold of 12.5º C for flight in England (Hamon *et al.* 1987).

Field pea and faba bean, *Vicia faba* L. (Fabales: Fabaceae), are the preferred hosts of post-diapause adults (Landon *et al.* 1995). After weevils arrive at host plants, mating occurs and females begin laying eggs. The duration of the oviposition period varies depending on the region but is generally in the range of 10 days (Williams *et al.* 1995). Jackson (1920) noted that egg-laying continued until a short time before death, or until the end of June to early July in England. Oviposition peaks at temperatures between 12 and 22°C (Hans 1959). The number of eggs laid ranges from 354 to 1,655 per female (Jackson 1920). At the beginning of the oviposition period, only one to five eggs are produced per female per day, but the oviposition rate later increases to approximately 24 eggs per female per day (Jackson 1920). Eggs are scattered over the soil surface by females as they feed (Jackson 1920; Hoebeke and Wheeler 1985).

The duration of the egg stage of *S. lineatus* varies depending on temperature and humidity. Jackson (1920) reported that eggs hatched in 20 to 21 days, but the incubation period can be as short as 18 days (Prescott and Reeher 1961; Hoebeke and Wheeler 1985). Research by Lerin (2004) indicated that eggs required 70 ± 2.5 (S.D.) days to hatch at 8°C, whereas only 6.2 ± 0.5 days were required at 29°C. At temperatures above 30°C, the time required for hatching and mortality increased significantly (Lerin 2004). At 33°C, 100% of eggs failed to hatch, although significant embryonic development had occurred (Lerin 2004). The results of Lerin's (2004) investigation are in close agreement with the earlier work of Andersen (1933, 1934). Relative humidity can also affect the development time of eggs, as Jackson (1920) noted that eggs subjected to excessive desiccation did not hatch.

After hatching, first-instar larvae burrow into the soil surrounding the host plant. In the soil, larvae chew a nearly undetectable hole in root nodules of the host plant and crawl inside (Jackson 1920; Hoebeke and Wheeler 1985). All five larval instars feed on *Rhizobium leguminosarum* Frank (Rhizobiales: Rhizobiaceae) (Johnson and O'Keeffe 1981), over a period ranging from 30 to 60 days depending on the geographical region and soil temperature (Jackson 1920;

Hoebeke and Wheeler 1985; Landon *et al.* 1995). Upon maturation, larvae create oval-shaped pupation cells in the soil (Jackson 1920). Pupation requires 16 to 19 days in Europe (Jackson 1920), or approximately 15 days in North America (Prescott and Reeher 1961; Hoebeke and Wheeler 1985). The adult remains in the soil until its exoskeleton has fully sclerotized (Jackson 1920).

New generation adults feed on the remaining green tissue of field peas and faba beans. When those food sources are exhausted or leaves have senesced, a period of extensive flight begins, in which the weevils feed on a variety of secondary leguminous host plants (Jackson 1920; Fisher and O'Keeffe 1979a; Hamon *et al.* 1987; Murray and Clements 1992). This feeding period continues until late summer or autumn, when adult weevils search for overwintering sites (Jackson 1920; Hoebeke and Wheeler 1985).

In North America and England, *S. lineatus* is univoltine (Jackson 1920; Hoebeke and Wheeler 1985); however, the species may be bivoltine in some regions (Hans 1959; Hoebeke and Wheeler 1985). In the field, *S. lineatus* adults can live up to eleven months (Schotzko and O'Keeffe 1988); longevity is limited by food availability and population density (Schotzko and O'Keeffe 1988). Female longevity is greater than male longevity at low population densities (Schotzko and O'Keeffe 1988).

1.4. *Sitona lineatus* Migration and Flight Activity

Sitona lineatus undergoes two distinct periods of migration: the migratory spring flight from overwintering sites to field peas or faba beans, and late summer or autumn flight for overwintering (Fisher and O'Keeffe 1979a). Using omnidirectional flight traps, Fisher and O'Keeffe (1979a) observed the flight patterns of *S. lineatus* in the United States for three years. The greatest numbers of weevils trapped in spring were collected at approximately the same time every year, regardless of weather conditions, *P. sativum* growth stage or *S. lineatus* population density. The timing of the autumn flight was more variable, and correlated to the timing of host plant senescence (Fisher and O'Keeffe 1979a). However, during both the spring and autumn flight periods, significantly more weevils were caught at heights over 1.5 m, while in the interval between the peak flight periods weevils were trapped only at lower elevations (Fisher and O'Keeffe 1979a). These results are largely in agreement with those from Europe (Stein 1972; Hamon *et al.* 1987), although Hamon *et al.* (1987) observed that the timing of the spring migration was temperature-dependent. Based on their results, Fisher and O'Keeffe (1979a) concluded that the pea leaf weevil migrates in the spring and late autumn, and that localized dispersal, for example from areas of high to low density in a production system, is achieved by low-elevation flight. Migratory flight may not always be necessary for the pea leaf weevil to locate its primary hosts, such as in small-scale farming operations or in systems where crop rotation is not practiced.

Several authors have noted that consumption of *V. faba* or *P. sativum* is required for reproductive system maturation (Hans 1959; Fisher and O'Keeffe 1979a; Hamon *et al.* 1987; Schotzko and O'Keeffe 1988; Landon *et al.* 1995). If this is indeed the case, spring migration would be required for *S. lineatus* to find its primary hosts, especially in regions where crop rotation is practiced, or where overwintering sites are far from legume fields. In mid-summer, in-field dispersal of the pea leaf weevil is achieved primarily by walking or occasional low-altitude flight (Hamon *et al.* 1987). Flight at significant elevation is believed to cease after the spring migration by the overwintered population as the flight muscles of *S. lineatus* females may be sacrificed for the production of reproductive material, as described by Johnson (1963) and termed the “oogenesis-flight syndrome.”

A number of factors influence the flight activity of the pea leaf weevil. Hamon *et al.* (1987) found that the timing of the peak spring migration was associated with a minimum temperature of 12.5°C. Light intensity was also associated with peak flight times (Hans 1959). Within a period of 24 h, peak flights occurred after 11:00 h, with peaks between 12:00 and 13:00 h, and no flight activity occurred between 02:00 to 11:00 h (Hamon *et al.* 1987). Stein (1972) recorded a similar pattern of no flight before 08:00 h or after 17:00 h for a number of curculionid species. In several tests, the maximum temperature at which flight occurred ranged from 26 to 30°C (Stein 1972; Stein and Rezwani 1973). Prevailing winds also affected pea leaf weevil flight patterns, as migratory flight tends to follow a downwind trajectory (Hamon *et al.* 1987). Peak flight altitude of *S. lineatus* was recorded at 152 and 183 cm by Fisher and O’Keeffe (1979a), and ranged from seven to 10 m, as recorded by Hamon *et al.* (1987).

Distinct migration periods for the pea leaf weevil have been identified, but the duration of these periods differs among regions. In England, the spring migration, based on significant levels of flight (more than 10 weevils caught during a period of seven days), extended from 25 March until 23 May, with the autumn migration occurring over a two-month period beginning in August (Hamon *et al.* 1987). The duration of spring migration varies with temperature. Hamon *et al.* (1987) postulated that the timing of migration is triggered by photoperiod but that the ability to migrate is limited by temperature. The timing and duration of the autumn migration period depends on the availability of food at the emergence site (Jackson 1920; Fisher and O'Keeffe 1979a; Hamon *et al.* 1987).

1.5. Host-plant Preferences

Field pea and faba bean are the preferred hosts of post-diapause *S. lineatus* adults (Landon *et al.* 1995). Both plant species have a symbiotic relationship with the nitrogen-fixing bacterium, *Rhizobium leguminosarum* biovar *viciae* (Spaink 1994; Mutch and Young 2004), which may explain the preference of *S. lineatus* for these species. Danthanarayana (1967) suggested that leghaemoglobin is associated with the tendency of all *Sitona* species to feed on nitrogen-fixing legumes.

Reproduction of *S. lineatus* has been investigated with respect to host plant consumption. Development of the female reproductive system is a gradual process that can be quantified by the lengths of the germaria and vitellarium-lateral oviducts (Schotzko and O'Keeffe 1986a). Reproductive development occurs from July through March during feeding on both primary and secondary hosts (Schotzko and O'Keeffe 1986a). However, oogenesis of *S. lineatus* is believed to require post-diapause feeding on either *P. sativum* or *V. faba* (Hans 1959; Fisher and O'Keeffe 1979a; Schotzko and O'Keeffe 1986a; Hamon *et al.* 1987; Landon *et al.* 1995). Specifically, Schotzko and O'Keeffe (1986a) suggest that early oviposition is facilitated by female feeding on *P. sativum,* as individuals collected before March did not oviposit when reared on alfalfa, *Medicago sativa* L. (Fabales: Fabaceae). There are no records of larvae completing their development on hosts other than field pea and faba bean (Fisher and O'Keeffe 1979b; Hoebeke and Wheeler 1985; Ferguson 1994; Murray and Clements 1995).

Chickpea, *Cicer arietinum* L. (Fabales: Fabaceae), has been investigated as an alternate primary host of the pea leaf weevil, as there has been some evidence of larval feeding on its root nodules (Williams *et al.* 1991). Williams *et al.* (1991) suggested that the consumption of chickpea is the result of host-range expansion in regions where both pea and chickpea occur simultaneously, but where peas are removed at the green stage. After the removal of the peas, the weevils may switch to the remaining chickpea crops to mate and oviposit (Williams *et al.* 1991). The potential for chickpea as a primary host requires further investigation.

Pea leaf weevil feeding can vary among genotypes of its primary hosts, and adults seem to feed preferentially on certain pea cultivars (Havlickova 1980; Cantot 1989; Kordan and Śledź 1994; Wojciechowicz-Zytko and Młynarczyk 2002). Wojciechowicz-Zytko and Młynarczyk (2002) note that the lifespan and survival rate of *S. lineatus* varies among faba bean cultivars. Pea nutrition, including carbohydrate, nitrogen and protein contents, varies with cultivar and the health of the plant (Havlickova 1980). This characteristic may be used to develop host plant resistance. Extrinsic resistance factors, such as epicuticular waxes, may also influence pea leaf weevil feeding rates on primary hosts (White and Eigenbrode 2000; Rutledge *et al.* 2003; Chang *et al.* 2004).

Several studies have noted host preferences and impacts on weevil longevity. Jaworska (1992) noted that in early spring, the population density on pea cultivars was 15.5 weevils m^{-2} compared with only 0.25 weevil m^{-2} on faba bean cultivars. Research by Grieb (1976) and Wiech (1977) yielded similar results. In May, during oviposition, faba beans were the more attractive hosts (Jaworska 1992). Alternating between hosts in this way is possibly due to changing nutrient requirements of the pea leaf weevil at different stages of its life cycle (Jaworksa 1992). Of the two primary hosts, peas were more attractive to weevils, but longevity was greatest on faba bean (Jaworska 1998). Longevity of the pea leaf weevil, however, is also dependent upon the population density and the number of eggs a female lays in her lifetime (Schotzko and O’Keeffe 1988). At low population densities, single mating pairs for example, longevity was greater on peas than on any other legume (Schotzko and O’Keeffe 1988).

A number of perennial legumes serve as secondary hosts to *S. lineatus* in spring before the emergence of annual legumes, in the late summer and fall when annual legumes have senesced, and during the overwintering period (Jackson 1920; Fisher and O’Keeffe 1979a; Wiech 1984; Hoebeke and Wheeler 1985; Hamon *et al.* 1987; Murray and Clements 1992; Murray and Clements 1995). Markkula and Köppä (1960) noted that in late summer, several *Sitona* species were collected on white clover, *Trifolium repens* L. (Fabales: Fabaceae), and of those, *S. lineatus* was the most abundant, accounting for 59 to 96% of the specimens collected. In England, *S. lineatus* can significantly damage white clover, consuming up to 30% of the photosynthetic area of plants during overwintering (Murray and Clements 1995). In field tests, weevils preferred sweet clover, *Melilotus albus* L. (Fabales: Fabaceae), and alfalfa (Krasnopol'skaya 1966; Zlatanov 1966; Lykouressis and Emmanouel 1991; Murray and Clements 1994). Lupin, *Lupinus albus* L. (Fabales: Fabaceae), incurred significant levels of feeding damage in Germany (Ferguson 1994).

In addition to *T. repens*, *M. albus*, *M. sativa*, and *L. albus*, other secondary hosts can include red clover (*Trifolium pretense* L., Fabales: Fabaceae), sweet clover, black medic (*Medicago lupulina* L., Fabales: Fabaceae), Siberian pea shrub (*Caragana arborescens* Lam., Fabales: Fabaceae) and black locust (*Robinia pseudoacacia* L., Fabales: Fabaceae) (Fisher and O'Keeffe 1979b, 1979c). Alfalfa, a longer-season crop than field pea, supported larger populations of weevils for a longer period than *P. sativum* (Schotzko and O'Keeffe 1988). There is some evidence that *S. lineatus* exhibits 'limited polyphagous behaviour' (Greib and Klingauf 1977), because in the absence of legumes prostrate knotweed (*Polygonum aviculare* L., Polygonales: Polygonaceae) and rose species (*Rosa* spp. Rosales: Rosaceae) have been consumed in laboratory and field experiments (Greib and Klingauf 1977, Fisher and O'Keeffe 1979c).

Only two legumes have been rejected in host suitability tests. The common bean, *Phaseolus vulgaris* L. (Fabales: Fabaceae), was never fed upon in greenhouse studies, was rejected in the greenhouse by gravid females in search of ovipositon sites and was rejected by larvae (El-Dessouki 1971); however, damage to *P. vulgaris* has been recorded in the field (Andersen 1934). Lentils, *Lens culinaris* Medik. (Fabales: Fabaceae), were never fed upon in choice tests (Fisher and O'Keeffe 1979b; Fisher and O'Keeffe 1979c; Schotzko and O'Keeffe 1988) or in the field (Fisher and O'Keeffe 1979b) and adult weevils starved if lentils were the only food choice offered (Schotzko and O'Keeffe 1988).

1.6. Pheromone and Chemical Signaling

Blight *et al.* (1984) demonstrated the use of an aggregation pheromone by *S. lineatus*. The pheromone, 4-methyl-3, 5-heptanedione, is produced by males when they feed upon *V. faba* (Blight *et al.* 1984; Blight and Wadhams 1987). The pheromone is produced in spring and is attractive to both male and female weevils (Blight and Wadhams 1987). Traps baited with an analogue of 4-methyl-3, 5-heptanedione caught significantly more individuals than those without the analogue (Blight *et al.* 1984; Blight and Wadhams 1987; Nielsen and Jensen 1993) and traps baited with higher concentrations trapped greater numbers of *S. lineatus* (Blight *et al*. 1984). Cone traps baited with the pheromone were much more species-specific than sticky traps (Nielsen and Jensen 1993), and pheromone-baited cone traps caught more weevils than sticky traps likely because weevils could access cone traps via the air or ground (Blight and Wadhams 1987). Weevils were observed to move upwind after detecting the pheromone, either by flight or by walking (Nielsen and Jensen 1993). The use of pheromone-baited cone traps has potential for both monitoring and mass trapping programs (Blight *et al.* 1984; Blight and Wadhams 1987; Nielsen and Jensen 1993).

There is some evidence that *S. lineatus* is sensitive to host plant odors during spring migration (Leroy *et al.* 1999), and that field pea releases volatile compounds that attract *S. lineatus*. Landon *et al.* (1997) found that *cis*-3-hexen-1-yl acetate made up 87% of the volatiles released by field pea. This compound occurs in other legumes but in much lower proportions (Landon *et al.* 1997). During spring migration, *cis*-3-hexen-1-yl acetate was significantly attractive to *S. lineatus* (Landon *et al.* 1997).

1.7. Effects of Pea Leaf Weevil on Agricultural Systems

Both adults and larvae damage field pea and faba bean. Adult foliage feeding can destroy seedlings and young plants in the spring (Jackson 1920). Characteristic ‘U’-shaped notches result from adult feeding and under severe infestations, growing shoots may be completely destroyed (Jackson 1920). Adult feeding limits the photosynthetic capacity of legumes, which reduces the ability of the plant to produce reproductive organs or support root nodules (Havlickova 1982; Williams *et al.* 1995). The extent of damage to primary host plants during adult feeding in the spring depends on the timing of attack and weevil density (Williams *et al.* 1995). For example, Williams *et al.* (1995) observed that losses of photosynthetic area due to defoliation in faba beans reached 50% at the time of plant emergence but declined to 35% when defoliation began a week later. In the absence of compensation, defoliation can reduce the number of pods produced per plant (Williams *et al.* 1995). Nielsen (1990) reported that reduction in pod number contributed to a yield loss of 28%. Yield losses would be expected to increase as defoliation rates increase, due in part to the poor sequestering of nutrients by pea reproductive organs (Hodgson and Blackman 1957). In chickpea, weevil feeding caused 50% defoliation (Williams *et al.* 1991).

Pea leaf weevil larvae can significantly damage root nodules (Jackson 1920; George 1962), and the highest rate of nodule destruction coincides with flowering of peas (Jackson 1920). In greenhouse trials, larvae damaged between 40 and 80% of nodules, mostly on the main root (Verkleij *et al.* 1992). Cantot (1986) observed that 90% of root nodules were destroyed when 12 larvae were found on a single plant. El-Dessouki (1971) determined that when infested with 100 *S. lineatus* eggs, 98% of root nodules were destroyed and that the number of pods produced by the plant was reduced by 27%. However, it is unlikely that 100 eggs result in a population of 100 larvae, due to intraspecific competition, plant carrying capacity, infertile eggs, predation or poor hatching rates due to abiotic conditions. In greenhouse trials using *S. hispidulus* eggs and red clover plants, El-Dessouki and Stein (1970) observed that when densities were 50 and 100 eggs per plant, only 44 and 46% of larvae, respectively, were recovered after four weeks. After 5.5 weeks, only 17 of a possible 100 larvae were recovered, and at the end of the experiment, the larval density was similar in both treatments (50 and 100 eggs added) (El-Dessouki and Stein 1970). Based on these results, the maximum carrying capacity of a plant is in the range of 17 larvae per plant (El-Dessouki and Stein 1970).

Although root nodule destruction affects yield indirectly, larval damage is expected to have a greater negative impact on yield than adult feeding (Hunter 2001; Corre-Hellou and Crozat 2005). Root damage can make plants more susceptible to secondary infection and may alter the nitrogen balance between roots and above-ground structures (Hunter 2001). Two major concerns related to root nodule destruction include the loss of nitrogen nutrition in seeds and reduced nitrogen inputs to the soil (Doré and Meynard 1995; Corre-Hellou and Crozat 2005), although it has been noted that in the Netherlands, economic yield losses occur when the larval density is as low as two larvae per plant (Ester and Jeuring 1992). As damage due to pea leaf weevil herbivory increases, the amount of nitrogen derived from fixation decreases (Corre-Hellou and Crozat 2005), which contributes to reduced residual soil nitrogen available for subsequent crops (Doré and Meynard 1995). This relationship is exacerbated in the presence of weeds (Corre-Hellou and Crozat 2005). When damage scores for defoliation were low, root nodule damage was also low and 72% of the total nitrogen was derived from nitrogen fixation; however, when damage scores and nodule damage increased, only 49% of nitrogen was derived from fixation (Corre-Hellou and Crozat 2005). When soil is fertilized, field pea and faba bean root nodulation may be significantly or completely suppressed (George 1962). Hence, in fertilized plots, 50% reduction in root nodule number had no negative impact on yield including reduction of root nodule number due to larval consumption (George 1962).

The relationship between pea leaf weevil damage and plant compensation requires further investigation. Quinn and Hall (1992, 1996) tested the compensatory potential of alfalfa, a perennial legume, when attacked by *Sitona hispidulus* Fabricus (Coleoptera: Curculionidae). They suggested that the compensatory ability of plants varied over time, as plants over-compensated when damaged at early growth stages compared to later growth stages. Although more nodules were produced than were lost at early plant stages, it is possible that new nodules are not as efficient at fixing nitrogen as older nodules (Quinn and Hall 1992).

During the late summer, autumn, winter and early spring, *S. lineatus* utilizes a number of alternate hosts (Jackson 1920; Hoebeke and Wheeler 1985). Murray and Clements (1992, 1995) found that up to 30% of the photosynthetic area of white clover, primarily the trifoliate leaves, was consumed by overwintering pea leaf weevils. Reduction in biomass was significantly greater than the controls when densities were 1.0 and 0.5 weevils per plant, referred to as ‘high density’ (Murray and Clements 1992). In spring, high density populations (ranging from 0.5 to 1.0 weevils per plant) of *S. lineatus* reduced dense stands of white clover seedlings by 50% in seven days (Murray and Clements 1992). When alfalfa plants were defoliated by pea leaf weevils, root biomass was 45% less than that of control plants (Havlickova 1982). The effects of *S. lineatus* on its secondary hosts may not be limited to defoliation. For example, *S. lineatus* has been identified as a vector of bacterial wilt (*Clavibacter michiganensis* ssp. *insidiosus*) in alfalfa (Kudela *et al.* 1984). The leguminous secondary hosts of *S. lineatus* also contribute nitrogen to the soil; therefore, damage to these plants cannot be disregarded.

1.8. Managing Pea Leaf Weevil Populations

The adult of *S. lineatus* is most readily collected and monitored in the field, but most crop damage is usually inflicted by its larvae. Consequently, considerable research effort regarding population monitoring and economic threshold development has focused on investigating the relationship between adult densities and nodule damage by larvae. Cantot (1986, 1989) developed a model to predict nodule damage by larvae based on adult feeding pressure using the numbers of box-trapped overwintered adults, leaf notches from adults and larvae collected per plant. Table 1.1 summarizes the rating system used by Cantot (1986), as well as the approximate range of larvae at each rank. A highly significant correlation was observed between the number of adult feeding notches on the first node of field peas and the resultant larval population (Cantot 1986). For example, a leaf-notch score of three indicated that the mean density was 12 larvae per plant and the amount of damage at this density resulted in destruction of 90% of root nodules (Cantot 1986).

However, leaf notching by adults may not be an accurate indication of larval or adult pea leaf weevil populations or the amount of nodule damage. Nielsen (1990) found that larval attack intensity was not dependent upon the extent of adult attack, despite a significant correlation between adult attack levels and the number of leaf notches. Landon *et al.* (1995) also found an increase in leaf notches with increasing population density or of increasing feeding intensity. El-Dessouki (1971) found that larval attack had no impact on leaf size or the number of flowers produced by the host plant. Cantot's model also does not consider leaf density per plant or the density of the plant stand (Nielsen 1990); however, both factors influence the overall ability of a crop to compensate for herbivory. A number of factors affect the size of pea leaf weevil larval populations, including adult population density, intraspecific competition, cultivar or host plant variety and host carrying capacity (El-Dessouki 1971; Nielsen 1990; Landon *et al.* 1995).

Due to the complexity of the relationship between host plants, weevil damage and abiotic factors, there are no comprehensive economic thresholds currently available for pea leaf weevil. Available thresholds include the presence of 10 eggs per plant (Doré and Meynard 1995), three or more adult weevils per 180° sweep (Quisenberry *et al.* 2000) and indirect measures based on the number of leaf notches (Cantot 1986; Cantot 1989; Landon *et al.* 1995).

Monitoring legume crops for *S. lineatus* is vital for appropriately timing control measures and developing economic thresholds. Research by Nielsen and Jensen (1993) suggested that cone traps baited with pea leaf weevil aggregation pheromone could be effective for monitoring population changes in low-density pea leaf weevil populations. The use of pheromone traps at field margins provides an earlier indication of infestation than counting leaf notches (Nielsen and Jensen 1993). Therefore, the use of pheromone traps may facilitate more efficient foliar insecticide usage. Similarly, pheromone traps baited with 4-methyl-3, 5-heptanedione effectively monitored population size, but the number of weevils trapped was not related to the amount of damage to pea seedlings (Quinn *et al.* 1999). The use of pea leaf weevil aggregation pheromone trapping systems is currently limited to monitoring. To be of greater use in a pea leaf weevil management system, a relationship must be established between the capture rate of weevils in pheromone traps and yield reduction (Nielsen and Jensen 1993).

Research investigating *S. lineatus* chemical control has focused on identifying the most efficacious insecticidal products and application procedures. Table 1.2 summarizes a variety of chemical products that have been investigated for use in the control of *S. lineatus*. Foliar insecticides can reduce adult pea leaf weevil populations and protect yields (Bardner *et al.* 1983). For example, cyhalothrin-lambda reduced adult populations by approximately 56% compared to untreated controls (Steene *et al.* 1999). Other foliar insecticides evaluated for *S. lineatus* control include imidacloprid, cypermethrin, aldicarb, permethrin, deltamethrin, parathion, cyfluthrin, benomyl and carbofuran (Bardner and Fletcher 1979; McEwen *et al.* 1981; Bardner *et al.* 1983; Arnold *et al.* 1984a, 1984b; Griffiths *et al.* 1986; Ester and Jeuring 1992; Doré and Meynard 1995; Sache and Zadoks 1996; Steene *et al.* 1999). Foliar applications have no direct effects on larvae, but timely application can decrease adult pea leaf weevil populations, egg production and eventual larval populations (Steene *et al.* 1999). Foliar sprays must be applied as soon as weevils are detected in order to control larval populations, as adult weevils mate and oviposit soon after arrival. If applied too late, egg production is not prevented and control efforts will not impact larval populations (King 1981; Bardner *et al.* 1983; Ester and Jeuring 1992).

Williams *et al.* (1998) investigated the effects of seed primers on germination rates and plant compensation to pea leaf weevil herbivory. Polyethylene glycol inhibited compensation and exacerbated the effects of herbivory so that overall plant development, including nodule development, decreased (Williams *et al.* 1998). Seed treatments to improve *P. sativum* and *V. faba* emergence and compensation for pea leaf weevil herbivory must be refined for optimal benefit in programs designed to limit the impact of pea leaf weevil.

Using insecticidal seed treatments to protect the roots of annual legumes and to provide systemic protection to the foliage has had some success. Steene *et al.* (1999) observed that seed treatments significantly decreased pea leaf weevil populations when infestation levels were low to moderate. Compounds such as bendiocarb and phorate had some impact on pea leaf weevil populations, but did not increase yields (Barder *et al.* 1983). Imidacloprid applied as a seed treatment reduced adult feeding activity by 40 and 60% in subsequent years, while furathiocarb and benfuracarb did not consistently reduce weevil populations (Steene *et al.* 1999). The benefits of seed treatments may be reduced as treated seeds tend to adhere to one another, which reduces the sowing rate and the subsequent density of plant stands (Bardner *et al.* 1983). Advances in seed treatment technology should enable producers to circumvent these difficulties.

Control of *S. lineatus* may be achieved via the application of seed treatments or seedbed treatments. For example, Ester and Jeuring (1992) found yields to be 10 to 20% greater when seeds were treated compared to the use of foliar sprays. Tested seed treatments include carbofuran, benfuracarb, fonofos, methiocarb and tefluthrin (Ester and Jeuring 1992). Similarly, Horak and Buryskova (1980) observed yield increases of 28% in plots with carbofuran, aldicarb or phorate as in-furrow treatments compared to control plots, and Taupin and Janson (1997) observed that furathiocarb as a seed treatment was more effective than foliar sprays.

Bardner *et al.* (1983) reported that phorate was effective for in-furrow treatment for reducing adult herbivory and larval numbers, but the effects degraded over several months, leaving subsequent crops prone to infestation. Both aldicarb and carbofuran as in-furrow treatments controlled pea leaf weevils equally well at high and moderate application rates (Bardner *et al.* 1983). In general, in-furrow treatments increased yields by 5 to 36% relative to untreated plots (Bardner *et al.* 1983). Dieldrin and lindane applied as seedbed treatments increased yields in 23 of 26 plots and reduced larval populations by 70 to 80% when applied at high rates (Bardner and Fletcher 1979). However, both dieldrin and lindane have extensive residual activity and pose significant threats to the environment (Bardner and Fletcher 1979; Bardner *et al.* 1983); therefore, these products have been de-registered in many countries worldwide, and have been completely banned in others (Voldner and Li 1995; Carvalho 2006).

Deregistration and restriction upon the usage of certain insecticidal compounds has led to the investigation of new products with novel modes of action. In the Czech Republic, for example, furathiocarb, a product used to coat legume seeds such as alfalfa for protection against *Sitona* weevils has recently been restricted (Rotrekl and Cejtchaml 2008). In response, the efficacy of products including Cruiser 350 FS (thiamethoxam) and Elado 480 FS (beta-cyfluthrin and clothianidin) have been investigated (Rotrekl and Cejtchaml 2008). In greenhouse trials, both products were applied at a rate of 10 L t^{-1} of seed and both were as efficacious as furathiocarb; at 20 L t^{-1} only 6.3% of treated plants had sustained damage three days after infestation when treated with beta-cyfluthrin plus clothianidin (Rotrekl and Cejtchaml 2008). In field trials, both products applied at 20 L t^{-1} of seed significantly reduced damage to alfalfa plants compared to the untreated control (Rotrekl and Cejtchaml 2008). Based on these results, Rotrekl and Cejtchaml (2008) recommend that these products replace furathiocarb.

Several factors can limit insecticide efficacy. Management of pea leaf weevil using insecticides requires accurate monitoring and timely spraying (King 1981; Bardner *et al.* 1983; Ester and Jeuring 1992). In the past, seed treatments and furrow treatments have been applied unnecessarily when weevil populations were low (Bardner and Fletcher 1979). Because *S. lineatus* invades crops over a period spanning several weeks in spring (Hamon *et al.* 1987), persistent insecticides have the best potential to control adult weevils (Hamon *et al.* 1987), but compounds with residual activity such as chlorpyrifos threaten non-target organisms (Hunter 2001) and the environment (Bardner and Fletcher 1979; Bardner *et al.* 1983). Correct application, using appropriate equipment in order to maximize coverage will enhance insecticide efficacy (Arnold *et al.* 1984a, 1984b) and help protect the environment from pesticide drift.

Ward and Morse (1995) reported that the negative effects of insecticides could be reduced by only applying insecticides to portions of the field. Ward and Morse (1995) sprayed areas of plots where pea leaf weevils were at high infestation levels and found that in untreated areas, weevil numbers were much lower than expected. Their results suggest that weevils redistribute from areas of high density to those where populations have been reduced by spraying. During redistribution, individual weevils may emigrate or come into contact with chemical residues and die. Ward and Morse (1995) suggest that by only spraying portions of the production area, weevil populations may be significantly reduced in both sprayed and unsprayed areas. Targeted spraying has the potential to decrease selection pressures for insecticide resistance and maintain natural enemy populations (Ward and Morse 1995).

A more sustainable *S. lineatus* management option involves breeding resistant legume varieties. Field pea genotypes vary in the amount of waxy bloom on their leaves (White and Eigenbrode 2000; Chang *et al.* 2004). Manipulation of the genes that control this characteristic may introduce an extrinsic resistance characteristic to the plant (Rutledge *et al.* 2003), given that more weevils occur on plants with reduced waxy bloom (White and Eigenbrode 2000; Chang *et al.* 2004). However, overall numbers of herbivores were greater on waxy plants because predator pressure was lower than on varieties with reduced waxy bloom (White and Eigenbrode 2000; Chang *et al.* 2004). Chang *et al.* (2004) postulated that *S. lineatus* will select peas with the reduced waxy bloom trait. Hence, there is some potential for the use of reduced waxy bloom plants as trap crops, as the pea leaf weevil is highly mobile and able to select its preferred hosts.

Breeding for *S. lineatus* resistance has been attempted numerous times. After examining all pea varieties in the breeding collection at the University of Idaho, one cultivar of Austrian winter pea was found with significant resistance to

S. lineatus relative to other cultivars. However, in a breeding experiment using the resistant 'Melrose' cultivar, no increase in resistance was observed in the progeny (Nouri-Ghanbalani *et al.* 1978). Nouri-Ghanbalani *et al.* (1978) observed that all progeny exhibited reduced resistance when compared to the parent cultivars. Wnuk and Wiech (1983) observed some differential preferences among pea cultivars in laboratory studies. The cultivars 'Alaska Ekspres' and 'Cukrowy Iłowiecki' were unacceptable hosts for consumption, while 'Cud Kelvedonu,' 'Rarytas,' and 'Nora' cultivars were acceptable hosts (Wnuk and Wiech 1983). At low or moderate *S. lineatus* population density, the cultivars 'Scotch Green,' 'Garfield,' and 'Latah' were damaged less than other cultivars (Auld *et al.* 1980). Tulisalo and Markkula (1970) noted that in field and greenhouse trials, 'English Sword' was the most susceptible cultivar, while 'Early Onward' was the most resistant. However, over the five years of their trial, no statistically significant differences were observed in the field (Tulisalo and Markkula 1970). Auld *et al.* (1980) suggested that due to additive gene action in existing pea populations, breeding programs will potentially yield a resistant cultivar. However, breeding attempts to date have yielded mixed results, and the observation that resistance is quickly lost in successive generations and crosses suggests that the potential for success of breeding programs is limited.

Sitona lineatus seems to exhibit varying levels of preference for field pea genotypes based on differences in biochemical content and phenotype. Havlickova (1980) noted that *S. lineatus* fed at different rates on different field pea cultivars. A comparison of three cultivars, 'Gorkovskij,' 'Lancet' and

‘Neuga,’ indicated that ‘Gorkovskij’ incurred the highest rates of injury when material of all three varieties was offered to the adults in a choice test. Biochemical analysis indicated that fructose, glucose, maltose and saccharose levels in the three cultivars varied (Havlickova 1980). High saccharose levels stimulated the most feeding, which may explain the preference of *S. lineatus* for the ‘Gorkovskij’ cultivar (Havlickova 1980). In addition, Havlickova (1980) found that the amino acid tyrosine inhibited herbivory, as did thick pea leaves (Landon *et al.* 1995). Understanding the impact of differences in nutritional levels of pea cultivars, as well as phenotypic differences such as leaf thickness, may aid in the selection of field pea cultivars for planting in regions prone to pea leaf weevil infestation.

Cultural methods to control *S. lineatus* have been investigated in most regions, with varying levels of success. McEwen *et al.* (1981) investigated the impact of nitrogen fertilization and irrigation on *S. lineatus* in a faba bean production system and found that nitrogen fertilization had no effect on overall yields, but did decrease the number of larvae, which is attributed to the production of fewer root nodules by fertilized plants, as observed by George (1962). Interestingly, bio-fertlizers composed of pig and poultry waste decreased the amount of *S. lineatus* feeding damage when compared to unfertilized control plots (Arkhipchenko *et al.* 2005). Larval populations were lower in irrigated plots, and the highest larval densities were observed in the driest year of the field trial, suggesting that larvae do not tolerate high levels of moisture well (McEwen *et al.* 1981). Irrigation also increased the yield and biomass of faba beans, including nodule biomass, but nitrogenase activity decreased (McEwen *et al.* 1981). The relationship between larval success and irrigation requires further investigation, as moist, humid conditions that result from irrigation should promote higher egg hatch rates (Jackson 1920; Lerin 2004), which would in turn result in higher larval population densities.

The impact of conventional and conservation tillage on *S. lineatus* colonization and success has recently been investigated in the United States. Using aerial traps, Hanavan *et al.* (2008) compared the densities of weevils arriving at tilled and un-tilled plots. Significantly more weevils were caught in the traps in conventionally tilled plots during the spring migration period, likely because cooler soil temperatures and surface residues delay pea emergence in no-till systems (Hanavan *et al.* 2008). Colonization of no-till plots occurred later and less feeding damage was incurred by peas in no-till plots (Hanavan *et al.* 2008).

Planting crops in no-till or conventional-till production systems is similar to sowing earlier or later in the growing season, which can uncouple the phenological relationship between pests and their hosts, and can be used to avoid periods of high pest pressure (Teetes 1981). This strategy has had some success in controlling the Hessian fly, *Mayetiola destructor* Say (Diptera: Cecidomyiidae), in wheat in the northern U.S.A (Wratten 1981), and flea beetles, *Phyllotreta* spp. (Coleoptera: Chrysomelidae), in canola in western Canada (Dosdall *et al.* 1999). Several researchers have found that annual legume crops sown early in the season undergo more damage than crops planted later (Doré and Meynard 1995). The benefits of this control method will depend on the length of the growing season

(Teetes 1981). For example, Doré and Meynard (1995) suggest that in France, late sowing is a viable option, but acknowledge that in other regions it is not. Williams *et al.* (1998) determined that plants were most susceptible to attack by adult weevils immediately after seedling emergence and that plants that emerged early in the season must become established quickly to prevent significant yield losses. If field pea and pea leaf weevil emergence coincide, seed treatments that promote rapid stand establishment may reduce yield losses under high pea leaf weevil pressure (Williams *et al.* 1998).

Intercropping legumes with cereal crops is a common practice in tropical regions and in small-scale operations (Singh and van Emden 1979; Vandermeer 1989), and may be a viable cultural method for the management of *S. lineatus*. Baliddawa (1984) examined the effects of a series of different *V. faba* and oat, *Avena sativa* L. (Poales: Poaceae), intercrop systems on pea leaf weevil populations. Results indicated that all mixtures of the two crops significantly reduced weevil numbers when compared to monocultures. Baliddawa (1984) suggested that oat in the intercrop system interfered with the ability of the weevil to find suitable hosts by 1) providing a physical obstruction or barrier between the weevils and their hosts, 2) adding plant volatiles to the immediate atmosphere that confused the weevils, and 3) altering the visual appearance of the crop system which prevented the identification of host plants. Pea leaf weevils were also observed to emigrate from dicultures or polycultures (Baliddawa 1984). In addition to controlling *S. lineatus*, intercropping may have other benefits, such as helping to control other legume pathogens and pests, such as crenate broomrape, *Orobanche crenata* Forsk. (Scrophulariales: Orobanchaceae) (Fernandez-Aparicio *et al.* 2007). Moreover, intercrops have been found to increase natural enemy populations by as much as 32% (Wiech and Wnuk 1991).

Intercrops have significant potential for reducing the impact of *S. lineatus* and other pests of legumes. However, the effect of intercropping on the other component(s) of the intercrop, and on yield may not always be positive. Due to the nitrogen-fixing capability of legumes, it is expected that legumes would provide nitrogen to the other component; however, this was not the case in a pea-barley (*Hordeum vulgare* L.) intercrop investigated by Jensen (1996). Rather, Jensen (1996) observed that the two crop components utilized soil and atmospheric nitrogen in a complementary fashion – meaning that the barley utilized the nitrogen present in the soil, while the peas fulfilled their nitrogen requirement via fixation. The yield of the pea-barley intercrop was less variable and greater than the yield of monocultured peas, but less than monocultured barley (Jensen 1996). The potential of intercropping for the control of *S. lineatus* is also limited by the willingness of growers to use intercrop systems and the technology available to harvest these crops.

Crop rotation may be of limited efficacy for the management of the pea leaf weevil, due to the migratory habits of the weevil and its flight potential. Distance between sites is important due to the migratory and dispersal habits of *S. lineatus*. For example, Kokorin (1964) observed that if field peas or faba beans were grown after clover (in the same field) or in sites adjacent to clover, severe feeding damage occurred. Similar rates of damage were also observed when field peas or faba beans were grown near forests (Kokorin 1964), or adjacent to alfalfa (Krasnopol'skaya 1966). The least degree of damage was observed in plots located the greatest possible distance from clover swards (Kokorin 1964). A significant distance will be required for crop rotation to be effective, especially in regions of large-scale production where monocultures cover large areas and support high weevil population densities.

Biological control, or the regulation of pest populations by natural enemies, can be very effective in some agroecosystems (Van Driesche and Bellow 1996). Natural enemies of *S. lineatus* include birds (Jackson 1920), Hymenoptera (Hans 1959) and carabid beetles (Hamon *et al.* 1990). Several parasitoid species can impact pea leaf weevil populations in Europe and northern Africa, including *Allurus muricatus* (Haliday), *Microctonus aethiopoides* (Loan), *Perilitus rutilus* (Nees) and *Pygostolus falcatus* (Nees) (Hymenoptera: Braconidae) (Aeschlimann 1980). The egg parasitoid *Patasson lameerei* Debauche (Hymenoptera: Mymaridae) is found throughout southern Europe (Aeschlimann 1980), and targets non-melanized eggs that are exposed on the soil surface (Schotzko and O'Keeffe 1986b). *Sitona lineatus* eggs and adults are the principal targets of hymenopteran parasitoids, as larvae live sheltered within root nodules (Aeschlimann 1980; Hamon *et al.* 1990).

Hamon *et al.* (1990) investigated the impact of carabid beetles (Coleoptera: Carabidae) on pea leaf weevil populations. Their results suggest that adults emerging from pupae are more susceptible to predation (2.6 to 23.8% mortality) than larvae (0.6 to 10.5% mortality) (Aeschlimann 1980; Hamon *et al.*

1990). Carabid beetles are more active near the soil surface, thus larvae may avoid predation due to their below-ground habitat (Hunter 2001). Periods of peak carabid beetle activity correspond with the peak time of new-adult pea leaf weevil emergence in late July to mid-August (Hamon *et al.* 1990). Tests using starved carabid beetles in the laboratory determined that a single beetle could consume 20 or more adult weevils per day, although in the field, only one or two weevils are expected to be consumed daily (Hamon *et al.* 1990). Overall, Hamon *et al.* (1990) predicted that 30% or more of the pre-overwintering pea leaf weevil population could be reduced by carabid beetle predation.

In England, several ground beetle species occurred at high densities in fields infested by *S. lineatus*, including *Pterostichus madidus* Fabricius, *Pt. melanarius* Illiger, *Harpalus rufipes* DeGeer and *Agonum dorsale* Pontoppidian (Coleoptera: Carabidae) (Hamon *et al.* 1990). In Poland, two carabid species were observed to coincide with *S. lineatus* (Ropek and Jaworska 1994). *Pterostichus cupreus* L. (Coleoptera: Carabidae) was active during the pea leaf weevil oviposition period, while *Bembidion properans* Stephens (Coleoptera: Carabidae) was more active in June and preyed upon both eggs and emerging *S. lineatus* larvae (Ropek and Jaworska 1994).

Several fungal species appear to have some potential for controlling populations of *S. lineatus* including *Beauveria bassiana* Vuilleman (Hypocreales: Clavicipitaceae), *Metarhizium flavoviride* Metschn. (Hypomycetes: Moniliales), *Metarhizium anisopliae* Metschn. (Hypomycetes: Moniliales), *Paecilomyces farinosus* Holmsk. (Eurotiales: Trichocomaceae) and *Paecilomyces fumosoroseus*

Wize (Eurotiales: Trichocomaceae) (Hans 1959; Müller-Kögler and Stein 1970; Poprawski *et al.* 1985). A strain of *B. bassiana* isolated in southern France is effective in the control of *Sitona discoideus* Gyllenhal (Aeschlimann *et al.* 1985). The efficacy of entomopathogenic fungi will vary with the virulence of the fungal strain, the concentration of fungi in the soil and the life stage of the weevil at the time of attack (Poprawski *et al.* 1985; Verkleij *et al.* 1992). Poprawski *et al.* (1985), for example, investigated five fungal species for their effects on pea leaf weevil eggs, larvae and pupae. Their results suggested that *S. lineatus* eggs were resistant to all except *M. flavoviride* which produced 32% mortality when applied at high concentrations. Newly eclosed *S. lineatus* larvae exposed to fungi were susceptible to all five fungi tested (*M. anisopliae, M. flavoviride*, *B. bassiana*, *P. farinosus* and *P. fumosoroseus*) (Poprawski *et al.* 1985). In an investigation of the efficacy of *B. bassiana* in central Europe, Müller-Kögler and Stein (1970) found that when soil was inoculated with 10^7 conidia cm^{-3} the number of emerging adults was 48% less than the number of weevils emerging from the control. The longevity of *S. lineatus* adults infected with *B. bassiana* decreased as the severity of the infection increased, and approximately 50% of infected adults died within 10 days of infection (Müller-Kögler and Stein 1970). These results suggest that if fungi are present at the time of hatching, pea leaf weevil larval populations may be significantly reduced.

Results of field studies by Verkleij *et al.* (1992) using *M. anisopliae* illustrate that the efficacy of fungal applications may be limited. Using indigenous *M. anisopliae* conidia, Verkleij *et al.* (1992) applied known concentrations of conidial suspensions to soil. The fungal application did not significantly reduce nodule damage or pea leaf weevil populations. The fungal application was expected to have a significant impact, as pea leaf weevils infected with *M. anisopliae* were found occurring naturally in nearby fields (Verkleij *et al.* 1992). The poor results of the field application were attributed to two factors. First, it was noted that the concentration of fungal conidia in the soil declined over time, rather than becoming established as expected. Second, it was possible that the fungal strain applied was not virulent (Verkleij *et al.* 1992). In addition to these factors listed by Verkleij *et al.* (1992), Müller-Kögler and Stein (1970) suggested that at low conidial concentrations, weevils must be exposed to the fungi for a longer period of time to become fatally infected. The degree of target organism specificity of entomopathogenic fungi requires further investigation, as *B. bassiana* may infect non-target beneficial arthropods such as Carabidae (Riedel and Steenberg 1998).

Trials using entomopathogenic nematodes have been successful in Europe. When *S. lineatus* larvae were exposed to *Steinernema carpocapsae* Weiser (Rhabditida: Steinernematidae), *Steinernema feltiae* Filipjev (Rhabditida: Steinernematidae) or *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) for six days, 100% of weevils died (Jaworska 1998). When adult weevils were exposed to *H. bacteriophora* at a rate of 300 nematodes per weevil, mortality reached 50% after six days and 100% after 14 days (Wiech and Jaworska 1990). *Heterorhabditis bacteriophora* infective juveniles penetrate the cuticle of their hosts (Bedding and Molyneux 1982), which explains the success of this species in infecting adult weevil hosts. Jaworska and Ropek (1994) noted that pea leaf weevils fed *V. faba* were less susceptible to infection than weevils fed *P. sativum*. Mortality of third-instar larvae fed *P. sativum* reached 100% in four days (Jaworksa and Ropek 1994). Nematode reproduction was greater in weevil hosts reared on faba bean compared to weevil hosts reared on field pea (Jaworska 1998).

Several factors must be considered before embarking upon a nematode-based biological control program for the pea leaf weevil. First, introducing nematodes or augmenting existing populations is costly and the overall impact of the nematodes is unlikely to compare favorably to the level of control provided by more cost-effective insecticides (Georgis *et al.* 2006). However, Nielsen and Philipsen (2004) suggested that upon introduction, nematode populations should persist if large numbers of potential hosts are present. Second, nematode populations increased between spring and autumn (Nielsen and Philipsen 2004), suggesting that winter conditions limit nematode populations. In North America, nematodes are currently used to control a number of soil-dwelling pests, but are limited to use in more temperate regions (Hominick 2002). The use of nematodes to control pea leaf weevil in northern regions where *P. sativum* and *V. faba* are grown is limited by abiotic conditions including relative humidity (Glazer 1992), moisture levels (Georgis and Gaugler 1991; Bilgrami and Gaugler 2007) and soil type (Georgis and Gaugler 1991).

Methods of increasing nematode efficacy have been explored in an attempt to equalize the impacts of nematodes and insecticides. The efficacy and pathogenicity of *S. carpocapsae* and *H. bacteriophora* increased after exposure to magnesium and manganese ions prior to release (Jaworska *et al.* 1999). The use of ions to stimulate nematode activity may be limited by effects on non-target organisms when applied to entire cropping systems (Jaworska *et al.* 1999).

The direct impact of nematodes on non-target organisms must also be considered. Ropek and Jaworska (1994) found that *S. carpocapsae* and *H. bacteriophora* had no significant impact on the longevity of carabid beetles. Carabid beetles should escape nematode attack because nematodes and carabids utilize different habitats, although there is also speculation that carabid beetles may be resistant to nematodes (Ropek and Jaworska 1994). The impact of nematodes on other beneficial insects in field pea and faba bean production areas warrants further investigation.

Pea leaf weevil aggregation pheromone has been investigated for its use in a stimulo-deterrent diversionary strategy (SDDS) (Pyke *et al.* 1987; Miller and Cowles 1990; Cook *et al.* 2007), designed to reduce pea leaf weevil populations in harvestable crops (Smart *et al.* 1994, Agelopoulos *et al.* 1999). SDDS is described as a push-pull system, and consists of several control measures with limited efficacy combined into one control program with increased efficacy (Miller and Cowles 1990; Agelopoulos *et al.* 1999, Cook *et al.* 2007). Smart *et al.* (1994) described several possible components of a push-pull control system for *S. lineatus*, including: 1) the male-produced aggregation pheromone, to ‘pull’ weevils away from a field pea or faba bean crop, 2) an antifeedant, such as neem oil produced by the neem tree, *Azadirachta indica*, A. Juss. (Sapindales:

Meliaceae), to ‘push’ weevils away from the crop, 3) a trap crop to which the weevils are attracted by the pheromone, and pushed to by the antifeedant and, 4) an insecticide applied to the trap crop to reduce weevil populations. Neem oil can effectively deter feeding by crop pests, and when applied to *P. sativum* and *V. faba* plants neem oil significantly reduced weevil damage and larval numbers relative to control plants (Smart *et al.* 1994). However, the use of neem oil is limited by its short residual efficacy and the need for frequent re-application (Smart *et al.* 1994). Crop cultivars may also be used to repel pea leaf weevils from *P. sativum* and *V. faba* crops (Agelopoulos *et al.* 1999). Applying insecticides such as deltamethrin to trap crops, in conjunction with the use of attractants to pull pests away from crops significantly reduced both feeding damage and larval populations (Smart *et al.* 1994). Using chemical control measures in conjunction with attractants requires that the chemical used not be a repellent (Smart *et al.* 1994).

Attempts have been made to control pea leaf weevil by genetically altering *R. leguminosarum* through the insertion of genes from *Bacillus thuringiensis* subsp. *tenebrionis* Berliner (Bacillales: Bacillaceae) that express endotoxins (Bezdicek *et al.* 1994; Skøt *et al.* 1994; Quinn and Bezdicek 1996). Expression of the *cryIII* gene for protein production in *R. leguminosarum* caused pea leaf weevil mortality, a decrease in the rate of pea leaf weevil development and a reduction in the rate of denodulation (Bezdicek *et al.* 1994; Quinn and Bezdicek 1996). Similar results were found for *S. hispidulus* larvae feeding on genetically altered *M. sativa* root nodules (Bezdicek *et al.* 1994; Quinn and Bezdicek 1996).

The competitiveness of *Rhizobium* sp. containing *cryIII* genes with unaltered *Rhizobium* sp., as well as the overall effect of altered nodules on plant biomass, may limit the use of *cryIII*-gene insertion in programs to control pea leaf weevils. Bezdicek *et al.* (1994) found that when altered *Rhizobium* bacteria were introduced to the soil, these occupied 40 to 97% of root nodules, suggesting that altered strains are competitive. The effects of altered *Rhizobium* bacteria on overall plant biomass have been harder to assess. Bezdicek *et al.* (1994) found that while denodulation by *S. lineatus* decreased significantly when altered bacteria were present in root nodules, the overall biomass of plants was significantly increased when plants were inoculated with the wild-type strain. Therefore, widespread use of this management strategy requires further investigation to better understand the nature of the symbiotic relationship between altered *R. leguminosarum* and the host plant. If larval damage to root nodules can be prevented using altered *R. leguminosarum,* lost biomass may not represent a significant loss compared to the potential losses by the weevil.

1.9. Research Needs for Pea Leaf Weevil Management

Improved integrated pest management (IPM) systems for the pea leaf weevil are necessary to maintain the economic and environmental viability of *P. sativum* and *V. faba* production. By definition (Dent 2000), this will require the incorporation of cultural practices and biological controls with chemical control strategies but to date, research in this direction is lacking. Two significant research gaps in need of immediate attention are: 1) development of simple and sufficiently accurate monitoring systems both for timing weevil arrival and estimating adult weevil densities, and 2) determination of reliable economic threshold estimates. In the absence of these, IPM programs, especially those with a significant chemical component, are almost certain to be inefficient.

Accurate population monitoring is a cornerstone principle of IPM (Dent 2000), and considerable research remains to be completed to improve monitoring of *S. lineatus* populations. A promising monitoring method involves use of the male-produced aggregation pheromone, 4-methyl-3, 5-heptanedione (Blight *et al.* 1984; Blight and Wadhams 1987; Nielsen and Jensen 1993). However, there is a need to correlate trap catches with damage levels (Nielsen and Jensen 1993). It would be beneficial to develop a pea leaf weevil forecasting strategy that utilizes 4-methyl-3, 5-heptanedione to estimate the size of the newly emerged population in late summer and autumn by determining if new generation adults are sensitive to aggregation pheromones in the fall. Such information may be instrumental for advising growers of risks associated with *S. lineatus* attack in the next year, and so enable them to alter crop selections depending on the *S. lineatus* risk assessment.

In addition to conducting research to improve pheromone monitoring systems, other monitoring approaches should also be assessed. Sweep netting is inappropriate early in the season when weevils are invading because field pea or faba bean plant stands would be too small to sample with this method. Another inherent difficulty involved with sweep sampling is the habit of *S. lineatus* to play dead and fall from the foliage when disturbed (Jackson 1920). The use of pitfall traps, located on field margins and within fields, may be an efficient method by which to detect the arrival of weevils in the spring, but would require that producers are able to easily identify *S. lineatus* in the traps. Extension programs for producer education regarding the identification and habits of the pea leaf weevil would be necessary if pitfall trapping is to be used as a monitoring tool. A pitfall trap monitoring approach could yield valuable information for producers, but would be somewhat impractical because of the considerable effort required to maintain traps and process the samples.

Degree-day models can be used to predict the occurrence and duration of emergence and flight peaks of *S. lineatus*, and so improve the timing of chemical, biological, and cultural control procedures as well as our understanding of egg and larval mortality. However, no such models currently exist for this species. Information on the degree-day requirements of *S. lineatus* can allow researchers and producers to explore and exploit relationships that might exist where cultural practices such as planting time could influence those pest populations.

Viable economic thresholds for the pea leaf weevil must be developed in a manner that includes the effects of plant health, plant density and above- and below-ground damage. Density-damage relationships that have been established to date have been criticized for ignoring such factors (El-Dessouki 1971; Cantot 1986; Nielsen 1990; Landon *et al.* 1995). Solving the complex relationship that exists between *S. lineatus* adult numbers and crop damage by larvae will be difficult, but nevertheless it is required to ensure that *P. sativum* and *V. faba* production remains profitable and environmentally sustainable.

Although developing crop plant resistance to *S. lineatus* has not yet proven fruitful, more research effort needs to be expended in this direction. This should begin with identification of such behavior-modifying chemicals as attractants, arrestants, deterrents, stimulants and repellents. Leaf wax contents have been investigated for conferring host plant resistance to the pea leaf weevil (White and Eigenbrode 2000; Chang et al. 2004), but other morphological characters like trichomes should also be investigated. Greater understanding of how the pea leaf weevil responds to such attributes can provide plant breeders with more specific targets in their breeding programs for resistance.

Several approaches to *S. lineatus* control have been explored worldwide, but in the future, these strategies, which are not mutually exclusive, must be employed in tandem. Advances in chemical control strategies, including the application of neonicotinoid compounds as seed-treatments, should be considered for incorporation in IPM programs because they use a relatively small amount of active material (Taylor and Harman 1990) and are more target organism-specific than the use of foliar sprays. Similarly, the development and implementation of resistant crop cultivars will reduce the need for insecticide application, and resistant cultivars would also support populations of natural enemies. In contrast to monocultures, intercrops will increase the diversity of production systems and promote the activity of natural enemies (Altieri and Letourneau 1982). To date, several potential natural enemies of *Sitona* species have been identified from the Mediterranean region, including *M. aethiopoides* (Aeschlimann 1980; Phillips *et al.* 2000), which has been introduced to Australia for biological control of *S.*

humeralis (Aeschlimann 1980). The introduction of classical biological control agents into North America will require significant investigation, but if successful, will add another crucial layer to an IPM program. If efficacious biological control agents can be identified, the benefits will outweigh the costs involved with implementing biological control. To date, one of the most promising integrated control strategies described in the literature is the push-pull system that has been outlined by Smart *et al.* (1994), combining the use of natural antifeedants with trap crops, pheromone traps and foliar insecticides, and more such innovative approaches need to be investigated.

1.10. Conclusions

Sitona lineatus is a successful field pea and faba bean pest that is expected to continue to expand its range, especially in North America where field peas and faba beans are produced on a large scale, for both yield and soil nitrogen augmentation. To date, few research initiatives have focused on the development of IPM programs for this important pest. Rather, biological, cultural and chemical control methods have been assessed and implemented independently. This single-strategy approach has not proven to be particularly effective, as *S. lineatus* continues to cause substantial damage to field pea and faba bean crops worldwide. However, the integrated use of intercropping, host plant resistance, predators, parasitoids, pathogens, and chemical insecticides has potential for the improved management of pea leaf weevil. In view of the shortcomings of existing control strategies, a renewed research initiative for the management of *S. lineatus* is required especially because many research avenues remain unexplored and the effectiveness of various management strategies will vary across the extremely broad geographical range of this species. In regions where *S. lineatus* has only become established recently, research projects must be undertaken to understand its biology, as well as to explore potential management options and develop economic thresholds. Such a project was initiated in the Canadian prairies in 2007 and the research projects described below were included in this research initiative.

Table 1.1. The range of leaf notches on the first pair of leaves and resultant larval populations used to rank the degree of *Sitona lineatus* feeding damage by Cantot (1986).

Leaf Notch Score	Number of Notches	Number of Larvae
0	0	0 to 1
1	1 to 5	2 to 6
2	6 to 15	7 to 11
3	15 and above	12 and above

Table 1.2. The chemical class, trade names, application method and application rate of selected insecticide products that have been investigated for use against *Sitona lineatus* L. (Coleoptera: Curculionidae).

Active Ingredient	Chemical Class	Application Format	Application Rate	Plant Stage/Month when Applied	Reference(s)
Phorate	Organophosphate	Foliar Spray	2.24 kg ha^{-1};	Late May	Horak and Buryskova 1980; King 1981; Bardner *et al.* 1983
Aldicarb	Carbamate	Seedbed	10 kg ha^{-1}	Sowing	McEwan *et al.* 1981; Bardner *et al.* 1983
Carbofuran	Carbamate	In Furrow	2.24 kg ha^{-1}	Sowing	Bardner *et al.* 1983; Griffiths *et al.* 1986
Carbosulfan	Carbamate	Granule (Foliar)	2.24 kg ha^{-1}	1 April to 31 May	Bardner *et al.* 1983; Griffiths *et al.* 1986
		Seed Treatment	0.05% Seed Wt.	Sowing	Bardner *et al.* 1983
		In Furrow	2.24 kg ha^{-1}	Sowing	Bardner *et al.* 1983
Benfuracarb	Carbamate	Seed Treatment	2.0 g kg^{-1} seed	Sowing	Ester and Jeuring 1992; Steene *et al.* 1999
Bendiocarb	Carbamate	Seed Treatment	0.2% Seed Wt.	Sowing	Bardner *et al.* 1983
Furathiocarb	Carbamate	Seed Treatment	2.0 g kg^{-1} seed	Sowing	Ester and Jeuring 1992; Steene *et al.* 1999; Rotrekl and Cejtchaml 2008
Cyhalothrin-Lambda	Pyrethroid	Foliar Spray	6.25 g ha^{-1}	N/A	Steene *et al.* 1999
Permethrin	Pyrethroid	Foliar Spray	0.15 kg ha^{-1}	Early May	McEwan *et al.* 1981; Bardner *et al.* 1983; Griffiths *et al.* 1986
Beta-cyfluthrin	Pyrethroid	Seed Treatment	20 L t^{-1}	Sowing	Rotrekl and Cejtchaml 2008
Imidacloprid	Neonicotinoid	Foliar Spray	100 g ha^{-1}	N/A	Steene *et al.* 1999
		Seed Treatment	1.75 g kg^{-1} seed	Sowing	Steene *et al.* 1999
Thiamethoxan	Neonicotinoid	Seed Treatment	20 L t^{-1}	Sowing	Rotrekl and Cejtchaml 2008
Clothianidin	Neonicotinoid	Seed Treatment	20 L t^{-1}	Sowing	Rotrekl and Cejtchaml 2008

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2. Chapter Two: Integrated management of *Sitona lineatus* L. (Coleoptera: Curculionidae) with nitrogen fertilizer, *Rhizobium* inoculation and thiamethoxam insecticide.

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2.1. Introduction

The production of field pea, *Pisum sativum* L. (Fabales: Fabaceae), constitutes a significant portion of agricultural grain production, with approximately 25 million ha sown annually worldwide (Schatz and Endres 2003), and approximately 1.5 and 0.34 million ha grown annually in Canada and the U.S.A., respectively (Statistics Canada, 2010; National Agricultural Statistics Service 2010). Field pea seed is high in protein and ideal for consumption by humans and livestock (Gibson 1974; Verkleij *et al.* 1992; Corre-Hellou and Crozat 2005). Field pea is favored in crop rotations and intercropping because plants contribute nitrogen (N) to the soil through bacterial fixation (Gibson 1974; Jensen 1996; McKenzie and Dunn 2008). Nitrogen from peas enters the soil via root exudates, sloughed root cells entering the rhizosphere (Jensen 1996), and decomposition when plant residues remain in the field after harvest (Liebman and Davis 2000; Geijersstam and Mårtensson 2006). The high cost of N fertilizers (Walley *et al.* 2007; Kumbhar *et al.* 2008) has contributed to an increase in the production of pulse crops worldwide; however, increased production has been accompanied by concurrent outbreaks of insect pests.

Sitona lineatus L. (Coleoptera: Curculionidae), the pea leaf weevil, is a serious pest of field peas and faba beans (*Vicia faba* L., Fabales: Fabaceae) throughout Europe, Asia, Africa and North America, and it has become established in Alberta and Saskatchewan, Canada in the past ten years (Vankosky *et al.* 2009). Over the course of the growing season, there are three key periods of *S. lineatus* feeding activity that have the potential to significantly decrease both yield and N fixation by field pea. First, severe defoliation can occur immediately after plant emergence, potentially killing seedlings. Second, prolonged and continual defoliation from adult feeding can occur throughout the growing season. Finally, root nodule consumption and destruction can occur during larval development. Early-season defoliation by adult weevils arriving from overwintering sites can cause losses of up to 50% of photosynthetic tissues (Williams *et al.* 1995) and when infestations are particularly severe, growing shoots may be completely consumed (Jackson 1920). Occasionally, complete crop losses due to foliar consumption have been recorded (Jackson 1920). When *S. lineatus* adults invade crops after seedling establishment, foliar consumption is usually less destructive than earlier invasions; however, adults of *S. lineatus* are long lived and may continue feeding until late summer (Hoebeke and Wheeler 1985). Feeding by the overwintered and new generations may overlap as new generation adults begin emerging in mid-summer (Hoebeke and Wheeler 1985). Such prolonged feeding activity can cause yield losses in the range of 30% (Nielsen 1990). All larval instars feed upon *Rhizobium leguminosarum* biovar *viciae* Frank (Rhizobiales: Rhizobiaceae) (Jackson 1920; Johnson and O'Keeffe

1981; Hoebeke and Wheeler 1985), the symbiotic nitrogen-fixing bacterium that form root nodules on field pea roots, by consuming root nodule tissues. Cantot (1986) observed that 12 larvae destroyed 90% of root nodules on a single plant, and when El-Dessouki (1971) infested individual field pea plants with 100 *S. lineatus* eggs, 98% of root nodules were destroyed and pod production by infested plants was reduced by 27%.

Managing *S. lineatus* has been a challenge in all regions where it reaches pest densities. This study was undertaken to identify options for *S. lineatus* management that are compatible with current field pea production strategies, and that can be utilized in an integrated pest management program. My objective was to investigate the effects of nitrogen fertilization, seed treatment with a systemic insecticide, and seed inoculation with *R. leguminosarum*, alone and in combination, to determine their effects on *S. lineatus* foliar feeding, root nodule feeding and plant yield under field conditions.

2.2. Materials and Methods

2.2.1. Sites and Treatments.

Plots of *P. sativum* were planted at Lethbridge (49°41’39”N, 112°49’58”W) and Vauxhall (50°04’08”N, 112°05’51”W), Alberta, Canada in 2007, 2008 and 2009. At each site, a randomized complete block experimental design was used with four blocks and eight treatments per block. At Lethbridge, each plot was 3.2 by 7.0 m; at Vauxhall, plots were 3.2 by 7.0 m in 2007 and 3.2 by 10.0 m in 2008 and 2009. The treatments were: 1) untreated control, 2) N fertilizer, applied as urea at a rate of 60 kg ha^{-1}, 3) the inoculant, N-Prove®, containing *Rhizobium leguminosarum* biovar *viciae* cells applied at the recommended rate of 1.6 g per kg of seed, 4) seed treatment with the neonicotinoid insecticide thiamethoxam (Cruiser®), applied at 30 g a.i. ha^{-1}, 5) inoculant plus N fertilizer as described above, 6) N fertilizer plus thiamethoxam, 7) inoculant plus thiamethoxam, and 8) inoculant plus N fertilizer plus thiamethoxam.

Prior to planting, seeds were treated with Apron Maxx® (3.75 g mefenoxam and 2.5 g fludioxonil per 100 kg seed), a broad spectrum fungicide to prevent seedling disease. Plots were also fertilized with phosphorous (P_2O_5 at 13 kg P ha^{-1}). At Vauxhall, the pre-emergent herbicide, Edge™ (ethalfluralin, 1.10 kg a.i. ha^{-1}), was applied prior to seeding and at Lethbridge, Odyssey® (imazamox 14.7 g a.i. ha^{-1} and imazethapyr 14.7 g a.i. ha^{-1}) was applied in-crop for weed control. Seeding dates for both sites in all years are given in Table 2.1. In 2007 and 2008, all plots were sown with ‘Cutlass’ semi-leafless field pea, which is resistant to powdery mildew and adapted to this ecoregion (Blade *et al.* 2004). In 2009, ‘Cutlass’ seed was not available and plots were sown with ‘CDC Bronco,’ a semi-leafless field pea variety bred to reduce lodging and provide powdery mildew resistance (Warkentin *et al.* 2005). Target field pea density was 107.6 plants m^{-2} (26.1 g seed m^{-2}) and row spacing was approximately 18 cm. Monthly temperature and precipitation data are summarized in Table 2.2 for each site in all three study years.

2.2.2. Plant Damage Assessments.

Plants were assessed for adult *S. lineatus* feeding damage on three dates in late May and early June when plants were at approximately the three-, five-, and eight-node growth stages (Table 2.1). Ten plants were assessed from each of two 1 m^2 quadrats in each plot on each date. Quadrats were randomly placed within each plot, with one quadrat in each half of the plot to encompass in-plot variability. Assessments at the three- and five-node stages were performed in the field, but the eight-node assessment was performed by harvesting 20 plants from each plot and assessing the damage on each plant in the laboratory. On each assessment date, the growth stage of each plant was determined using the Biologische Bundesanstalt Bundessortenamt and Chemical Industry (BBCH) Phenological Growth Stage key, which ranks growth stages from 00 (dry seed) to 99 (harvested product) (Weber and Bleiholder 1990; Feller *et al.* 1995). For each plant, the number of ‘U’-shaped feeding notches formed along the leaf margins on each node and on the terminal leaf (most recently emerged leaf) were recorded; these notches are characteristic of adult *S. lineatus* feeding (Jackson 1920; Nielsen 1990).

Mean values per plant were recorded for all plots for both the total damage per plant and terminal leaf damage per plant. The proportion of seedlings with damage on the terminal leaves was calculated for all plots and compared to the economic threshold of 30% of plants with terminal leaf feeding damage as determined by El-Lafi (1977) to evaluate the efficacy of each plot treatment.

2.2.3. Root Nodule Assessments.

Plants were harvested for root nodule assessment at the flowering stage (BBCH growth stages 60 to 67), which coincides with the timing of maximum root nodule activity (Depret and Laguerre 2008). Also, all larvae that will reach the adult stage should be present when plants are flowering (Doré and Meynard 1995). Sampling dates are given in Table 2.1. Three samples of three plants each were randomly selected from all plots treated with inoculant, inoculant plus N fertilizer, and inoculant plus thiamethoxam and were harvested with the surrounding soil. Roots and soil were enclosed in plastic bags secured at the base of the plant stems. Harvested plants were stored at approximately 4°C until assessment in the laboratory, to prevent plant and nodule senescence.

All plant samples were gently washed to remove soil from the roots. Two of the three samples were assessed for root nodule damage (Samples A and B). These samples were washed, and all larvae, pupae, and root fragments were collected from the soil. Larvae and pupae were counted and preserved in 70% (v/v) ethanol. Above-ground plant material was assessed for foliar damage as described in the previous section. Every nodule on each plant was examined with a dissecting microscope. The total number of nodules per plant was recorded. For each nodule, the type of nodule (single-lobed or tumescent with multiple lobes), the presence or absence of larval damage, and the presence or absence of larvae and of leghaemoglobin expression were recorded. Leghaemoglobin expression was used to determine if nodules were actively fixing nitrogen and was indicated by pink-colored nodules (Allen and Allen 1981). If nodules were classified as tumescent, the total number of lobes was recorded. Larvae in root nodules were carefully removed using a scalpel, their size was recorded and they were preserved in 70% ethanol. Mean totals per plant were reported for each plot. The third sample of three plants (collected in 2008 and 2009 only) was dried and weighed using an OHAUS TS4KD balance. Foliar biomass, root biomass, and total biomass per plant were recorded. Root biomass was not recorded for Sample A and B plants due to the destructive nature of the root nodule assessment.

2.2.4. Yield.

Plots were harvested in August (Table 2.1) using a small-plot combine, with one exception. At Lethbridge, 10.5 m^2 were harvested per plot; 15.0 m^2 were harvested at Vauxhall in 2008 and 2009. In 2007 at Vauxhall, weed density was extremely high, prohibiting the use of the small-plot combine. Therefore, four 0.5 m^2 quadrats were harvested by hand in each plot. These plants were threshed and the seeds were collected by hand. For each plot, percent moisture, percent protein, and yield (kg $plot^{-1}$) were recorded. Yield was corrected for 14% moisture content and converted to tonnes per hectare (t ha^{-1}), as reported in Blade *et al.* (2004) and Warkentin *et al.* (2005).

2.2.5. Statistical Analysis.

Mean values for above- and below-ground parameters were analyzed using the mixed-model analysis of variance for randomized complete block experimental designs (SAS Institute 2004) to determine the effect of site, treatment and site by treatment interactions. All parameters were analyzed on a yearly basis using data combined from the two sites, Lethbridge and Vauxhall. In the model, treatment was considered a fixed effect, and replicate (block) and site were considered random. For all analyses $\alpha = 0.05$. Where significant effects were observed, the data were further analyzed using the LSMEANS procedure with the PDIFF function in SAS, to compare results from each treatment with all other treatments and error was controlled using the Bonferroni adjustment of alpha (SAS Institute 2004). In the literature, the effects of *Rhizobium* inoculation on pest response to insecticides have not been documented (see: McEwen *et al.* 1981; Bardner *et al.* 1983; Nielsen 1990; Steene *et al.* 1999). There are few references regarding the efficacy of thiamethoxam against *S. lineatus* (one example is Rotrekl and Cejtchaml 2008) and although the effects of N fertilization can be predicted the effect of N fertilizer and *Rhizobium* inoculation or N fertilizer and thiamethoxam on *S. lineatus* is uncertain. Therefore, no *a priori* contrasts were included in the experimental analysis to allow for a full exploration of the treatments when applied alone or in combination. Where significant site by treatment interactions were observed, the nature of the interaction was further investigated using the SLICE function (SAS Institute 2004) to determine if there was a significant treatment effect at either site, then the PDIFF function was used to compare each treatment to all other treatments where results were significant.

Regression analysis was used to determine if the proportion of plants with terminal leaf damage can be used to predict the impact of *S. lineatus* damage on yield. All plots from 2008 and 2009 that were not treated with thiamethoxam were pooled for the regression analysis. Using PROC REG (SAS Institute 2004), the relationship between the proportion of plants per plot with terminal leaf damage (at the three- and five-node stages) and yield (t ha^{-1}) was assessed.

2.3. Results

2.3.1. Above-ground Plant Damage.

In 2007, *S. lineatus* population densities were extremely high and extensive foliar damage prevented plants from being assessed for adult feeding. Assessments were performed in 2008 and 2009. Treatment did not significantly affect growth stage of *P. sativum* plants (i.e., plants were at approximately the same stage in all plots at each assessment date) (Table 2.3). Site did have an effect on plant growth stage in 2009 at the three- ($F_{1, 6}$ = 6.76, p = 0.0407) and five-node stages ($F_{1, 6}$ = 23.50, p = 0.0029), with plants slightly more advanced at Vauxhall. No significant interactions of site and treatment were observed (Table 2.3).

In both 2008 and 2009, feeding damage severity on the whole plant was greater at Lethbridge than at Vauxhall, with significant differences observed at the fifth- and eighth-node assessments in both years (Table 2.4). In 2008, treatment had a significant effect on the number of *S. lineatus* feeding notches observed in each plot at the three- ($F_{7, 42}$ = 4.08, p = 0.0017) and five-node stages ($F_{7, 42}$ = 2.83, p = 0.0166), but not at the eight-node stage ($F_{7, 42}$ = 0.73, p = 0.6490). In 2009, treatment had a significant effect on the degree of *S. lineatus* feeding in each plot at all three growth stages (Table 2.4). At the three- and five-node stages, all plots to which thiamethoxam was applied had significantly less feeding damage than control plots, N fertilizer only and inoculant only plots (Figure 2.1). The effect of thiamethoxam on *S. lineatus* feeding was not as pronounced at the eight-node stage, as no difference between thiamethoxam and inoculant only plots was observed (Figure 2.1). Plants treated with N fertilizer alone had significantly more feeding notches than those treated with thiamethoxam alone (i.e. 20.06 ± 2.81 (S.E.) and 2.89 ± 0.75, respectively at the three-node stage). At the eight-node stage, plants receiving thiamethoxam only continued to have less *S. lineatus* damage than plants given N fertilizer only (39.81 ± 11.82 and 93.31 ± 12.05, respectively).

An effect of site was observed on feeding notches on the terminal leaves at the fifth-node stage ($F_{1,6} = 9.66$, $p = 0.0209$) in 2008 and at all assessment stages in 2009 (Table 2.4). Again, damage was greater to plants at Lethbridge than to plants at Vauxhall. Plot treatment had a significant effect on the number of *S. lineatus* feeding notches on the terminal leaves at the five-node stage in 2008 ($F_{7,42} = 2.24$, $p = 0.0493$), and on damage at the three- ($F_{7,42} = 9.32$, $p < 0.0001$) and five-node stages ($F_{7,42} = 6.03$, $p < 0.0001$) in 2009 (Figure 2.2). Plants treated with N fertilizer only had an average of 1.94 ± 0.58 (± S.E.) terminal leaf notches at the three-node stage, compared to a mean of less than 0.20 terminal leaf notches on plants treated with thiamethoxam, either alone or in combination. At the eight-node stage, *P. sativum* plants in all treatment groups had less than 0.50 terminal leaf notches per plant, on average (Figure 2.2), and differences were no longer observed among treatments. No significant site by treatment interactions were observed at any growth stage in either year.

Site had an effect on the proportion of plants with terminal leaf damage at the five-node stage in 2008 ($F_{1, 6} = 20.02$, $p = 0.0042$) and at the three-node stage in 2009 ($F_{1, 6} = 10.62$, $p = 0.0173$) (Table 2.4). As observed for mean feeding notches on the whole plant and on terminal leaves, a greater proportion of plants had terminal leaf damage at Lethbridge than at Vauxhall. In 2008, no effect of treatment was observed at either growth stage (Table 2.4). In 2009, significant treatment effects occurred at both the three- ($F_{7, 42} = 14.95$, $p < 0.0001$) and five-node ($F_{7, 42} = 9.71$, $p < 0.0001$) stages, as plots treated with thiamethoxam (alone or in combination) usually had fewer plants with terminal leaf damage relative to plots without thiamethoxam (Figure 2.3). At the three-node stage, for example, approximately 62% of plants growing in N-fertilized plots and control plots suffered terminal leaf damage. In plots treated with inoculant plus N fertilizer plus thiamethoxam, approximately 7% of plants had terminal leaf damage (Figure 2.3).

An analysis by node was used to determine which nodes received protection from *S. lineatus* herbivory at the three-, five-, and eight-node growth stages. In 2008, significant differences in feeding notches were observed on the second and third nodes at the three-node assessment, on all nodes at the five-node assessment and on the second and third nodes at the eight-node assessment (Table 2.5). In 2009, significant differences in feeding notches were observed for all nodes at the three- and five-node assessment, and up to and including the seventh node at the eight-node assessment (Table 2.5). Where significant differences in the number of feeding notches among treatments were observed, plants treated with thiamethoxam had significantly fewer notches on each node than those without thiamethoxam. For example, plants from N-fertilized plots had 6.53 ± 1.01 (± S.E.) notches on the fifth node at the five-node growth stage in 2009, relative to 0.41 ± 0.31 notches on the same node of plants treated with inoculant plus N fertilizer plus thiamethoxam. Plants reached the eight-node stage approximately 40 to 50 days after planting (Table 2.1), depending on the year.

2.3.2. Below-ground Plant Damage.

Treatment means (± S.E.) and ANOVA *p*-values for root nodule parameters are given in Table 2.6. In 2007, total nodules ($F_{2, 10} = 5.08$, $p = 0.0300$) and tumescent nodules ($F_{2, 10} = 14.57$, $p = 0.0011$) were affected by treatment but nodules expressing leghaemoglobin ($F_{2, 10} = 1.62$, $p = 0.2466$) and damaged nodules ($F_{2, 10} = 3.22$, $p = 0.0832$) were not. Where significant treatment effects were observed in 2007, plants receiving inoculant plus thiamethoxam had the most root nodules, most tumescent root nodules, and the most nodules expressing leghaemoglobin relative to inoculant plus N fertilizer-treated plots and this trend was observed in 2008 and 2009 as well (Table 2.6). Treatment did not have a significant effect on damaged root nodules in either 2008 ($F_{2, 12} = 2.01$, $p = 0.1771$) or 2009 ($F_{2, 12} = 3.29$, $p = 0.0726$), which was similar to the 2007 results. In 2008 and 2009, a significant effect of treatment was observed for nodules expressing leghaemoglobin ($F_{2, 12} = 7.91$, $p = 0.0065$ and $F_{2, 12} = 11.81$, $p = 0.0015$ respectively), tumescent nodules ($F_{2, 12} = 4.18$, $p = 0.0420$ and $F_{2, 12} = 15.49$, $p = 0.0005$ respectively) and total nodules ($F_{2, 12} = 8.81$, $p = 0.0044$ and $F_{2, 12} = 6.53$, $p = 0.0121$ respectively) per plant. Site effects were observed for several root nodule parameters (total nodules, leghaemoglobin expressing nodules and damaged nodules) in 2007 and 2008, but only for damaged nodules in 2009, as plants growing at Lethbridge had more root nodules than plants at Vauxhall.

In 2008 treatment had a significant effect on foliar ($F_{2, 12} = 4.88$, $p = 0.0282$), root ($F_{2, 12} = 6.17$, $p = 0.0144$) and total biomass ($F_{2, 12} = 5.36$, $p = 0.0217$) per plant (Table 2.7). For all parameters, plants treated with inoculant plus thiamethoxam had significantly greater biomass than plants treated with inoculant plus N fertilizer (Table 2.7). No significant treatment effects were observed in 2009, for any biomass parameter.

2.3.3. Yield.

Two yield parameters were considered for statistical analysis, *P. sativum* yield from each plot (t ha^{-1}) and the protein concentration (%) of seeds. Protein concentration of seeds was not measured in 2007. Site effects on yield were observed in 2008 only ($F_{1, 6} = 51.07$, $p = 0.0004$) as were effects of treatment ($F_{7, 41} = 4.69$, $p = 0.0006$). In 2009, a significant effect of site was observed on protein concentration ($F_{1, 6} = 97.48$, $p < 0.0001$), as seeds from Vauxhall plots (24%) were richer in protein than seeds from Lethbridge (21%). Treatment had a significant effect on protein concentration in 2008 ($F_{7, 41} = 4.60$, $p = 0.0007$). No significant site, treatment or site by treatment interactions were observed for yield in 2007 or 2009 (Table 2.3), but for both yield parameters in 2008, a significant site by treatment interaction was observed (Table 2.3). When the interaction was investigated further, treatment was observed to have a significant effect on yield

($F_{7, 21} = 16.03$, $p < 0.0001$) and seed protein concentration ($F_{7, 21} = 12.21$, $p < 0.0001$) at Lethbridge only.

Yield at Lethbridge in 2008 was greatest when plants were treated with inoculant plus N fertilizer, which significantly exceeded yield of all plots except those treated with inoculant plus N fertilizer plus thiamethoxam and inoculant only (Figure 2.4). Control plants and plants treated only with thiamethoxam had the lowest yields. Seed protein concentration was significantly greater when plants were treated with inoculant plus N fertilizer plus thiamethoxam (20.1 ± 0.5 %), inoculant plus N fertilizer (19.3 ± 0.4 %) and inoculant (18.7 ± 0.4 %) than in thiamethoxam only (17.2 ± 0.5 %) and control (17.2 ± 0.2 %) plots (Figure 2.5).

A significant linear relationship was observed between the proportion of plants with terminal leaf damage and yield at both the three- ($p < 0.0001$, $R^2 = 0.2401$) and the five-node stages ($p = 0.0015$, $R^2 = 0.1528$). At both growth stages, the relationship between damage on the terminal leaves and yield was negative. At the three-node stage the following equation can be used to predict yield loss: $y = 4.42 – 2.38x$, where y = yield (t ha^{-1}) and x = proportion of plants with terminal leaf damage. If assessed for damage at the five-node stage, the equation to predict yield loss is $y = 4.36 – 2.03x$.

2.4. Discussion

A significant effect of site was observed for most above- and below-ground plant damage parameters, as well as yield (t ha^{-1}) and percent seed protein concentration. The site effect may be explained by differences in *S. lineatus* population density between sites. Although weevil populations were not estimated directly, the degree of plant notching is routinely used as an indicator of adult weevil density (Cantot 1986; Doré and Meynard 1995). More feeding notches were consistently recorded at Lethbridge than at Vauxhall in all study years. The significant impact of site upon parameters such as yield and percent protein concentration may have been the result of varying climatic factors between the two sites, especially soil type, precipitation and mean temperature. For example, a number of crop species growing in drought conditions are known to have higher seed protein concentrations than plants with adequate available moisture (Dornbos and Mullen 1992; García del Moral *et al.* 2007). It is unlikely that the change of *P. sativum* cultivar explains the absence of treatment effects on yield in 2009. Assessment of *P. sativum* cultivars for resistance to *S. lineatus* has shown some differences in *S. lineatus* feeding preferences between cultivars (Nouri-Ghanbalani *et al.* 1978; Havlickova 1980); however, breeding programs for *S. lineatus* resistance have not been successful (Nouri-Ghanbalani *et al.* 1978).

On all assessment dates, above-ground feeding damage to plants treated with N fertilizer alone was comparable to plants in untreated control plots. In fact, the total number of feeding notches per plant in plots receiving only N fertilizer exceeded that of control plants at the three-node and eight-node stages. Moreover, in N-fertilized plots, the proportion of damaged seedlings exceeded the recommended economic threshold of 30% of plants with terminal leaf damage as described by El-Lafi (1977) at the three- and five-node stages. According to the Plant Vigor Hypothesis of Price (1991), plants with high nutrient contents are susceptible to increased levels of insect herbivory relative to plants with low nutrient contents. The results of this study appear to concur with the Plant Vigor Hypothesis. Plants in the N-fertilized plots had greater access to soil N following germination, which should in turn have led to greater foliage N concentration. These seedlings were apparently selected more frequently by adults for feeding.

As expected, plants fertilized with N and treated with *Rhizobium* inoculant had the fewest root nodules, the fewest tumescent root nodules and the fewest nodules expressing leghaemoglobin per plant in all years. These plants had the lowest root biomass and total biomass per plant. When soil N reserves are sufficiently high, root nodule initiation, formation, and development are inhibited (George 1962; Allen and Allen 1981; Nelson and Edie 1991; Abdel Wahab *et al.* 1996), explaining the trend observed in this study. Root nodules are a limiting resource for *S. lineatus* larvae, which means that plants have a larval carrying capacity (McEwen *et al.* 1981; Nielsen 1990). This carrying capacity would be further limited in plants with reduced numbers of root nodules, as observed for N-fertilized plants in this study and that of McEwen *et al.* (1981). It appears that N-fertilized plots were attractive to adult weevils for feeding, but were less suitable as larval hosts.

In 2008 when treatment had a significant impact on yield, plots treated with N fertilizer alone yielded poorly relative to plots treated with *Rhizobium* inoculant. Therefore, N fertilization alone, applied at 60 kg ha^{-1} does not appear to be a viable control option for *S. lineatus* in field pea. Perhaps an effect would be observed in N fertilizer was applied at greater rates. Because of the increasingly high cost of N fertilizers (Walley *et al.* 2007; Kumbhar *et al.* 2008), and the inconsistent results of these trials, using N fertilizer to enhance pea leaf weevil control is not recommended. However, N-fertilized field pea may be useful as a trap crop, by attracting adult weevils away from unfertilized plants, especially if oviposition rates are equivalent on fertilized and unfertilized plants. Trap crops have been investigated as a component of a stimulo-deterrent diversionary strategy for *S. lineatus* by Smart *et al.* (1994) in Europe and the use of spring-planted peas as trap crops should be investigated in North America. Using winter peas as a trap crop has been investigated in southern Alberta, with mixed results (Cárcamo *et al.* 2010).

Thiamethoxam seed coating reduced *S. lineatus* foliar feeding for up to 40 to 50 days after planting or until plants reached the eight-node growth stage. Plots receiving thiamethoxam, alone or in any combination with inoculant and N fertilizer had fewer feeding notches relative to plots not treated with thiamethoxam. These results are similar to those of Koch *et al.* (2005), who observed a significant decrease in damage ratings for the bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae) on common bean, *Phaseolus vulgaris* L. (Fabales: Fabaceae) and Rotrekl and Cejtchaml (2008) who observed less feeding by adult *Sitona* weevils on thiamethoxam-treated alfalfa, *Medicago sativa* L. (Fabales: Fabaceae). Moreover, thiamethoxam as a seed treatment prevented levels of feeding from exceeding the economic threshold of 30% of seedlings with terminal leaf damage as recommended by El-Lafi (1977), at the three-, five-, and eight-node growth stages. Where plots were not treated with thiamethoxam, the proportion of damaged plants exceeded this economic threshold. By the eight-node stage, all plots showed approximately equal proportions of plants with terminal leaf damage, with no plot exceeding more than 5% of plants with terminal leaf damage. This indicates that the efficacy of thiamethoxam at the eight-node stage may have been reduced due to dilution via plant growth, as observed by Nault *et al.* (2004). However, by the eight-node stage, the height of the plants may also dissuade weevils from climbing to the terminal leaves, which would also explain why the proportion of plants with terminal leaf damage is reduced at the eight-node stage. In addition to the suppressive effects on *S. lineatus* feeding activity, plants treated with inoculant plus thiamethoxam had more root nodules than plants treated with inoculant only and significantly more nodules than plants treated with inoculant plus N fertilizer. This relationship has not been observed previously in field peas. Improved plant vigor has been reported when canola seeds (*Brassica napus* L., Brassicales: Brassicaceae) were treated with thiamethoxam (Doyle *et al.* 2001). It is possible that increased nodulation in field pea plants treated with thiamethoxam may be the result of improved below-ground plant vigor, providing more root hairs which increases root nodule initiation sites (Allen and Allen 1981). Further investigation of this trend is required in order to determine if thiamethoxam effects root vigor.

The systemic nature of thiamethoxam when applied as a seed treatment ensures that newly emerging leaves are protected (Maienfisch *et al.* 2001; Elbert *et al.* 2008). Leaf tissue concentration of the active ingredient in systemic insecticides is expected to decline over time, providing protection only early in the growing season (Nault *et al.* 2004). Studies testing the efficacy of thiamethoxam against potato leafhoppers, *Empoasca fabae* (Harris) (Hemiptera: Cicadellidae), on snap beans found that an application rate of 30 g per 100 kg of seed controlled leafhoppers for 38 days, but by 42 days after planting, no significant effects were observed relative to controls (Nault *et al.* 2004). Results of this study are comparable to those of Nault *et al.* (2004), as thiamethoxam protected field pea plants from *S. lineatus* feeding for approximately 50 days after planting in 2008 and 43 days after planting in 2009. Williams *et al.* (1995) observed that significant yield losses to field pea were incurred when the most severe foliar feeding damage occurred at the four- to six-node stages. Therefore, my results suggest that thiamethoxam provided protection to plants during their most vulnerable growth period, even under high *S. lineatus* pressure.

Seed treatments with systemic insecticides such as those containing thiamethoxam are generally compatible with biological control and IPM practices (Ripper *et al.* 1949; Maienfisch *et al.* 2001; Elbert *et al.* 2008). In Europe and North America, a number of predators of *S. lineatus* eggs, adults and larvae have been identified, principally ground beetles (Coleoptera: Carabidae) (Hamon *et al.* 1990; Ropek and Jaworska 1994; Vankosky *et al.* 2010), and parasitoids of *S. lineatus* have been identified by Aeschlimann (1980). If natural enemy populations can be protected from insecticides by using systemic products (Ripper *et al.* 1949), natural control of *S. lineatus* populations should be enhanced.

Timing insecticide application is one of the greatest challenges in managing *S. lineatus* populations. To have an impact on oviposition, foliar insecticides must be applied immediately after weevils are detected in field pea crops (King 1981; Steene *et al.* 1999), as Williams *et al.* (1995) observed that the pre-oviposition feeding period for adult female *S. lineatus* was 10 days for weevils collected before spring migration. If applied after oviposition begins, insecticides will not prevent establishment of larval populations (King 1981; Bardner *et al.* 1983; Ester and Jeuring 1992). This is a very narrow window of time in which to act and requires accurate monitoring at short time intervals. However, if insecticides are applied too early, subsequent sprays may be required if the residence time of the product is poor, or if weevils re-invade sprayed fields. By treating seeds with an insecticidal product, such as thiamethoxam, issues pertaining to crop monitoring and foliar insecticide application can be avoided. The use of seed treatments has been advised for producers in regions where pest pressure is expected to be high (Koch *et al.* 2005), and this should also be the case for *S. lineatus*.

Planting field peas with *Rhizobium* inoculant is a standard agronomic practice used to ensure that sufficient bacterial populations are present in the soil to maximize root nodule formation and the rate of N fixation (Rennie *et al.* 1993). In all study years, inoculated field peas had more root nodules than the inoculant plus N fertilizer treatment, but fewer than plants in inoculant plus thiamethoxam plots. This trend may be explained if thiamethoxam has an effect on the vigor of field pea root systems, similar to the above-ground effect observed in canola (Doyle *et al.* 2001), providing more root nodule initiation sites on field pea roots. Inoculating field peas did not significantly suppress foliar consumption by adults, except when used in combination with thiamethoxam. When both inoculant and thiamethoxam were applied, the proportion of plants with *S. lineatus* feeding on the terminal leaves was below the economic threshold proposed by El-Lafi (1977). At Lethbridge in 2008, when a significant effect of treatment was observed on yield, those with the highest yields were treated with *Rhizobium* inoculant, including inoculant only plots where levels of terminal leaf damage exceeded the economic threshold. For all other site-years, no effect of plot treatment on yield was observed. Therefore, results of this study suggest that although thiamethoxam prevents *S. lineatus* damage from exceeding the proposed economic threshold, *Rhizobium* inoculant is required to maintain yields, whether or not the economic threshold is exceeded.

In 2008, inoculant plus thiamethoxam-treated plants yielded well relative to plants receiving thiamethoxam only, in addition to having higher numbers of root nodules. No reference to *Rhizobium* inoculation of field pea or faba beans was found in any literature pertaining to *S. lineatus* management and yield impacts (for example: Bardner and Fletcher 1979; McEwen *et al.* 1981; Bardner *et al.* 1983; Nielsen 1990; Steene *et al.* 1999). However, literature regarding legume production strongly advocates the use of *Rhizobium* inoculants, especially in soils that are acidic or where legumes have not been recently grown (Allen and Allen 1981; Fettell *et al.* 1997). Results of this study suggest that well-nodulated plants, in the presence or absence of thiamethoxam, are better able to tolerate *S. lineatus* feeding damage and that these plants yield better than control plants and plants receiving only N fertilizer or only thiamethoxam.

Integrated pest management programs can incorporate multiple control strategies to limit insecticide use and promote sustainable agricultural practices (Kogan 1998). When applied simultaneously, N fertilizer, *Rhizobium* inoculant, and thiamethoxam significantly reduced feeding damage relative to untreated controls. However, this reduction was not significantly different from the level of damage observed for thiamethoxam alone, thiamethoxam plus inoculant or thiamethoxam plus N fertilizer. Similar results were observed for yield, where the inoculant plus N fertilizer plus thiamethoxam treatment was among the best yielding treatments, but was not significantly better than the other treatments including inoculant. If the goal of producers is to suppress adult *S. lineatus* feeding activity, then including thiamethoxam as a seed treatment may be advised. However, thiamethoxam does not appear to impact larval populations, which are considered more damaging to *P. sativum* plants (Doré and Meynard 1995; Hunter 2001; Corre-Hellou and Crozat 2005). Thiamethoxam, in this study, did not protect yields when applied in the absence of *Rhizobium* inoculant. Therefore, in terms of increasing yield and maximizing profits, thiamethoxam does not appear to be an effective management option for *S. lineatus* unless applied in conjunction with *Rhizobium* inoculant. Where *Rhizobium* inoculation is standard practice for producers the additional cost of thiamethoxam seed treatment may be justified, as thiamethoxam was observed to improve nodulation and inoculant plus thiamethoxam-treated plots yielded as well as plots treated with inoculant plus N fertilizer.

Integrated pest management programs are reliant upon the economic threshold and economic injury level concepts (Higley and Pedigo 1993). El-Lafi (1977) recommended that control measures for *S. lineatus* should be taken when 30% of plants had damage on the terminal leaves. Thiamethoxam applied as a seed coating prevented foliar damage from exceeding this threshold, although thiamethoxam alone does not appear to protect yield. A linear relationship was observed between the proportion of plants with terminal leaf damage and yield, indicating that as the proportion of damaged plants increased, yield decreased. This relationship will help to determine an economic threshold for *S. lineatus*, which will be useful to producers in determining whether or not insecticide applications for *S. lineatus* are required on their fields.

One issue related to systemic insecticide application is the increased cost to producers. Systemic products are generally applied at planting, and may be unnecessary if pest outbreaks do not occur. To prevent economic losses related to unnecessary seed treatment applications, accurate forecasting and monitoring systems, both regional and local, are needed in order to predict where *S. lineatus* will be a threat. A more thorough understanding of the impact of larval *S. lineatus* on field pea yield is also needed, and it must be determined if systemic products can directly impact larval populations.

Table 2.1. Dates of plot seeding, plant damage assessments, and harvest at Lethbridge and Vauxhall, Alberta, in 2007, 2008 and 2009. Interim periods between planting and assessments and harvest are given as the number of days.

Site/Year	Activity	Calendar Date	Julian date	Interim from seeding (days)
Lethbridge 2007	Plots Seeded	10 May 2007	130	
	Collection for Root Nodule Assessment	13 July 2007	194	64
	Plot Harvest	02 Aug 2007	214	114
Vauxhall 2007	Plots Seeded	27 Apr 2007	117	
	Collection for Root Nodule Assessment	5 July 2007	186	69
	Plot Harvest	25 July 2007	206	89
Lethbridge 2008	Plots Seeded	30 Apr 2008	121	
	3-node assessment	28 May 2008	149	28
	5-node assessment	05 June 2008	157	36
	8-node assessment	19 June 2008	171	50
	Collection for Root Nodule Assessment	04 July 2008	186	65
	Plot Harvest	11 Aug 2008	224	103
Vauxhall 2008	Plots Seeded	29 Apr 2008	120	
	3-node assessment	29 May 2008	150	30
	5-node assessment	09 June 2008	161	41
	8-node assessment	18 June 2008	170	50
	Collection for Root Nodule Assessment	03 July 2008	185	64
	Plot Harvest	13 Aug 2008	226	106
Lethbridge 2009	Plots Seeded	04 May 2009	124	
	3-node assessment	27 May 2009	147	23
	5-node assessment	02 June 2009	153	29
	8-node assessment	16 June 2009	167	43
	Collection for Root Nodule Assessment	07 July 2009	188	64
	Plot Harvest	20 Aug 2009	232	108
Vauxhall 2009	Plots Seeded	06 May 2009	126	
	3-node assessment	28 May 2009	148	22
	5-node assessment	04 June 2009	155	29
	8-node assessment	16 June 2009	167	41
	Collection for Root Nodule Assessment	06 July 2009	187	61
	Plot Harvest	18 Aug 2009	230	104

Table 2.2. Mean temperature (May through August) and total precipitation (April to August) for Lethbridge and Vauxhall, Alberta in 2007, 2008 and 2009 (Environment Canada 2010). Precipitation is given as the growing season total (snow or rainfall) for each site-year.

	Daily mean temperature		
Site year	Minimum (°C)	Maximum (°C)	Precipitation (mm)
Lethbridge 2007	7.68	25.03	189.5
Lethbridge 2008	6.94	22.57	345.5
Lethbridge 2009	4.76	22.53	223.5
Vauxhall 2007	9.09	24.83	203.3
Vauxhall 2008	8.16	23.18	261.0
Vauxhall 2009	7.50	22.82	234.0

Table 2.3. Mixed model ANOVA results for growth stage on three assessment dates, *Pisum sativum* yield (t ha^{-1}), and seed protein concentration (%), for Lethbridge and Vauxhall, Alberta in 2007, 2008 and 2009. Values shown in bold font are significant where $\alpha = 0.05$.

Effect		Assessment 1 growth stage	Assessment 2 growth stage	Assessment 3 growth stage	Yield (t ha^{-1})	Seed Protein (%)
			2007			
Site (S)	$F_{(1, 6)}$	.[‡]	.	.	0.00	.
	p-value	.	.	.	0.9782	.
Treatment (T)	$F_{(7, 40)}$	.	.	.	1.44	.
	p-value	.	.	.	0.2175	.
S*T	$F_{(7, 40)}$	.	.	.	1.23	.
	p-value	.	.	.	0.3111	.
			2008			
Site	$F_{(1, 6)}$	3.60	0.14	3.59	**51.07**	**144.86**
	p-value	0.1066	0.7194	0.1071	**0.0004**	**<0.0001**
Treatment	$F_{(7, 42)}$ †	1.49	0.93	1.01	**4.69**	**4.60**
	p-value	0.1957	0.4914	0.4389	**0.0006**	**0.0007**
S*T	$F_{(7, 42)}$ †	1.64	0.99	0.85	**5.68**	**7.36**
	p-value	0.1516	0.4539	0.5497	**0.0001**	**<0.0001**
			2009			
Site	$F_{(1, 6)}$	**6.76**	**23.50**	0.53	1.57	**97.48**
	p-value	**0.0407**	**0.0029**	0.4926	0.2574	**<0.0001**
Treatment	$F_{(7, 42)}$ †	0.67	1.01	2.07	0.48	0.80
	p-value	0.6923	0.4364	0.0679	0.8436	0.5923
S*T	$F_{(7, 42)}$ †	0.68	0.61	1.20	0.48	1.00
	p-value	0.6905	0.7454	0.3256	0.9582	0.4474

† Yield (t ha^{-1}) and seed protein in 2008, and yield (t ha^{-1}) in 2009 had only 41 denominator degrees of freedom.

[‡] Growth stage assessments and seed protein content were not measured in 2007.

Table 2.4. Mixed model ANOVA results for mean *Sitona lineatus* feeding damage, evaluated by counting feeding notches (FN), to *Pisum sativum* plants at the three-, five-, and eight-node growth stages, on the whole plant and on the terminal leaf (TL) and for the proportion of plants with terminal leaf damage at the three- and five-node stages, for Lethbridge and Vauxhall, Alberta in 2008 and 2009. Feeding damage was not evaluated in 2007. Values shown in bold font are significant where $\alpha = 0.05$.

Effect		FN at 3rd-node stage	FN at 5th-node stage	FN at 8th-node stage	3-node TL damage	5-node TL damage	8-node TL damage	Proportion TL damage 3-node	Proportion TL damage 5-node
					2008				
Site	$F_{(1, 6)}$	5.78	**22.34**	**113.34**	1.59	**9.66**	2.89	1.84	**20.02**
	p-value	0.0529	**0.0032**	**<0.0001**	0.2544	**0.0209**	0.1398	0.2240	**0.0042**
Treatment	$F_{(7, 42)}$	**4.08**	**2.83**	0.73	1.66	**2.24**	0.37	1.74	1.98
	p-value	**0.0017**	**0.0166**	0.6490	0.1453	**0.0493**	0.9131	0.1258	0.0801
Site*Treatment	$F_{(7, 42)}$	0.16	0.57	0.81	1.98	1.52	1.07	2.20	1.26
	p-value	0.9915	0.7772	0.5813	0.0815	0.1884	0.3985	0.0535	0.2909
					2009				
Site	$F_{(1, 6)}$	0.05	**6.85**	**60.05**	**10.97**	**3.66**	**15.65**	**10.62**	5.35
	p-value	0.8301	**0.0396**	**0.0002**	**0.0162**	**0.1042**	**0.0075**	**0.0173**	0.0600
Treatment	$F_{(7, 42)}$	**20.66**	**22.76**	**7.72**	**9.32**	**6.03**	1.37	**14.95**	**9.71**
	p-value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.2420	**<0.0001**	**<0.0001**
Site*Treatment	$F_{(7, 42)}$	0.39	0.72	1.28	1.49	0.80	1.83	0.59	0.63
	p-value	0.9030	0.6562	0.2835	0.1975	0.5898	0.1072	0.7586	0.7286

Table 2.5. Mixed model ANOVA *p*-values for *Sitona lineatus* feeding damage to *Pisum sativum* plants at Lethbridge and Vauxhall in 2008 and 2009, when plants were assessed by node at approximately the three-, five- and eight-node growth stages. Significant *p*-values (< 0.05) are given in bold font. No significant site by treatment interactions were observed for any node.

		p-values	
Growth Stage	Node	2008	2009
Thee-node stage (Assessment 1)	1	0.0912	**<0.0001**
	2	**0.0001**	**<0.0001**
	3	**0.0002**	**<0.0001**
	4	.	**<0.0001**
Five-node stage (Assessment 2)	1	**0.0359**†	**<0.0001**
	2	**0.0323**†	**<0.0001**†
	3	**0.0033**†	**<0.0001**†
	4	**0.0413**†	**<0.0001**
	5	**0.0045**†	**<0.0001**
Eight-node stage (Assessment 3)	1	0.2480†	**0.0015**
	2	**0.0238**†	**0.0002**†
	3	**0.0412**†	**<0.0001**†
	4	0.0575†	**<0.0001**†
	5	0.3659†	**0.0001**†
	6	0.1470†	**0.0032**†
	7	0.1317†	**0.0095**†
	8	0.3402†	0.0718†
	9	0.7296†	0.3121

† Indicates significant effects of site on feeding damage.

. Indicates that data for a given node was not available.

Table 2.6. Mixed model ANOVA results and treatment means (± S.E.) for total root nodules, tumescent root nodules, leghaemoglobin (LH) expressing nodules and root nodules damaged by *Sitona lineatus* at Lethbridge and Vauxhall, Alberta in 2007, 2008 and 2009. Significant *p*-values are given in bold font. For each parameter, means with the same letters are not significantly different (*p* >0.05).

			Treatment Means (± S.E.)		
Nodule Parameter	Treatment $F_{(2, 12)}$	Treatment *p*-value	Inoculant	Inoculant + nitrogen (N)	Inoculant + thiamethoxam
			2007		
Total	5.08	**0.0300**†‡	7.32 ± 1.27	9.58 ± 2.19	15.90 ± 3.14
Tumescent	14.57	**0.0011**†	1.03 ± 0.43**a**	1.17 ± 0.51**a**	5.61 ± 1.09**b**
LH Expressing	1.62	0.2466	0.39 ± 0.32	0.40 ± 0.16	1.27 ± 0.54
Damaged	3.22	0.0832†	5.44 ± 1.28	8.34 ± 2.16	12.59 ± 3.00
			2008		
Total	8.81	**0.0044**†	29.01 ± 2.95**ab**	25.78 ± 2.49**a**	39.77 ± 4.13**b**
Tumescent	4.18	**0.0420**†	14.05 ± 2.35**ab**	13.67 ± 2.65**a**	18.44 ± 2.51**b**
LH Expressing	7.91	**0.0065**	15.56 ± 2.71**a**	13.22 ± 1.54**a**	23.13 ± 2.31**b**
Damaged	2.01	0.1771†	14.20 ± 5.37	16.02 ± 4.06	19.21 ± 6.57
			2009		
Total	6.53	**0.0121**	25.08 ± 2.80**ab**	19.73 ± 2.22**a**	35.22 ± 4.99**b**
Tumescent	15.49	**0.0005**	10.00 ± 1.63**a**	6.43 ± 1.55**a**	23.70 ± 4.00**b**
LH Expressing	11.81	**0.0015**	19.45 ± 2.48**a**	14.84 ± 2.60**a**	30.26 ± 4.31**b**
Damaged	3.29	0.0726†	16.83 ± 3.98	11.16 ± 3.38	17.77 ± 5.73

†Indicates a significant effect of site (Lethbridge or Vauxhall) when analyzed with the mixed model ANOVA ($p < 0.05$).

‡ When alpha was adjusted using the Bonferonni adjustment to prevent error, no significant contrasts were observed.

Table 2.7. Mixed model ANOVA results for the effect of plot treatment on foliar, root and total biomass per plant at Lethbridge and Vauxhall, Alberta in 2008 and 2009. Mean biomass per plant is given for each treatment (± S.E.). Significant results ($p < 0.05$) are given in bold font. For each parameter, means with the same letters are not significantly different ($p > 0.05$).

				Treatment Means (± S.E.)		
Year	Biomass (plant^{-1})	Treatment F$_{(2, 12)}$	Treatment *p*-value	Inoculant	Inoculant + nitrogen (N)	Inoculant + thiamethoxam
2008	Foliage	**4.88**	**0.0282**	2.20 ± 0.34**ab**	1.77 ± 0.18**a**	3.31 ± 0.55**b**
	Root	**6.17**	**0.0144**†	0.16 ± 0.03**ab**	0.13 ± 0.03**a**	0.24 ± 0.04**b**
	Total	**5.36**	**0.0217**	2.36 ± 0.36**ab**	1.89 ± 0.20**a**	3.55 ± 0.58**b**
2009	Foliage	0.66	0.5323	3.83 ± 0.35	3.53 ± 0.34	4.02 ± 0.42
	Root	1.92	0.1884	0.20 ± 0.01	0.16 ± 0.02	0.23 ± 0.03
	Total	0.79	0.4771	4.04 ± 0.35	3.68 ± 0.36	4.25 ± 0.42

† Indicates parameters where site had a significant effect on mean biomass per plant.

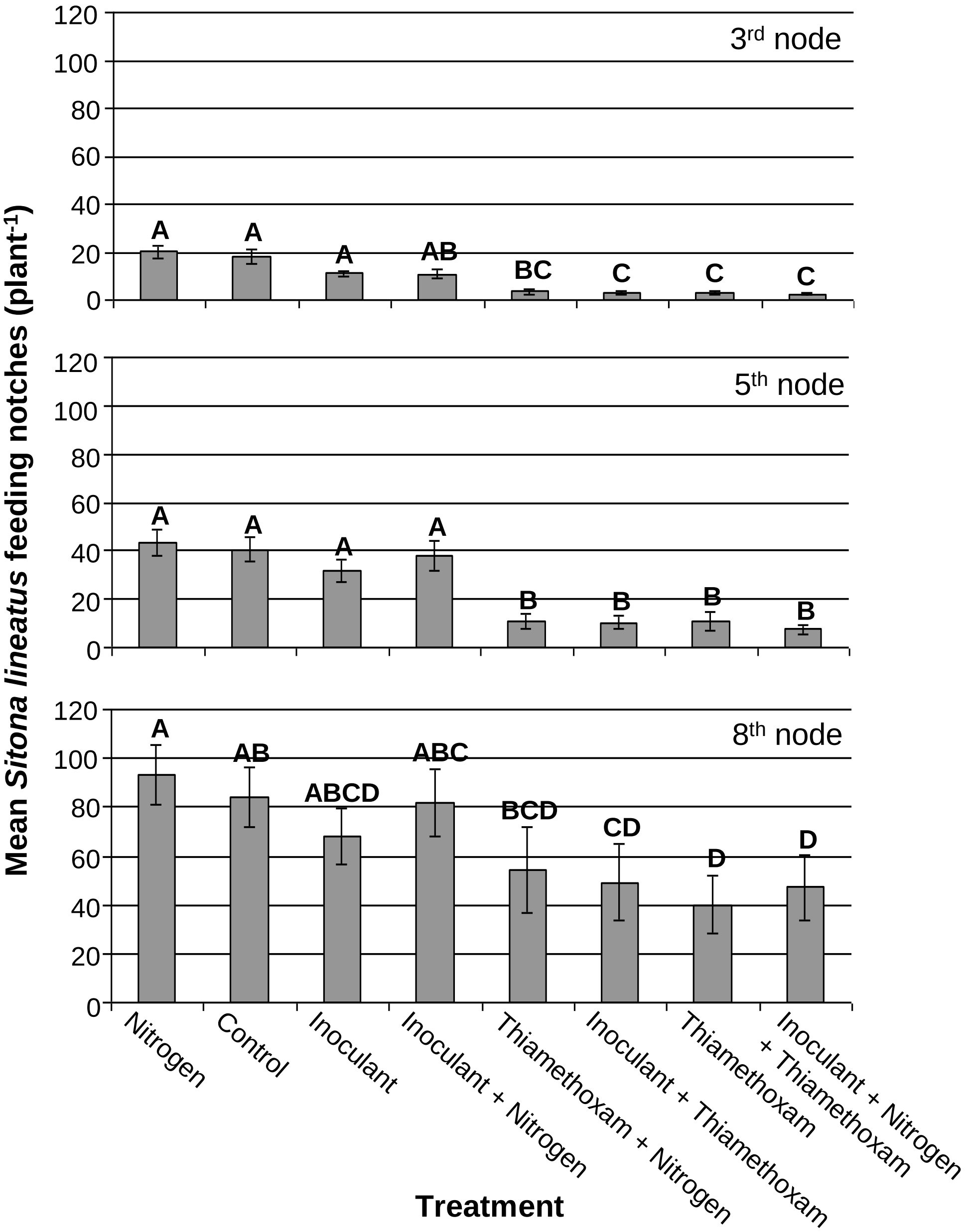

Figure 2.1. Mean *Sitona lineatus* feeding notches per plant (± S.E.) on *Pisum sativum* at Lethbridge and Vauxhall, Alberta in 2009, given for assessments at the three-, five-, and eight-node growth stages. Means with the same letters are not significantly different ($p > 0.05$).

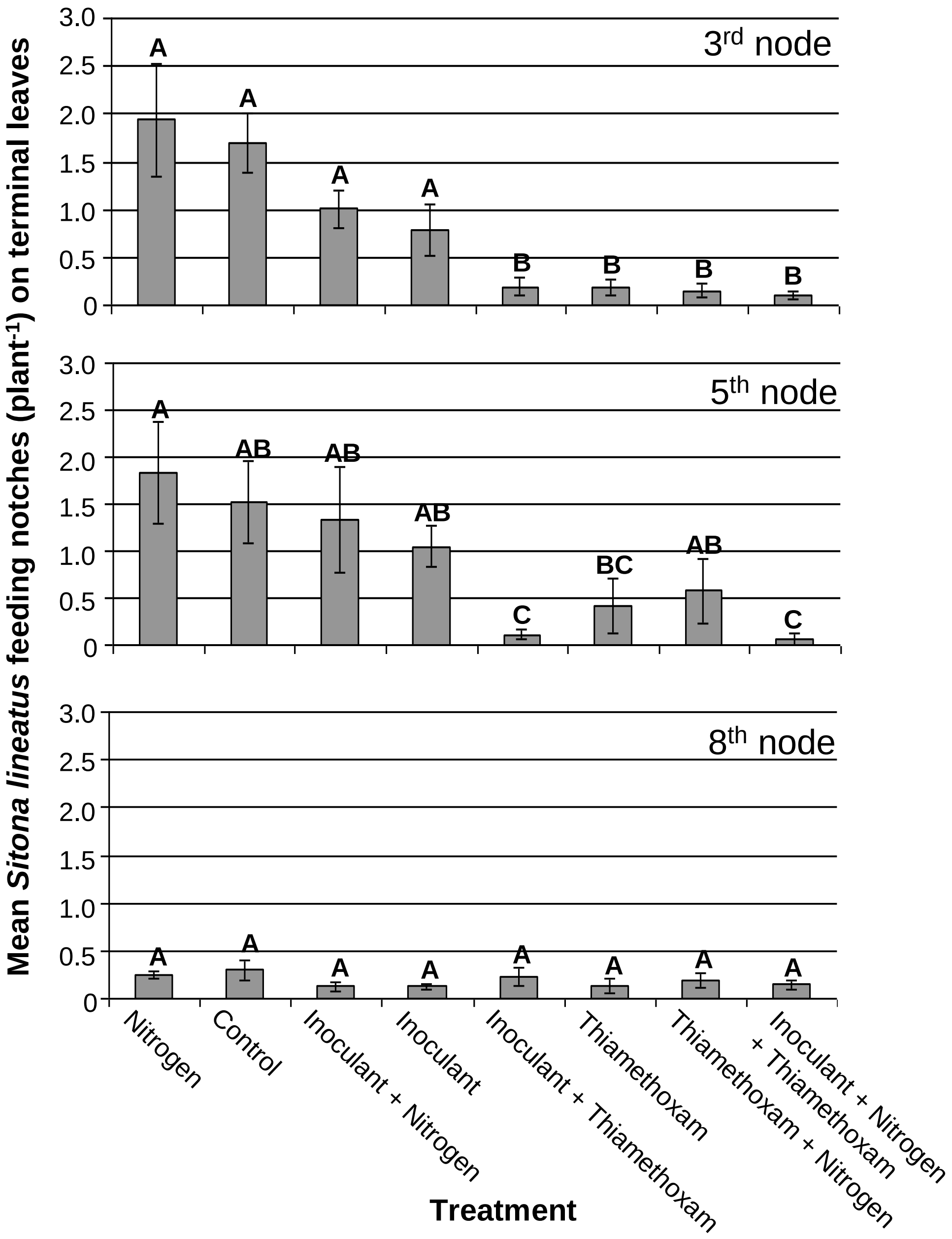

Figure 2.2. Mean *Sitona lineatus* feeding notches on the terminal leaves (± S.E.) of *Pisum sativum* plants at Lethbridge and Vauxhall, Alberta in 2009, given for assessments at the three-, five-, and eight-node growth stages. Means with the same letter are not significantly different ($p > 0.05$).

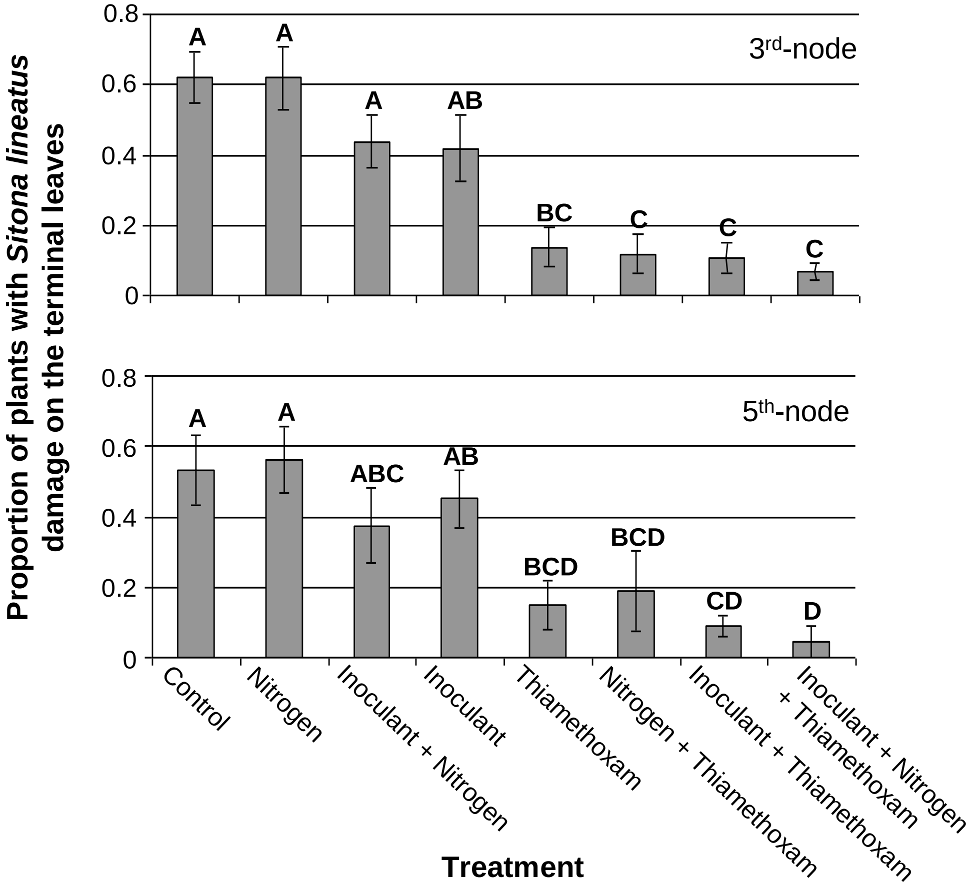

Figure 2.3. Mean proportion of *Pisum sativum* plants with *Sitona lineatus* damage on the terminal leaves (± S.E.) when plants were assessed at the three- and five-node growth stages at Lethbridge and Vauxhall, Alberta in 2009. Means with the same letters are not significantly different (*p* > 0.05).

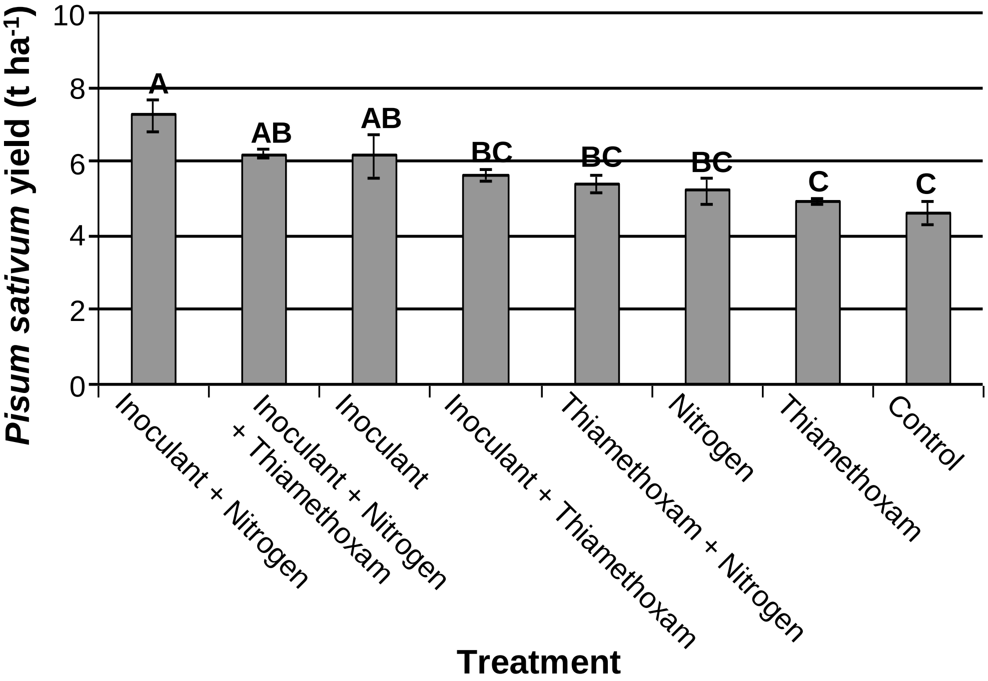

Figure 2.4. Mean yield (± S.E.) of *Pisum sativum* plants under attack by *Sitona lineatus*, given for all plot treatments at Lethbridge, Alberta in 2008, the only site-year where plot treatment had a significant impact on yield. Means with the same letters are not significantly different ($p > 0.05$).

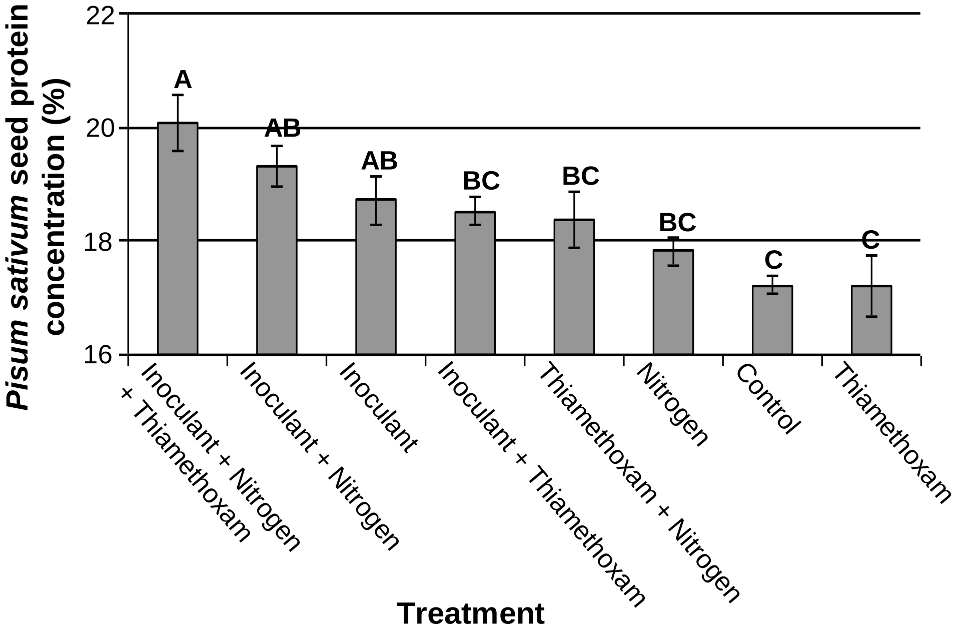

Figure 2.5. Mean protein concentration (± S.E.) of *Pisum sativum* seeds from plants under attack by *Sitona lineatus*, given for all plot treatments at Lethbridge, Alberta in 2008, the only site-year where plot treatment had a significant effect on seed protein concentration. Means with the same letters are not significantly different ($p > 0.05$).

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3. Chapter Three: Response of *Pisum sativum* L. (Fabales: Fabaceae) to *Sitona lineatus* L. (Coleoptera: Curculionidae) infestation: effect of adult weevil density on damage, larval population and yield loss.

3.1. Introduction

The pea leaf weevil, *Sitona lineatus* L. (Coleoptera: Curculionidae), is a pest of field peas, *Pisum sativum* L., and faba beans, *Vicia faba* L. (Fabales: Fabaceae), in Europe, Africa and North America (Jackson 1920; Hoebeke and Wheeler 1985; Vankosky *et al.* 2009). Overwintered *S. lineatus* migrate to field pea or faba bean fields in early spring after temperatures reach a threshold of 12°C and the photoperiod is appropriate (Hans 1959; Hamon *et al.* 1987). Adults consume field pea and faba bean foliage, leaving "U"-shaped notches along the margin of the leaves (Jackson 1920; Nielsen 1990). After feeding begins, weevils mate and females oviposit over the soil surface (Jackson 1920; Hoebeke and Wheeler 1985). Larvae burrow into the soil and feed upon *Rhizobium leguminosarum* Frank (Rhizobiales: Rhizobiaceae), the symbiotic bacterium of field pea and faba bean root nodules that fix nitrogen (Jackson 1920; Johnson and O'Keeffe 1981; Hoebeke and Wheeler 1985). *Sitona lineatus* feeding rarely kills the host plant. However, El-Dessouki (1971) observed that severe damage to root nodules by larvae decreased pod production by 27% and Nielsen (1990) observed that foliar damage by adults reduced photosynthetic area which in turn reduced pod production of faba beans by 28%.

To prevent yield loss and ensure that nitrogen inputs to the soil remain high in legume production systems, integrated pest management (IPM) programs for *S. lineatus* are under investigation. Economic injury levels and economic thresholds are pivotal concepts of IPM, designed to reduce reliance upon insecticide applications (van den Bosch and Stern 1962; Pedigo *et al.* 1986; Higley and Pedigo 1993). A number of factors should be considered when developing economic thresholds, including the response of the plant to pest pressure and plant compensation (Cammell and Way 1987). Economic threshold estimates should be developed for the insect stage that induces the greatest damage and can be controlled by insecticides (Stern 1973). However, not all pests are most damaging to their hosts at a time when they can be controlled, and control can be further complicated when more than one life stage causes economic damage. This is the case for *S. lineatus*, as adults and larvae can both reduce yield, and the larvae, which are more devastating to the plant (Doré and Meynard 1995; Hunter 2001; Corre-Hellou and Crozat 2005), are concealed in root nodules beneath the soil (Jackson 1920; King 1981; Bardner *et al.* 1983; Ester and Jeuring 1992).

Developing an accurate economic threshold for *S. lineatus* poses a significant challenge, as reflected by current economic threshold estimates. Examples include thresholds of 10 eggs per plant (Doré and Meynard 1995), three or more adult weevils per 180° sweep net sample (Quisenberry *et al.* 2000) and 30% of plants with damage on the terminal leaf (El-Lafi 1977). The practicality of the first two economic threshold estimates is arguable, as weevils are known to “play dead” thus avoiding sweep net capture (Jackson 1920) and based on my experience in the laboratory it is not practical to count eggs in the field. Relative to these methods, counting feeding notches is simple, especially if counts are restricted to the terminal leaves as recommended by El-Lafi (1977). Cantot (1986), for example, developed a ranking system for leaf notching with four levels (zero to three, three being severe and representing 15 or more notches on the first node leaves), and found that severe leaf notching corresponded to 12 larvae per plant, which destroyed 90% of root nodules.

Although Cantot's work yielded a strong predictive relationship between foliar consumption and root nodule damage, his work has been criticized for failing to consider factors such as plant density in determining that relationship (Nielsen 1990). Plant response to pest pressure is affected by several factors, including the density of the pest population, pest regulation by natural enemies, intraspecific competition, host variety, host carrying capacity and the size and health of the plant stand being attacked (El-Dessouki 1971; Nielsen 1990; Landon *et al.* 1995). Two important considerations for *P. sativum* production are the concentration of available soil nitrogen and the presence of symbiotic *Rhizobium* bacteria, which are required for root nodule development (Allen and Allen 1981). High levels of soil nitrogen inhibit root nodule formation (George 1962; Allen and Allen 1981); however, nitrogen availability can protect yield when nodule losses occur (George 1962). The objective of this research was to investigate the relationship between adult *S. lineatus* density, larval density, above- and below-ground damage and yield loss when *P. sativum* plants were inoculated with *R. leguminosarum* bacteria and fertilized with nitrogen (N). I also investigated the economic threshold estimate of 30% of plants with terminal leaf damage as described by El-Lafi (1977) in Alberta, Canada, where *S. lineatus* has recently become an established pest (Vankosky *et al.* 2009).

3.2. Methods and Materials

3.2.1. Sites and Treatments.

In 2008 and 2009, exclusion cages were placed on field pea plots at two sites to manipulate *S. lineatus* population density. Study sites were located at Lethbridge (49°41’39”N, 112°49’58”W) and Vauxhall (50°04’08”N, 112°05’51”W), Alberta, Canada. In 2008, the field pea cultivar ‘Cutlass’ was sown; in 2009, ‘Cutlass’ seed was unavailable and plots were sown with ‘CDC Bronco.’ Both cultivars were developed for Canadian agroecosystems and exhibit powdery mildew resistance (Blade *et al.* 2004; Warkentin *et al.* 2005). Planting dates are given in Table 3.1. The mean minimum and maximum temperature (°C), total precipitation (mm) and extreme wind speed (km h^{-1}) for the growing season, 01 May to 31 August, are given for each site and year in Table 3.2. Cages were placed on field pea plots that were treated with *R. leguminosarum* biovar *viciae* inoculant only and *Rhizobium* inoculant plus N fertilizer. N-Prove® inoculant, containing *R. leguminosarum* was applied as a seed treatment at 1.6 g per kg of seed and nitrogen was applied as urea at 60 kg ha^{-1}. Edge ™ (ethalfluralin, 1.1 kg a.i. ha^{-1}), a pre-emergent herbicide, was applied at Vauxhall where weeds were historically present in high densities. For the remainder of the growing season, weeds were controlled by hand weeding at both sites. All plots were fertilized with phosphorous as P_2O_5 at 16.8 kg ha^{-1} and seeds were treated with a broad spectrum fungicide, Apron Maxx® (3.75 g mefenoxam and 2.5 g fludioxonil per 100 kg seed), to prevent disease infection.

Cages were placed on field pea plots immediately after seeding to exclude *S. lineatus* adults (Table 3.1). Cages enclosed 1.0 m^2 and were held upright using rebar stakes at each corner. To prevent movement of weevils and other insects into and out of the cages, canvas flaps around the bottom of each cage were placed flush on the ground and covered with at least 10 cm of packed soil. Plant density was maintained at 32 plants per cage following plant emergence (after Nielsen 1990). Weevils were added to the cages at the two- to three-node growth stage, following the Biologische Bundesanstalt Bundessortenamt and Chemical Industry (BBCH) Phenological Growth Stage key (Weber and Bleiholder 1990; Feller *et al.* 1995), on the dates given in Table 3.1. *Sitona lineatus* adults to be placed in the cages were collected by hand from field pea plots near Lethbridge and held on alfalfa (*Medicago sativa* L., Fabales: Fabaceae) in the laboratory. Weevils were sexed following Jackson (1920) and equal proportions of males and females were added to each cage. At Lethbridge, five weevil densities were tested: 1) uncaged control, 2) caged control, 3) 0.25 weevils $plant^{-1}$, 4) 0.50 weevils $plant^{-1}$, and 5) 1.00 weevils $plant^{-1}$. At Vauxhall a sixth weevil density of 0.13 weevils $plant^{-1}$, was included. No weevils were added to the caged control and the uncaged control reflected the ambient *S. lineatus* population density at each site.

3.2.2. Above-Ground Plant Damage Assessment.

Sitona lineatus adult feeding upon pea foliage was assessed twice in June of both years when plants were at approximately the five- and eight-node growth stages. All assessment dates are provided in Table 3.1, and on each date all 32 plants in each cage were assessed. For each plant, the growth stage and the number of feeding notches on each node and on the terminal leaf was recorded. Feeding notches were defined as crescent or “U”-shaped notches along the leaf margin. The mean number of feeding notches on each plant and the proportion of plants with terminal leaf damage was determined for each cage. Using the proportion of plants with terminal leaf damage at the five-node stage, each cage was ranked as follows: 0 = zero plants with damage, 1 = less than 10% of plants with damage, 2 = 10 to 19%, 3 = 20 to 29%, 4 = 30 to 39%, 5 = 40 to 49%, 6 = 50% or greater.

3.2.3. Root Nodule Assessment.

One sample of three whole plants (shoots and roots) and the surrounding soil was harvested from each cage in early July of 2008 and 2009 (Table 3.1). Plants were harvested at peak flower (BBCH stage 60 to 67), which corresponds to peak nodule activity (Depret and Laguerre 2008) and when all larvae that are expected to reach the adult stage are present (Doré and Meynard 1995). Roots and the surrounding soil were enclosed in plastic bags that were sealed around the stems, leaving the foliage free. Each sample was labeled and stored at approximately 4°C for one to two weeks while awaiting assessment in the laboratory.

Just prior to assessment, each sample was washed to clean the soil from the root nodules. Any loose larvae and pupae were collected, enumerated and stored in 70% (v/v) ethanol. After washing, foliage was separated from the roots. Foliar damage was assessed as described above. Using a dissecting microscope, every root nodule on each plant was examined. Parameters of interest included the type of nodule (single-lobed or tumescent with multiple protuberances), the number of protuberances on tumescent nodules, nodule color, feeding damage and the presence or absence of larvae inside nodules. The total number of root nodules on each plant was recorded. Root nodule color was recorded as an indicator of leghaemoglobin expression, where pink nodules express leghaemoglobin and fix nitrogen, while green or white nodules do not (Allen and Allen 1981). Therefore, the number of nodules fixing nitrogen was estimated based on leghaemoglobin expression. Larvae found in root nodules were carefully removed using a scalpel and preserved in 70% (v/v) ethanol.

3.2.4. Cage Harvest.

Upon plant senescence in August, cages were harvested by hand (Table 3.1). Plants were clipped off at the soil surface, counted, placed in harvest sacs and allowed to dry for several days at 35°C until completely dehydrated. When *P. sativum* plants were dry, pea biomass was measured and harvest sacs were threshed by hand. Total seed weight and total aboveground biomass were reported per plant for each cage. After plants were harvested in 2009, new generation adult *S. lineatus* were collected from all cages except the uncaged controls. Weevil collections were performed for a standard period of 5.00 min and enumerated in the laboratory to give an estimate of the total population of new generation adults produced in each cage.

The yield loss (%) for all cage treatments was calculated by comparing yields from cages with 0.13, 0.25, 0.50 and 1.00 weevil plant^{-1} with yield of control cages. The economic impact associated with yield loss for each cage treatment was calculated based on the projected yield of 'Cutlass' field pea of 4.79 t ha^{-1}, as estimated by Blade *et al.* (2004) and the average price of field pea seed in June 2010 (\$179.00 t^{-1}, see Alberta Canola Producer's Commission, 2010). An example calculation is given below for the yield of 0.50 weevils plant^{-1} at Lethbridge. All monetary values are given in Canadian currency.

Yield Loss = 1 - (Yield of cage treatment / Yield of control)

= [1 - (9.87 / 10.83)]

= 0.0886 or 8.86%

Projected yield loss = Yield loss x Projected yield

= 0.0886 x 4.79 t ha^{-1}

= 0.4244 t ha^{-1}

Projected monetary loss (ha-1) = Projected yield loss x Field pea price

= 0.4244 t ha^{-1} x \$179.00 t^{-1}

= \$75.97 ha^{-1}

3.2.5. Statistical Analysis.

Regression and correlation models were used to investigate the relationships between damage parameters, weevil density and yield, using the PROC REG and PROC CORR functions (SAS Institute 2004). A split-plot ANOVA analysis was used to determine the effect of plot treatment (inoculant and inoculant plus N fertilizer), weevil density and the interaction therein using the PROC MIXED function (SAS Institute 2004) for damage and yield parameters. Where significant effects of weevil density were observed for any damage or yield parameter, contrasts were used to compare each density to the control. Significant differences in the degree of weevil damage or yield resulting from treatment (inoculant or inoculant plus N fertilizer) were determined directly using the LSMEANS function (SAS Institute 2004). Cage data were combined from 2008 and 2009 for each site and each site was analyzed independently.

To investigate the validity of the economic threshold of 30% of plants with terminal leaf damage (El-Lafi 1977) for the Canadian prairies, the proportion of plants with terminal leaf damage was calculated for each cage at the five-node growth stage. All cages from both years were pooled and a rank was assigned to each cage based on the proportion of plants damaged in that cage. Regression analyses (see above) were used to determine if a relationship between cage rank and yield or damage parameters existed. The PROC GLM function for ANOVA analysis (SAS Institute 2004) was used to determine if cage rank had a significant effect on damage and yield parameters. Where cage rank was significant, contrasts were used to compare the degree of damage or amount of yield observed when 30 to 39% of plants had terminal leaf damage to all other ranks.

3.3. Results

3.3.1. Above-ground Plant Damage Assessment.

Plot treatment (inoculant or inoculant plus N fertilizer) had no significant effect on *S. lineatus* foliar feeding damage at the five- or the eight-node growth stages at either site. At Lethbridge, weevil density had a significant effect on *S. lineatus* feeding notches per plant at the five- ($F_{4, 64} = 20.56$, $p < 0.0001$) and the eight-node stages ($F_{4, 64} = 18.53$, $p < 0.0001$); similar results were observed at Vauxhall (five-node: $F_{5, 83} = 9.08$, $p < 0.0001$; eight-node: $F_{5, 83} = 11.24$, $p < 0.0001$) (Figure 3.1). At Lethbridge, plants in control cages had significantly less *S. lineatus* feeding damage than was observed in cages with 0.50 and 1.00 weevil plant^{-1} and the uncaged controls at the five-node stage. At the eight-node stage, control cages had significantly less damage than the 0.25, 0.50 and 1.00 weevil plant^{-1} treatments and the uncaged controls. Uncaged control plants had the most *S. lineatus* feeding damage at the five-node stage with a mean of 27.96 ± 3.42 (± S.E.) notches per plant. At Vauxhall, significantly greater feeding damage was observed on plants in cages where the adult weevil density was 0.50 and 1.00 weevil plant^{-1} and the uncaged controls, relative to damage in the control cages ($p < 0.05$) at both the five- and eight-node stages (Figure 3.1). Plants in the caged controls (no weevils added) experienced minor feeding damage at both sites.

At Lethbridge, plot treatment (inoculant or inoculant plus N fertilizer) had no effect on the proportion of plants with damage on the terminal leaves at either growth stage. At Vauxhall, plot treatment had a significant effect on the proportion of plants with terminal leaf damage when assessed at the eight-node stage ($F_{1, 81} = 4.24$, $p = 0.0428$). The proportion of plants with terminal leaf damage was greater when plants were treated with inoculant plus N fertilizer (6.70 ± 1.37 %) than in cages with inoculant only (3.30 ± 1.01 %). At Lethbridge, weevil density had a significant effect on the proportion of plants with terminal leaf damage at the five- ($F_{4, 64} = 10.84$, $p < 0.0001$) and eight-node stages ($F_{4, 64} = 16.36$, $p < 0.0001$; Figure 3.2). On both assessment dates, fewer plants in control cages had damage on the terminal leaves relative to plants in the uncaged control, and at the five-node stage, control cages had fewer damaged plants than cages with 1.00 weevil plant^{-1} (Figure 3.2). No significant effect of weevil density was observed for either assessment stage at Vauxhall (five-node: $F_{5, 83} = 2.05$, $p = 0.0805$; eight-node: $F_{5, 83} = 1.92$, $p = 0.0993$).

3.3.2. Below-ground Plant Damage Assessment.

At Lethbridge only, plot treatment had a significant effect on total root nodules ($F_{1, 64} = 4.66$, $p = 0.0347$), tumescent root nodules ($F_{1, 64} = 9.05$, $p = 0.0038$) and nodules expressing leghaemoglobin ($F_{1, 64} = 10.29$, $p = 0.0021$). Plants treated with inoculant only had significantly more root nodules (46.76 ± 3.51) than those treated with inoculant plus N fertilizer (38.64 ± 2.81). Similar results were observed for tumescent root nodules (inoculant = 31.28 ± 3.30, inoculant plus N fertilizer = 22.00 ± 2.34). More nodules expressed leghaemoglobin on plants treated with inoculant plus N fertilizer (16.65 ± 1.23) than with inoculant only (14.95 ± 1.09).

Adult weevil density did not have a significant effect on any root nodule parameter at Vauxhall, including the mean number of damaged root nodules per plant ($F_{5, 82} = 1.79$, $p = 0.1237$) and larvae per plant ($F_{5, 82} = 0.65$, $p = 0.6599$). At Vauxhall, 39% of root nodules, on average, were damaged by *S. lineatus* larvae when adult density was 1.00 weevil plant^{-1}, relative to 29% damaged by 0.50 weevils plant^{-1} and 25% or less damaged in the remaining density treatments. The number of larvae per plant ranged from 0.78 ± 0.16 where the adult density was 0.13 weevil plant^{-1} to 1.57 ± 0.68 in control cages.

At Lethbridge, weevil density had significant effects on the total nodules per plant, tumescent nodules per plant, and nodules expressing leghaemoglobin (Table 3.3). Weevil density did not significantly affect damaged nodules or the number of larvae per plant (Table 3.3). Plants in control cages had the most root nodules and significantly more nodules than the uncaged control. Plants in control cages also had more tumescent nodules and more nodules expressing leghaemoglobin than plants exposed to the other weevil densities. Although the differences were not significant, 62% of root nodules were damaged by *S. lineatus* larvae on average in the control cages, relative to approximately 85% of root nodules in all other density treatments.

3.3.3. Cage Harvest.

At Lethbridge, plot treatment had a significant effect on plant biomass ($F_{1, 64} = 14.85$, $p = 0.0003$); mean biomass was greater where plants were treated with inoculant plus N fertilizer (18.82 ± 0.56 g plant^{-1}), relative to those treated with inoculant only (15.71 ± 0.55 g plant^{-1}), a difference of 3.11 g plant^{-1} or 16.52%.

Seed weight was also affected by plot treatment ($F_{1, 64}$ = 12.10, *p* = 0.0009), with greater mean yields observed on inoculant plus N fertilizer plots (10.38 ± 0.34 g plant^{-1}) relative to inoculant only plots (8.70 ± 0.38 g plant^{-1}), a difference of 1.68 g plant^{-1} or 16.18%. At Vauxhall, plot treatment did not have a significant effect on biomass ($F_{1, 83}$ = 1.47, *p* = 0.2290) or on seed weight ($F_{1, 83}$ = 0.77, *p* = 0.3834).

At Lethbridge, adult weevil density had a significant effect on foliar biomass ($F_{4, 64}$ = 2.25, *p* = 0.0496) and seed weight ($F_{4, 64}$ = 4.97, *p* = 0.0015). The mean biomass and seed weight (± S.E.) are given in Table 3.4. When means for each cage treatment were compared to the control using contrasts, the uncaged controls had significantly less biomass and seed weight (Table 3.4). At Vauxhall, adult weevil density had a significant effect on foliar biomass ($F_{5, 83}$ = 3.06, *p* = 0.0138), but not on seed weight ($F_{5, 83}$ = 2.10, *p* = 0.0735) (Table 3.4). Foliar biomass in the uncaged controls was the lowest of all density treatments, but no significant contrast results were observed when alpha (0.05) was adjusted to prevent errors in statistical interpretation. No significant effects of weevil density were observed on yield parameters, at either site, when the uncaged control data were excluded from the analysis.

At Lethbridge, seed weight in the uncaged control was 27.70% lower than that of the control cages. Compared to the control cages, seed weight was 8.22, 8.86 and 7.02% lower when adult weevil density was 0.25, 0.50 and 1.00 weevil plant^{-1}, respectively. A yield loss of 8.86% (0.50 weevil plant^{-1}) would cost a producer \$75.97 ha^{-1}. At Vauxhall, seed weight in the uncaged control was 25.38% lower relative to the seed weight of the control cages. Relative to the control, seed weight per plant was 9.96% lower in cages where the adult weevil density was 1.00 weevil plant^{-1} and 1.75 and 2.84% lower in the 0.13 and 0.25 weevil plant^{-1} cages, respectively. A yield loss of 9.96% would cost a producer $85.40 ha^{-1}. Seed weight was 1.08% greater when the adult weevil density was 0.50 weevil plant^{-1}, relative to the control, an increase of 0.10 g plant^{-1}.

No effects of plot treatment or adult weevil density were observed on the number of new-generation adult weevils collected at either Lethbridge or Vauxhall in 2009. At Lethbridge, between five and nine larvae were collected per plant, compared to only one to two per plant at Vauxhall. Approximately 50.71 ± 4.77 and 42.90 ± 4.88 new-generation adults were collected from each cage at Lethbridge and Vauxhall respectively.

3.3.4. Regression and Correlation Analyses for Above- and Below-Ground Damage Parameters and Cage Yield.

Regression analyses for Lethbridge are summarized in Table 3.5. At Lethbridge, increasing adult weevil density was positively related to feeding notches per plant at both the five- and the eight-node stages (Figure 3.3). Weevil density had a significant, but weak positive relationship with the proportion of plants with terminal leaf damage per cage at the five- and eight-node stages (Figure 3.4); similar results were observed at Vauxhall. No significant relationships were observed between adult weevil density and below-ground parameters (total nodules, tumescent nodules, damage nodules, nodules expressing leghaemoglobin and larvae per plant) at either site (Table 3.5). Seed weight and biomass were negatively related to adult weevil density, but not significantly (Vauxhall, seed weight: $p = 0.3310$, $R^2 = 0.0121$, $y = 9.1789 – 0.7654x$; biomass: $p = 0.4860$, $R^2 = 0.0062$, $y = 16.614 – 0.9605x$).

Results of correlation analyses used to investigate the relationship between all possible combinations of above- and below-ground parameters at both Lethbridge and Vauxhall are summarized in Table 3.6, where significance was defined as $p < 0.05$ and/or $r > 0.40$. At Lethbridge, feeding notches at both growth stages were significantly correlated to the proportion of plants with terminal leaf damage, but not to any root nodule parameter. Plant biomass was significantly correlated with seed weight ($p < 0.0001$, $r = 0.8661$), but neither yield parameter was significantly correlated with any other parameter. Total nodules, tumescent nodules, damaged nodules and nodules expressing leghaemoglobin were all significantly correlated with one another, and all but nodules expressing leghaemoglobin were correlated with the mean number of larvae plant^{-1}. Similar results were observed at Vauxhall, as well as a significant correlation between damaged nodules and feeding notches at the five- and eight-node stages (Table 3.5). Nodules expressing leghaemoglobin were also significantly correlated with larvae per plant at Vauxhall.

3.3.5. Ranked Proportion of Plants with Terminal Leaf Damage.

Only four cages had more than 50% of plants with terminal leaf damage. Cage rank had a significant effect on feeding notches at the five- (df = 6, F = 18.15, $p < 0.0001$) and eight-node stages (df = 6, F = 7.84, $p < 0.0001$). Cages where 30 to 39% of plants had terminal leaf damage at the five-node stage had the most feeding notches relative to all other ranks, and significantly more feeding damage than cages with 0, less than 10, and 10 to 19% of plants with damage (Figure 3.5). At the eight-node stage, feeding notches per plant peaked in cages where 30 to 39% of plants had terminal leaf damage and decreased as the proportion of plants with terminal leaf damage increased (Figure 3.5). Cages with no notches on the terminal leaves and with less than 10% of plants with terminal leaf damage had significantly fewer feeding notches than cages with 30 to 39% of plants with damage at the eight-node stage (Figure 3.5). Cage rank did not have a significant effect on yield or root nodule parameters ($p > 0.05$). However, yield in cages with 30 to 39% of plants with terminal damage was 15.98% less than cages where no plants had damage on the terminal leaves, representing a loss of \$137.01 ha^{-1}.

Regression analyses were used to test if cage rank was related to adult and larval *S. lineatus* feeding damage, plant nodulation, and yield (Table 3.7). Feeding notches per plant significantly increased as the proportion of plants with terminal leaf damage increased at the five- (Figure 3.6) and eight-node stages. All other tested relationships were non-significant, although increasing levels of terminal leaf damage did have a slightly negative effect on seed weight, total nodules, tumescent nodules (Figure 3.7), nodules expressing leghaemoglobin and larvae per plant. Increasing levels of terminal leaf damage had a slightly positive effect on the number of damaged nodules per plant.

3.4. Discussion

The extent of foliar damage to *P. sativum* plants in cages at Lethbridge and Vauxhall was significantly affected by adult weevil density, as levels of foliar feeding increased with adult weevil density. My observations agree with the results of Nielsen (1990), who observed a similar strong linear relationship between weevil density and foliar damage in faba beans. Lohaus and Vidal (2010) did not observe this relationship with field peas, which may reflect a lack of resolution as their treatments included only a control, weevil infestation and an uncaged control treatment. Both my study and that of Nielsen (1990) included at least four or five discrete weevil densities.

Evidence of *S. lineatus* adult and larval feeding was observed in control cages, indicating that the cages did not completely exclude weevils. However, I expect that this was the case for all of the cages. Weevils may have entered the control cages during plant assessments, and/or may have been present on the plots when the cages were first installed, even though they were established before plant emergence in the spring. At Lethbridge, the ambient *S. lineatus* population was particularly high, as evidenced by the extreme number of plant notches in the uncaged controls, making complete exclusion of weevils from control cages difficult. It must therefore be assumed that all cages had similar numbers of ambient weevils as the controls, but nevertheless the density-related effects observed here (e.g., Figures 3.2, 3.4 and 3.5) indicate that populations in control cages were still comparatively minor.

The proportion of plants with terminal leaf damage at the five- and eight-node stages increased as adult weevil density increased and was strongly correlated with the total number of feeding notches observed per plant. Assessing *P. sativum* plants for terminal leaf damage to predict adult density and yield loss is more feasible than assessing feeding damage on entire plants, especially if a substantial number of replicate plants are required. For example, 54 notches per plant, on average, were observed in the uncaged controls at the five-node stage, with upwards of 75 notches observed on some individual plants. On the terminal leaves, the number of feeding notches rarely exceeded 10 per plant. Moreover, proportions were determined based on the presence or absence of feeding notches on the terminal leaves of the 32 plants in each cage, which can be determined much quicker than counting all of the notches on 32 plants.

At Lethbridge, control cages had significantly more root nodules, including tumescent and leghaemoglobin expressing nodules, than uncaged controls, which had the fewest nodules relative to all other cage treatments. Plants in uncaged controls had approximately 50% fewer root nodules than plants in the caged controls, which could be attributed to a cage or microclimate effect, differing levels of adult *S. lineatus* feeding pressure or rates of plant compensation. I observed that the soil inside the cages was normally wetter than the soil outside the cages (i.e., in the uncaged controls). Drought conditions inhibit root nodule formation (Allen and Allen 1981; Whitehead 1983) and it is possible that drier conditions outside of the cages significantly inhibited root nodule development. It is also possible that *S. lineatus* consumption of foliar tissues contributed to differences in root nodule numbers between the caged treatments and the uncaged controls. When 50% of white clover, *Trifolium repens* L. (Fabales: Fabaceae), foliage was lost, nodule weight was reduced, the rate of nodule senescence increased and N-fixation was reduced by more than 70% (Ryle *et al.* 1985). Although *S. lineatus* foliar consumption did not reach levels approaching 50%, plants in the uncaged controls, especially at Lethbridge, were exposed to long-term defoliation by large populations of adult weevils, which may have contributed to the difference in root nodule numbers between uncaged and caged treatments.

The number of damaged nodules and larval density per plant were not affected by adult *S. lineatus* density at either site. At Vauxhall, one to two larvae were observed per plant, relative to five to nine larvae per plant at Lethbridge. The difference in larval populations between the two sites possibly reflects the higher concentration of sand in the soil at Vauxhall, which made it difficult to extract intact root systems and all of the surrounding soil (the total number of nodules sampled per plant at Vauxhall was also lower than at Lethbridge). As a result, larval populations at Vauxhall were likely underestimated due to sampling error, as emerging new generation adults at the two sites were not significantly different. Although larval populations at Vauxhall appeared to be low compared to those at Lethbridge, it should be noted that Ester and Jeuring (1992) observed that as few as two larvae per plant caused economic yield losses and that larval damage to *P. sativum* plants is considered to be more damaging to plants than foliar consumption (Doré and Meynard 1995; Hunter 2001; Corre-Hellou and Crozat 2005).

The carrying capacity of an environment is determined by the number of individuals that can be supported by that environment (Ricklefs 2001). The availability of root nodules has been hypothesized to exert a carrying capacity effect on larval populations of *Sitona* weevils, including *S. lineatus* and *S. hispidulus* Gyllenhal (Coleoptera: Curculionidae) (Goldson and French 1983; Quinn and Hower 1986; Nielsen 1990; Lohaus and Vidal 2010). Nielsen (1990), for example, observed that larval density on faba beans reached a maximum of 13 per plant, regardless of the number of eggs laid or adult density, and attributed this effect to intraspecific competition among larvae due to carrying capacity restraints. Similar results were observed in greenhouse experiments with *S. hispidulus*, as El-Dessouki and Stein (1970) observed that when 50 or 100 eggs were added to individual plants, there was no significant difference in the number of larvae recovered. *Sitona lineatus* females can produce more than 1655 eggs per female (Jackson 1920). In my study, only 32 plants were available for larval habitat in each cage, and it appears that even at the lowest density of 0.13 weevils plant^{-1}, the carrying capacity of each plant was overwhelmed, as I did not observe significantly greater levels of larval damage to root nodules as adult weevil density increased. Thus, I did not observe a relationship between adult and larval *S. lineatus* density per plant as adult density increased, nor did I observe a significant correlation between above-ground damage parameters and larval density.

At both Lethbridge and Vauxhall, uncaged controls had less biomass and seed weight than plants growing in cages. A microclimate effect was not unexpected, as Williams *et al.* (1995) observed a cage effect in a similar study. However, Williams *et al.* (1995) observed that yield in cages was reduced relative to uncaged controls, and attributed this to increased shading and increased humidity within the cages. In southern Alberta, summers are dry and windy, with winds reaching extremes of up to 95 km h^{-1} during the growing season (Table 3.2). Cages would have protected plants from the wind, as well as creating shady and humid conditions that would have favored growth relative to conditions in uncaged controls. Plants inside cages were taller and had larger leaves and stems than plants in the uncaged controls.

Biomass and seed weight were affected by plot treatment at Lethbridge, as plants treated with inoculant plus N fertilizer had greater biomass and seed weight. For both parameters, plants treated with inoculant plus N fertilizer yielded approximately 16% more than plants treated with inoculant alone, suggesting that nitrogen had a positive impact on plant development, at least within the cages. Based on these results, and those of George (1962), fertilizing *P. sativum* with nitrogen may help prevent yield losses when plants are under attack by *S. lineatus*. Whether N fertilizer improved the compensatory ability of pea plants is unclear, as is the effect of N fertilizer on uncaged plants. In open plot studies, no significant differences in yield were observed between plots receiving inoculant plus N fertilizer and inoculant only (Chapter 2). Moreover, plants receiving only N fertilizer yielded 0.92 t ha^{-1} less than plants treated with inoculant only (Chapter 2). It is possible that the effect of N fertilizer is only significant when coupled with a microclimatic effect as discussed above.

Plot treatment had a significant effect on total nodules, tumescent nodules and leghaemoglobin expressing nodules, which was expected as nitrogen is known to inhibit root nodule formation (George 1962; Allen and Allen 1981). Interestingly, N-fertilized plants had more nodules expressing leghaemoglobin. It is possible that N fertilizer delayed nodulation as noted by Gibson and Harper (1985), resulting in more functioning nodules on N-fertilized plants at maximum flowering, while nodules on plants treated with inoculant only were senescing at that time. At Lethbridge, plants receiving N fertilizer had fewer damaged nodules and supported fewer larvae than plants treated with inoculant only. Delayed nodule formation, delayed peak nodule activity and the ability to support fewer nodules may have an impact on yield, as greater seed weights were also observed on inoculant plus N fertilizer plots, as discussed above.

Foliar consumption increased as weevil density increased, but no significant negative relationship was observed between increasing adult weevil density and yield parameters. Although George *et al.* (1962) observed that at least 75% of foliage must be lost to defoliation for a significant yield loss to be observed, other work has shown that defoliation does contribute to yield loss. For example, Nielsen (1990), working with faba beans observed that pods, harvestable seeds and yield decreased as weevil density in cages increased. Lohaus and Vidal (2010) observed that seed production decreased by 18% when weevil density was 0.2 individuals plant^{-1} relative to the control. At Lethbridge, when weevil density was 0.50 individuals plant^{-1}, seed weight was reduced by 8.86% relative to the control, which was not significant, but represents a loss of \$75.90 ha^{-1}, based on the projected yield of 'Cutlass' peas and field pea prices in June 2010. A loss of \$75.90 ha^{-1} may be a significant economic loss for producers.

When cages were ranked based on the proportion of plants with terminal leaf damage, only feeding notches at the five- and eight-node stages showed a significant linear relationship with rank. Foliar damage peaked when 30 to 39% of plants had feeding notches on the terminal leaf, suggesting that intraspecific competition among adult weevils for foliage occurs at very high population densities. However, the number of tumescent nodules did decrease slightly as the proportion of terminal leaf damage increased, suggesting that foliar damage does impact nodule development. No relationship was observed between the proportion of plants with terminal damage and root nodule or yield parameters, suggesting that field peas have a carrying capacity for *S. lineatus* larvae and above-ground parameters cannot be used to predict larval populations or the extent of larval damage to root nodules.

The size of *S. lineatus* larval populations and the degree of root nodule damage does appear to have a greater impact on yield than above-ground damage parameters, as proposed by Doré and Meynard (1995), Hunter (2001) and Corre-Hellou and Crozat (2005). In addition, the results of this study support the conclusion that root nodules are a limited resource for *S. lineatus* larvae (El-Dessouki and Stein 1970; Nielsen 1990). Results from this cage experiment did not provide any evidence of strong relationships that could be used to predict larval densities or yield losses. However, when 30 to 39% of plants had terminal leaf damage, yield was reduced by 15.98% relative to plants with no terminal damage, representing a loss of \$137.01 ha^{-1}. El-Lafi (1977) observed economic losses when more than 30% of plants had terminal leaf damage and a loss of \$137 ha^{-1} is substantial for producers. To prevent substantial economic losses, action against *S. lineatus* must be taken before terminal leaf damage reaches 30% when measured at the five-node growth stage and before adults begin to oviposit, as suggested by Nielsen (1990) and Lohaus and Vidal (2010). Therefore, to control adult foliar damage and prevent larval establishment, foliar insecticides must be applied within one week of weevil arrival (King 1981; Bardner *et al*. 1983; Ester and Jeuring 1992). This approach is reliant upon frequent and effective crop monitoring and the effectiveness of foliar insecticide applications may still be compromised by re-invasions after initial treatment. Because larval damage appears to drive yield responses, a less labor-intensive and perhaps more effective strategy would be to utilize a systemic insecticidal product that can be applied as a seed dressing. Once the active ingredient is translocated throughout the plant, this strategy has the potential to cause mortality of adults feeding on foliage as well as larvae consuming root nodules.

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Table 3.1. Dates of seeding, weevil cage stocking, damage assessment and harvest for Lethbridge and Vauxhall, Alberta in 2008 and 2009.

		Lethbridge		Vauxhall	
Year	Activity	Date	Julian Date	Date	Julian Date
2008	Plot Seeding	30.IV.2008	121	29.IV.2008	120
	Cages Placed	07.V.2008	128	05.V.2008	126
	Cages Stocked	02.VI.2008	154	04.VI.2009	156
	Assessment at 5th-node	06.VI.2008	158	10.VI.2008	162
	Assessment at 8th-node	16.VI.2008	168	17.VI.2008	169
	Collection for Root Nodule Assessment	04.VII.2008	186	03.VII.2008	185
	Cage Harvest*	11.VIII.2008	224	13.VIII.2008	226
2009	Plot Seeding	04.V.2009	124	06.V.2009	126
	Cages Placed	11.V.2009	131	15.V.2009	135
	Cages Stocked	27.V.2009	147	29.V.2009	149
	Assessment at 5th-node	03.VI.2009	154	08.VI.2009	159
	Assessment at 8th-node	15.VI.2009	166	17.VI.2009	168
	Collection for Root Nodule Assessment	07.VII.2009	188	06.VII.2009	187
	Cage Harvest*	18.VIII.2009	230	17.VIII.2009	229

*Cage harvest began on the dates given. Not all cages were harvested on the same date as not all plants were fully senesced. Cage harvest was completed within one week of the start date.

Table 3.2. Mean daily temperature, total precipitation and extreme wind velocity for Lethbridge and Vauxhall, Alberta in 2008 and 2009 from 01 May to 31 August in each year (Environment Canada 2010). Temperature values are given as means for the entire growing season; precipitation is given as the total from May to August.

		Mean Daily Temperature		Total	Extreme
Site	Year	Maximum (°C)	Minimum (°C)	Precipitation (mm)	Wind Speed (km h^{-1})
Lethbridge	2008	22.58	6.94	325	83
	2009	22.53	6.20	204	89
Vauxhall	2008	23.19	8.16	261	95
	2009	22.82	7.51	243	91

Table 3.3. ANOVA results to determine the effect of weevil density on root nodule characteristics, root nodule damage and larval density at Lethbridge, Alberta. Mean values (± S.E.) are given for all below-ground parameters. Significant results are given in bold font ($p < 0.05$). For parameters significantly affected by weevil density, means with the same letters are not significantly different ($p > 0.05$).

			Treatment Means (± S.E.) by Weevil Density (plant^{-1})				
Parameter	$F_{4, 64}$	*p*-value	Control	0.25 plant^{-1}	0.5 plant^{-1}	1.0 plant^{-1}	Uncaged
Total Root Nodules†	3.72	**0.0088**	52.18 ± 4.63a	39.84 ± 5.49ab	41.58 ± 5.34ab	46.32 ± 4.58ab	29.52 ± 3.12b
Tumescent Nodules†	5.99	**0.0004**	38.22 ± 4.35a	24.79 ± 4.68ab	24.93 ± 4.50ab	28.34 ± 3.94a	13.33 ± 2.37b
Nodules Expressing LH†‡	7.32	**<0.0001**	44.56 ± 4.41a	30.86 ± 5.39ab	29.21 ± 3.56ab	30.54 ± 4.35ab	14.41 ± 3.07b
Damaged Nodules†	1.56	0.1944	32.52 ± 5.90	33.58 ± 4.65	35.58 ± 4.77	39.50 ± 4.23	24.93 ± 2.12
Larvae plant^{-1}	1.37	0.2538	5.11 ± 1.18	7.23 ± 1.64	7.84 ± 1.48	8.72 ± 1.42	6.57 ± 1.26

† Indicates parameters were significant effects of plot treatment (inoculant or inoculant plus N fertilizer)
‡ LH = leghaemoglobin

Table 3.4. ANOVA results to determine the effect of adult weevil density on yield parameters, biomass plant^{-1} and seed weight plant^{-1}, at Lethbridge and Vauxhall, Alberta. Mean biomass and seed weight (± S.E.) are given for each density treatment. Significant results are given in bold font (*p* < 0.05). Where significant effects of weevil density were observed on yield parameters, means that are marked with "a" were significantly different from the control.

Parameter (g plant^{-1})	F-value	p-value	Treatment means (± S.E.) by Weevil Density (plant^{-1}) Control	0.13 plant^{-1}	0.25 plant^{-1}	0.50 plant^{-1}	1.00 plant^{-1}	Uncaged
Lethbridge								
Biomass	2.25	**0.0496**	18.63 ± 0.90	.	18.06 ± 0.91	17.87 ± 0.85	18.16 ± 0.82	15.17 ± 1.20**a**
Seed Weight	4.97	**0.0015**	10.83 ± 0.66	.	9.94 ± 0.48	9.87 ± 0.48	10.07 ± 0.47	7.83 ± 0.66**a**
Vauxhall								
Biomass	3.06	**0.0138†**	15.61 ± 1.06	16.59 ± 1.18	16.86 ± 0.85	17.25 ± 1.33	14.96 ± 0.96	12.66 ± 1.02
Seed Weight	2.10	0.0735	9.14 ± 0.61	8.98 ± 0.64	8.88 ± 0.50	9.24 ± 0.84	8.23 ± 0.48	6.82 ± 0.59

† When alpha was adjusted using the Bonferonni adjustment to prevent statistical error, no significant contrasts were observed.

Table 3.5. Summary of regression statistics used to test the relationship between adult weevil density (per plant, *x*-axis) and above- and below-ground feeding damage and yield parameters for all cages combined from 2008 and 2009 at Lethbridge, Alberta. Similar results were observed for cages at Vauxhall, Alberta (not shown). All parameters were tested with one degree of freedom. Uncaged controls were excluded from the regression analysis, as the population density was not controlled.

	Regression Statistics			
y-axis	**F-value**	**p-value**	**R2**	**Equation**
Feeding notches, 5-node stage	20.68	<0.0001	0.2628	y=5.33+13.48x
Feeding notches, 8-node stage	48.87	<0.0001	0.4573	y=16.57+41.40x
Biomass (g plant^{-1})	0.11	0.7427	0.0019	y=18.35-0.38x
Seed weight (g plant^{-1})	0.69	0.4110	0.0117	y=10.44-0.59x
Plant proportion with terminal damage, 5-node stage	6.21	0.0156	0.0967	y=0.04+0.11x
Plant proportion with terminal damage, 8-node stage	7.14	0.0098	0.1132	y=0.03+0.06x
Total nodules (plant^{-1})	0.19	0.6642	0.0033	y=46.29-3.00x
Tumescent nodules (plant^{-1})	1.36	0.2479	0.0229	y=32.17-7.08x
Nodules expressing leghaemoglobin (plant^{-1})	3.46	0.0680	0.0563	y=38.81-11.47x
Damaged nodules (plant^{-1})	1.20	0.2784	0.0202	y=32.16+7.17x
Larvae (plant^{-1})	2.95	0.0914	0.0484	y=5.78+3.30x

Table 3.6. Summary of significant correlation analysis statistics, given for Lethbridge and Vauxhall, Alberta during 2008 and 2009 for studies investigating weevil density effects in caged plants on feeding damage, foliar biomass and root nodule parameters. Only the significant results are presented from the 55 possible combinations of parameters that were tested. The level of significance, α=0.05, was adjusted using the Bonferonni adjustment to prevent statistical errors (adjusted α=0.00091). All parameters are expressed per plant and all r-values are positive.

		Lethbridge		**Vauxhall**	
Variables		**p-value**	**r-value**	**p-value**	**r-value**
Feeding notches, 5-node	Feeding notches, 8-node	<0.0001	0.643	<0.0001	0.893
Feeding notches, 5-node	Proportion with terminal damage, 5-node	<0.0001	0.743	<0.0001	0.465
Feeding notches, 5-node	Damaged nodules	NA	NA	0.0002	0.407
Feeding notches, 8-node	Proportion with terminal damage, 5-node	0.0001	0.481	<0.0001	0.538
Feeding notches, 8-node	Proportion with terminal damage, 8-node	<0.0001	0.555	<0.0001	0.487
Feeding notches, 8-node	Damaged nodules	NA	NA	0.0002	0.410
Biomass (g plant^{-1})	Seed weight (g plant^{-1})	<0.0001	0.866	<0.0001	0.825
Proportion with terminal damage, 5-node	Proportion with terminal damage, 8-node	0.0005	0.446	<0.0001	0.464
Tumescent nodules	Total nodules	<0.0001	0.920	<0.0001	0.833
Tumescent nodules	Nodules expressing leghaemoglobin	<0.0001	0.892	<0.0001	0.835
Tumescent nodules	Damaged nodules	<0.0001	0.745	<0.0001	0.551
Tumescent nodules	Larvae	<0.0001	0.513	<0.0001	0.434
Total nodules	Nodules expressing leghaemoglobin	<0.0001	0.880	<0.0001	0.916
Total nodules	Damaged nodules	<0.0001	0.797	<0.0001	0.603
Total nodules	Larvae	<0.0001	0.532	<0.0001	0.499
Nodules expressing leghaemoglobin	Damaged nodules	<0.0001	0.666	<0.0001	0.501
Nodules Expressing leghaemoglobin	Larvae	NA	NA	<0.0001	0.427
Damaged nodules	Larvae	<0.0001	0.625	<0.0001	0.704

Table 3.7. Summary of regression statistics for above- and below-ground plant damage parameters for cages combined from the Lethbridge and Vauxhall, Alberta sites in 2008 and 2009 and ranked based upon the proportion of plants per cage with terminal leaf damage (*x*-axis). Ranks used were as follows: 0 = 0 plants, 1 < 0.1 plants, 2 = 0.1 to 0.19, 3 = 0.2 to 0.29, 4 = 0.3 to 0.39, 5 = 0.4 to 0.49, and 6 ≥ 0.5 plants. All parameters were tested with one degree of freedom.

	Statistics			
y-axis	**F-value**	**p-value**	**R^2**	**Equation**
Feeding notches, 5-node stage	93.21	<0.0001	0.4031	y=5.66+3.88x
Feeding notches, 8-node stage	44.96	<0.0001	0.2457	y=17.95+7.52x
Biomass (g plant^{-1})	1.51	0.221	0.0108	y=16.75+0.29x
Seed weight (g plant^{-1})	1.26	0.2637	0.0090	y=9.62-0.16x
Total nodules (plant^{-1})	2.36	0.1268	0.0169	y=36.14-1.71x
Tumescent nodules (plant^{-1})	3.61	0.0596	0.0257	y=20.47-1.75x
Nodules expressing leghaemoglobin (plant^{-1})	0.65	0.4227	0.0047	y=26.65-0.81x
Damaged nodules (plant^{-1})	0.05	0.8213	0.0004	y=19.03+0.26x
Larvae (plant^{-1})	1.22	0.2704	0.0089	y=4.15-0.32x

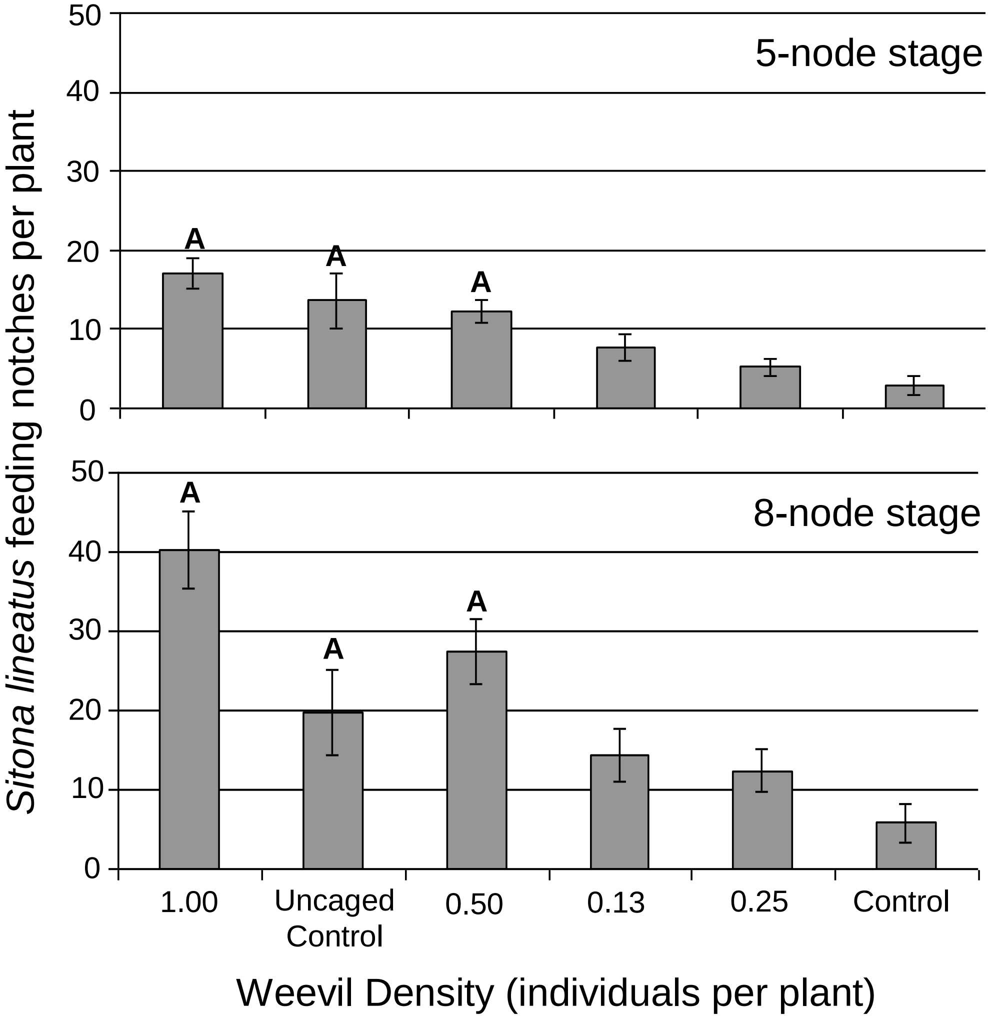

Figure 3.1. Mean *Sitona lineatus* feeding notches per plant (± S.E.) assessed at the five- and eight-node growth stages for various adult weevil densities at Vauxhall, Alberta in 2008 and 2009. Treatment means marked with "A" are significantly different from the control ($p < 0.05$).

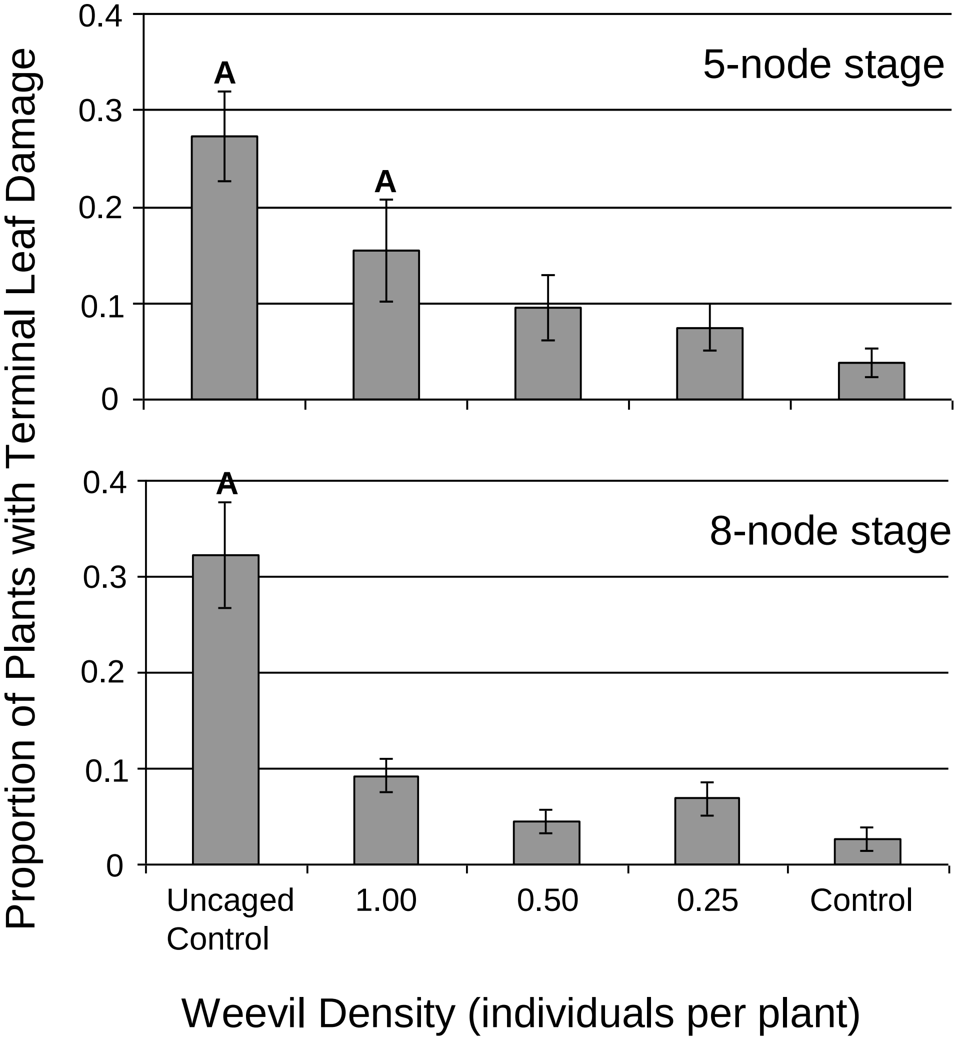

Figure 3.2. The mean proportion (± S.E.) of *Pisum sativum* plants with *Sitona lineatus* feeding notches on the terminal leaves for various adult weevil densities in cages at Lethbridge, Alberta in 2008 and 2009. Treatment means marked with "A" are significantly different from the control ($p < 0.05$).

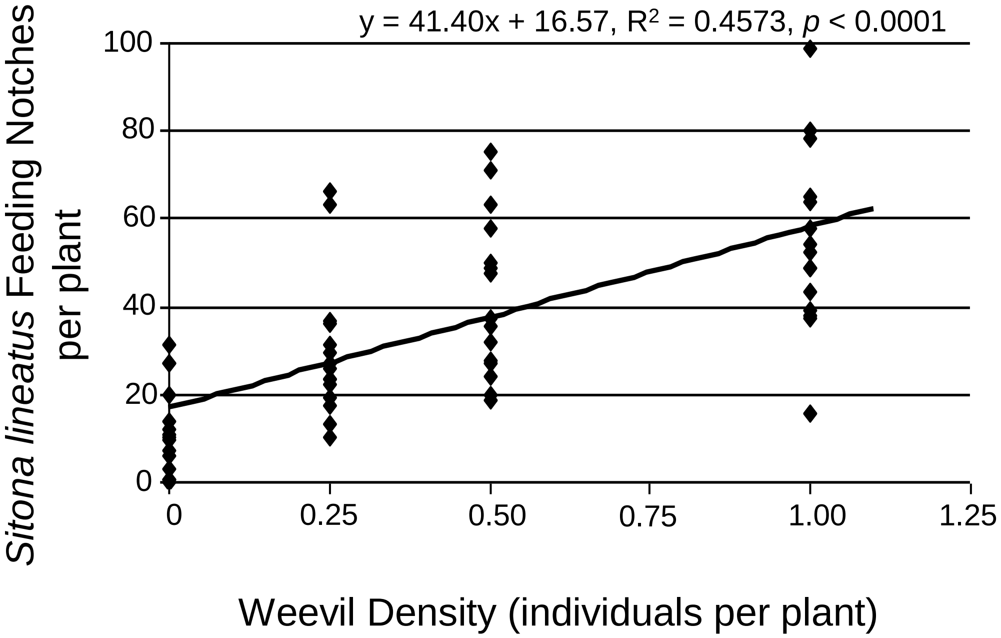

Figure 3.3. The relationship between *Sitona lineatus* foliar damage at the eight-node stage (feeding notches per plant) and adult weevil density (individuals per plant) for cages at Lethbridge, Alberta in 2008 and 2009 when feeding damage was assessed at the eight-node growth stage of *Pisum sativum*.

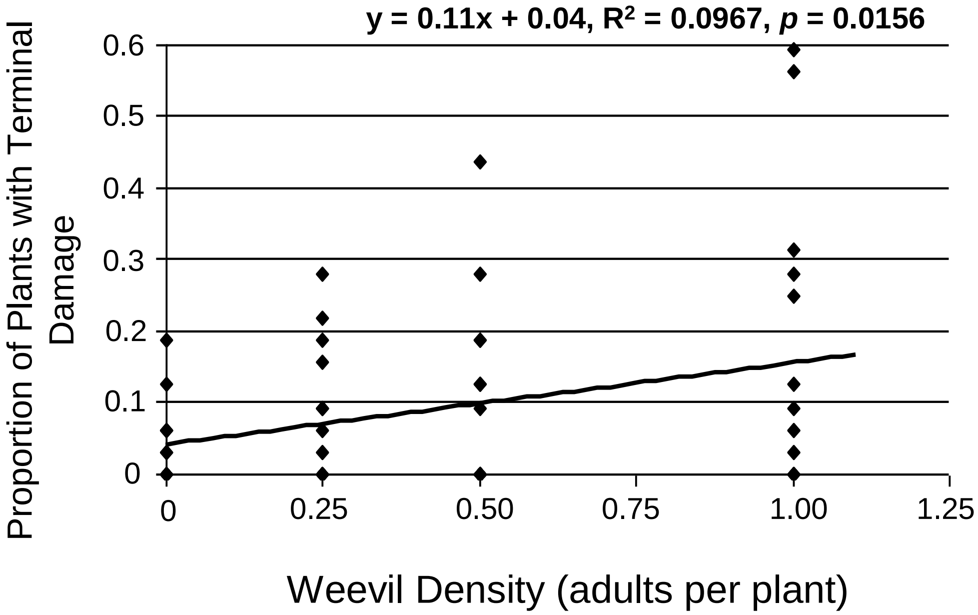

Figure 3.4. The relationship between the proportion of plants with terminal leaf damage at the five-node stage and adult *Sitona lineatus* density for cages at Lethbridge, Alberta in 2008 and 2009 when *Pisum sativum* plants were assessed at the five-node growth stage.

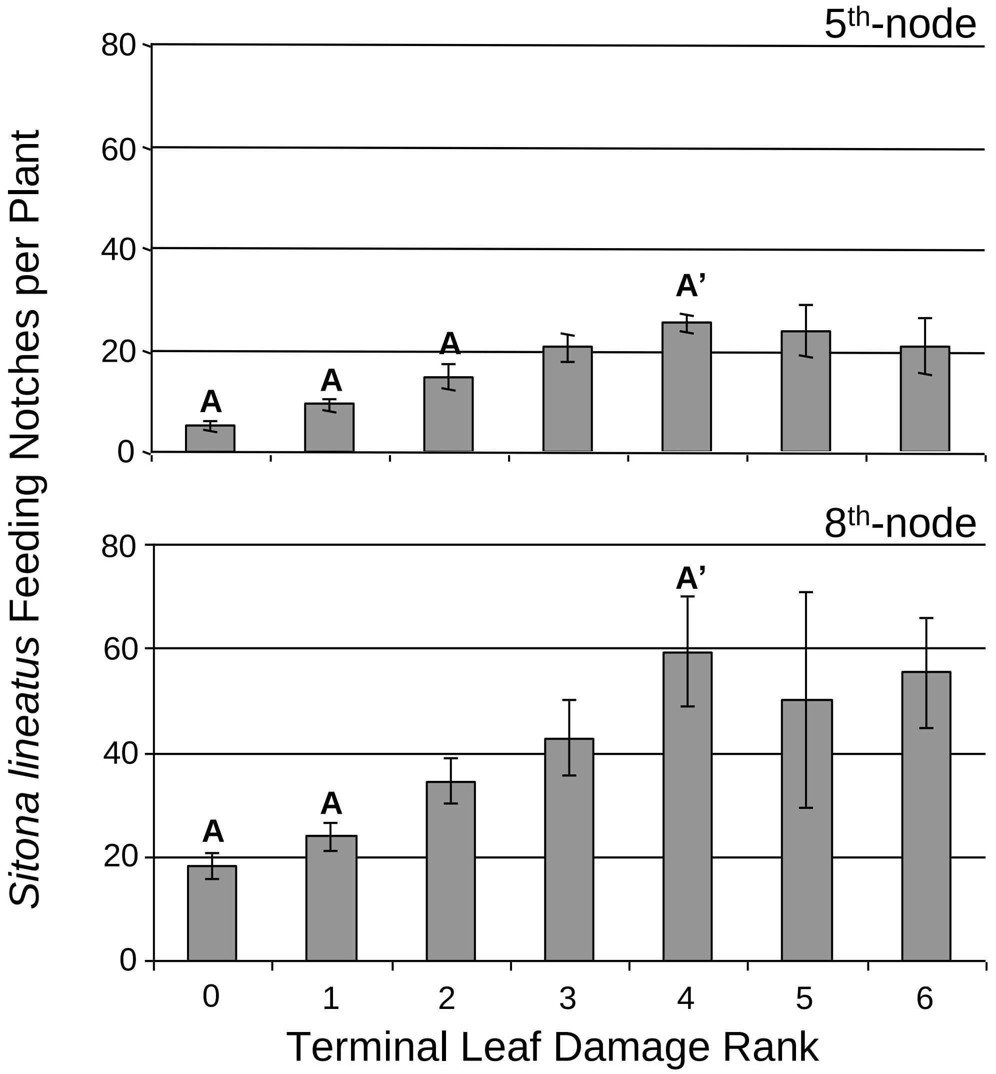

Figure 3.5. Mean *Sitona lineatus* feeding notches on *Pisum sativum* plants (± S.E.) when cages were ranked by the proportion of plants with terminal leaf damage within each cage. Cages from both sites and years were pooled for ANOVA analysis. Ranks were: 0 = zero plants with damage, 1 = less than 10% of plants with damage, 2 = 10 to 19%, 3 = 20 to 29%, 4 = 30 to 39%, 5 = 40 to 49%, 6 = 50% or greater. Treatment means marked with 'A' are significantly different from the economic threshold estimate of 30% of plants with terminal damage given by Rank 4, marked with A' ($p < 0.05$).

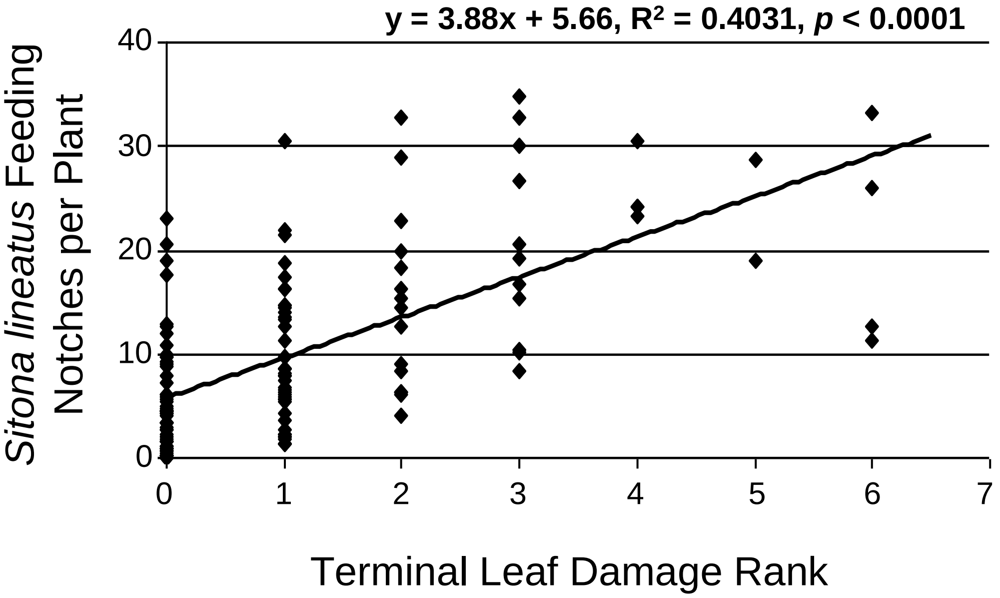

Figure 3.6. The linear relationship between mean *Sitona lineatus* feeding damage at the five-node stage and the rank of each cage as determined by the proportion of plants within each cage with *Sitona lineatus* damage on the terminal leaves. Ranks were as follows: 0 = zero plants with damage, 1 = less than 10% of plants with damage, 2 = 10 to 19%, 3 = 20 to 29%, 4 = 30 to 39%, 5 = 40 to 49%, 6 = 50% or greater.

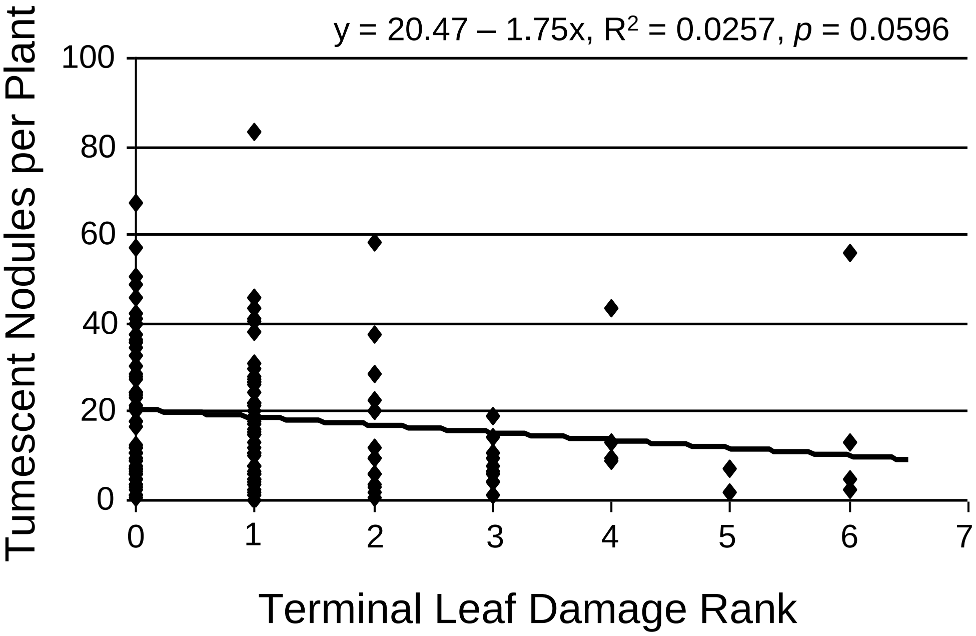

Figure 3.7. The linear relationship between mean tumescent nodules on *Pisum sativum* plants and the rank of each cage as determined by the proportion of plants within each cage with *Sitona lineatus* damage on the terminal leaves. Ranks were as follows: 0 = zero plants with damage, 1 = less than 10% of plants with damage, 2 = 10 to 19%, 3 = 20 to 29%, 4 = 30 to 39%, 5 = 40 to 49%, 6 = 50% or greater.

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4. Chapter Four: Identification of potential natural enemies of the pea leaf weevil, *Sitona lineatus* L. in western Canada.

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4.1. Introduction

The pea leaf weevil, *Sitona lineatus* L. (Coleoptera: Curculionidae), is a serious pest of field pea, *Pisum sativum* L., and faba bean, *Vicia faba* L. (Fabales: Fabaceae), in Europe, Africa and North America (Jackson 1920; Hoebeke and Wheeler 1985). Within the past ten years, *S. lineatus* has become established in southern Alberta, Canada, and has caused substantial economic damage (Vankosky *et al.* 2009). In autumn, *S. lineatus* adults migrate to shelter belts where they consume foliage of secondary leguminous hosts like alfalfa (*Medicago sativa* L., Fabales: Fabaceae) before overwintering (Jackson 1920; Schotzko and O'Keeffe 1988). In early spring, adults migrate to their primary hosts, field pea and faba bean crops, and feed on seedlings (Stein 1972; Fisher and O'Keeffe 1979; Hamon *et al.* 1987; Landon *et al.* 1995). After mating, females oviposit over the soil surface (Jackson 1920; Hoebeke and Wheeler 1985). Larvae burrow through the soil, penetrate root nodules (Jackson 1920; Hoebeke and Wheeler 1985) and feed upon the nitrogen-fixing bacteria, *Rhizobium leguminosarum* Frank (Rhizobiales: Rhizobiaceae) (Johnson and O'Keeffe 1981). Defoliation by adult weevils can account for losses of up to 50% of photosynthetic tissues, limiting the ability of plants to reproduce and support root nodules (Havlickova

1982; Williams *et al.* 1995). Yield losses of up to 28% were attributed to decreased pod production by Nielsen (1990). Larval damage to root nodules can range from 40 to 98% of nodules (El-Dessouki 1971; Cantot 1986; Verkleij *et al.* 1992). Larval feeding reduces seed protein content, especially in nutrient-poor soils, and the amount of nitrogen returned to the soil (Doré and Meynard 1995; Corre-Hellou and Crozat 2005). Control of *S. lineatus* using insecticides is difficult as a result of its high fecundity (Jackson 1920), migratory behavior (Fisher and O'Keeffe 1979; Hamon *et al.* 1987), and concealed larval habitat (Jackson 1920); however, the egg stage of *S. lineatus* is prone to mortality.

Employing biological control agents against *S. lineatus* populations has been explored extensively in Europe. Aeschlimann (1980) identified several potential parasitoids including *Allurus muricatus* (Haliday) and *Microctonus aethiopoides* (Loan), (Hymenoptera: Braconidae). *Patasson lameerei* Debauche (Hymenoptera: Mymaridae) was identified as a parasitoid of non-melanized *S. lineatus* eggs (Aeschlimann 1980; Schotzko and O'Keeffe 1986). Adult ground beetles (Coleoptera: Carabidae), including *Pterostichus cupreus* L., were shown to prey upon adult weevils and *Bembidion properans* (Stephens) was found to prey upon eggs and hatching larvae in Poland (Ropek and Jaworska 1994). Biological control of *S. lineatus* in North America using its natural enemies from Europe may be a viable option.

Before launching studies to assess potential classical biological control agents for introduction to southern Alberta, an evaluation of indigenous predators should be undertaken to determine if any of these species impact *S. lineatus* populations. If successful, a conservation biological control program may be initiated as a significant component of an integrated pest management (IPM) program for *S. lineatus* in Alberta. Conservation biological control programs are designed to promote populations of beneficial species by providing refugia, alternative hosts, and corridors between populated patches (Dent 1995; Van Driesche and Bellows 1996; Eilenberg *et al.* 2001). In agroecosystems, additional conservation methods can include using selective insecticides, implementing favorable tillage regimes, strip harvesting, cover crops, intercropping, winter seeding, crop rotation and installing shelter belts (Van Driesche and Bellows 1996; Eilenberg *et al.* 2001). A constraint of conservation biological control is intraguild predation (see review by Polis *et al.* 1989), which occurs commonly between carabid beetles of different size classes (Polis *et al.* 1989; Prasad and Snyder 2004; Prasad and Snyder 2006).

The objective of this research was to identify potential indigenous predators of *S. lineatus* eggs, a life stage susceptible to predation, as well as parasitoids of adult *S. lineatus* weevils, from southern Alberta for future use in an IPM program. The potential for intraguild predation in southern Alberta field pea production systems was investigated. I hypothesize that members of the beetle family Carabidae can play a key role in *S. lineatus* predation, both of eggs and adults, as several ground beetle species or their congeners identified as *S. lineatus* predators in Europe also occur in Alberta, for example, *Pterostichus melanarius* (Illiger) and *Bembidion properans* (Larochelle and Larivière 2003). Parasitoids, either indigenous or introduced, may also be present in Canadian agroecosystems, where they can be utilized against *S. lineatus* eggs and adults.

4.2 Materials and Methods

4.2.1. Egg Collection.

Adult weevils, collected from field peas in late May were sexed and mating pairs were reared in Petri dishes provisioned with fresh pea foliage that was replaced every 48 h. Weevil eggs were collected from the Petri dishes every 48 h. Eggs were placed on moist filter paper in Petri dishes and stored at 4°C until needed. Female weevils continued to oviposit until mid-August.

4.2.2. Egg Parasitism.

Twenty to 50 *Sitona lineatus* eggs, less than 48 h old, were exposed to potential parasitoids in the field on sentinel cards made of white index paper. Sentinel cards were anchored to the soil surface at the base of pea plants, using 10-cm-long nails, in plots of *P. sativum* near Lethbridge, AB (49°41’39”N; 112°49’58”W). Four cards were placed in the field on each of five dates between 10 June and 27 June 2009 when weather was favorable. Cards were exposed for 48 h, collected and placed in cardboard rearing boxes with alcohol vials to collect any emerging adult insects (after Dosdall *et al.* 2006). Rearing boxes were incubated at 20°C (16:8 light: dark photoperiod, controlled humidity) for three to four weeks. During that time, the collection vials were checked periodically for insects and refilled if necessary.

4.2.3. Adult Parasitism.

Adult *S. lineatus* weevils were dissected to determine the presence or absence of parasitoids. Overwintered adult weevils were collected from three sites within 100 km from Lethbridge, AB, between 22 May 2009 and 27 May 2009 (n = 129) and 27 July and at Vauxhall (50°04’08”N; 112°05’51”W) on 22 July 2009 (n = 12). New generation weevils were also collected from Lethbridge and Vauxhall in July (n = 44). In the laboratory, weevils were killed with 70% ethanol. The head and thorax were separated from the abdomen and the body cavity was opened. Using a dissecting microscope, the haemocoel, internal organs and tissues of each weevil were examined for the presence of parasitoid larvae.

4.2.4. Egg Predation.

Potential ground beetle predators of *S. lineatus* eggs were collected from field pea plots near Lethbridge and Vauxhall, Alberta. Plots at Lethbridge were planted on 04 May 2009 and at Vauxhall on 06 May 2009. At Lethbridge, eight dry pitfall traps (1.0 L containers), provisioned with moist paper towel, rocks and soil were placed around the perimeter of the field pea research plot on 14 May 2009. Traps were emptied every 24 to 48 h and traps remained in the field until the plots were harvested. Carabidae and Staphylinidae were collected from the traps while spiders and other insects were released. At Vauxhall, small beetle species, such as *Bembidion quadrimaculatum* L. (Coleoptera: Carabidae), were collected by hand from the soil surface using aspirators when field peas were at the six to nine leaf stage in June, at the late flowering stage in July and before harvest in August. In the laboratory, beetles were sorted by morphospecies and placed in rearing dishes containing moist soil, leaf litter and rocks for refugia. Rearing dishes were stored at 10°C (photoperiod 16:8 light: dark).

The potential of ground beetles to prey upon *S. lineatus* eggs was investigated in three no-choice tests with different substrates. In each test, one beetle (starved for 48 h) was confined for 48 h (20°C, 16:8 light: dark photoperiod, controlled humidity) in a Petri dish or rearing dish provisioned with a known number of *S. lineatus* eggs. The number of remaining eggs, presence or absence of egg debris, beetle location and egg location were recorded upon conclusion of the exposure period. After the experiment, each beetle was killed, preserved and determined to species using Lindroth (1961-1969). Voucher specimens were deposited in the Strickland Museum of the University of Alberta. Each beetle was used only once and replication varied for each species depending on the number of individuals collected (Table 4.1). The specific conditions of each test are described below and control trials were set up using the same conditions in the absence of a predator.

In Petri dish tests, 20 melanized *S. lineatus* eggs were placed on a single layer of moistened filter paper. No other organic matter was added to the Petri dishes aside from any soil that was transferred to the dish at the time of beetle addition.

In rearing dish tests, soil was placed to a depth of approximately 2 cm in 250 mL rearing containers and moistened. Filter paper (4.25 cm diameter) was placed on top of the soil in the center of the dish and moistened with distilled water. Twenty eggs were placed on the filter paper. Soil was added to the dishes in order to provide the beetles with a more natural habitat in which their behaviors, for example, burrowing by *Pt. melanarius* (Wallin 1988), could be observed in relation to their tendency to prey upon *S. lineatus* eggs.

In egg card tests, weevil eggs were glued to sandpaper egg cards following the procedure for seed cards (Westerman *et al.* 2003a, 2003b; O'Rourke *et al.* 2006; Saska 2008). Medium grain sandpaper was cut into 5 x 6 cm rectangles and sprayed with Krylon® repositionable glue using a plastic template marked out into a 4 x 5 grid with five open spaces. Five *S. lineatus* eggs were placed along the edges of each square of glue on the card after the glue had partially set. Cards were prepared within an hour of their use in each predation trial; prior to commencement of each trial one egg card was placed in each rearing dish, as described above. Egg placement on egg cards was designed to mimic the deposition of weevil eggs over the soil surface and was intended to determine if beetles would search out eggs in these conditions and to obtain an estimate of egg predation rates under field conditions.

4.2.5. Beetle Competition.

The potential for intraguild predation and competition among small and large beetle species for prey was investigated by exposing *S. lineatus* eggs to single specimens of *Pt. melanarius* and *B. quadrimaculatum* simultaneously using the rearing dish experimental design as described above. After 48 h, beetles were removed, killed, identified and the length and width of all surviving beetles was determined using digital calipers. The number of eggs remaining in each dish was recorded.

4.2.6. Statistical Analysis.

Predation upon *S. lineatus* eggs by each ground beetle species was analyzed based on the percentages of missing eggs using the PROC GLM procedure (SAS Institute 2004). Each test described above was analyzed separately. Beetle species were included if at least four replicate trials were performed using that species. If the ANOVA results were significant ($\alpha<0.05$), the contrast function (with Bonferroni adjustment) was used to identify differences in egg removal between beetle species of interest. Where data were non-normal and did not benefit from logarithmic transformation, the Kruskal-Wallis test was used and the Chi-square statistic was reported. For tests investigating intraguild predation and beetle competition, the mean percentage of eggs removed with two beetles was compared to data from the rearing dish test, for individual species and the control.

4.3. Results

4.3.1. Egg and Adult Parasitism.

No evidence of *S. lineatus* egg parasitism was observed in 2009. No evidence of parasitism was observed in dissected adult weevils from any site.

4.3.2. Predators.

Thirteen species of carabid beetles were collected at Lethbridge and Vauxhall. If more than four individuals of a species were used in a predation test, the number of individuals collected is given in Table 4.1, where the number of replicates is equivalent to the number of individuals collected. Large species, including *Pt. melanarius*, *Poecilus lucublandus* (Say), and *Poecilus scitulus* LeConte were most commonly found in dry pitfall traps. *Pterostichus melanarius* accounted for the vast majority of beetles collected in pitfall traps. At Vauxhall, where beetles were collected by hand, *B. quadrimaculatum* accounted for the majority of beetles captured. *Microlestes linearis* (LeConte) and *Bembidion timidum* (LeConte) were collected in low numbers at Vauxhall, and low numbers of *Amara littoralis* Mannerheim and *Agonum placidum* (Say) were collected with dry pitfall traps and by hand. Few staphylinid beetles were collected from dry pitfall traps. The majority of these individuals belonged to two species of the genus *Philonthus* Stephens (Coleoptera: Staphylinidae). Petri dish test results for Staphylinidae were pooled for statistical analysis.

4.3.3. Egg Predation.

In all predation arenas (Petri dish, rearing dish, egg card), *B. quadrimaculatum* removed the greatest percentage of *S. lineatus* eggs. In dishes to which *B. quadrimaculatum* had been added, *S. lineatus* egg debris was often observed. Egg debris was also observed in dishes with *M. linearis, Bembidion rupicola* (Kirby), *B. timidum*, Staphylinidae, and occasionally in *P. scitulus* trials.

No evidence of egg debris was observed in *Pt. melanarius* or *P. lucublandus* trials, although both species disturbed and moved eggs within the dishes.

Egg removal in Petri dishes differed significantly among species ($F_{6, 102}$ = 67.65, $p < 0.0001$) and ranged from 98% by *B. quadrimaculatum* to 11.75% by *Pt. melanarius* (Figure 4.1). Staphylinid beetles and *P. scitulus* removed intermediate numbers of eggs (Figure 4.1). *Bembidion quadrimaculatum* consumed significantly more pea leaf weevil eggs than all other beetle species. Staphylinidae consumed more eggs, on average, than *P. lucublandus, M. linearis*, and *Pt. melanarius*. *Poecilus scitulus* did not remove significantly more eggs than the staphylinids, *P. lucublandus, M. linearis* or *Pt. melanarius*, but did differ significantly from the control. *Poecilus lucublandus, M. linearis* and *Pt. melanarius* beetles did not remove significantly more eggs than were missing from the control, to which no beetle was added.

Only two beetle species, *B. quadrimaculatum* and *Pt. melanarius* were tested in the rearing dish arena. After 48 h, significantly more eggs were missing from *B. quadrimaculatum* rearing dishes (94.6%) than from *Pt. melanarius* dishes (17.4%) ($F_{2, 96}$ = 367.92, $p < 0.0001$) (Figure 4.2). Compared to the control, both species removed significant numbers of eggs.

Under conditions designed to simulate field conditions using egg cards in rearing dishes, 45% of eggs were missing from dishes with *B. quadrimaculatum*, which was significantly greater than the 3.4% of eggs missing from *Pt. melanarius* dishes (Kruskal-Wallis test: Chi-square$_2$ = 15.65, $p = 0.0004$). There was no difference in egg consumption between *Pt. melanarius* and the control

(Figure 4.3).

4.3.4. Beetle Competition.

Bembidion quadrimaculatum (1.60 x 5.60 mm) specimens were significantly smaller than *Pt. melanarius* (3.5 x 15.4 mm) specimens in both length ($F_{1, 69} = 3428.96$, $p < 0.0001$) and width ($F_{1, 69} = 2025.11$, $p < 0.0001$). Simultaneous exposure of *S. lineatus* eggs to both species significantly reduced the rate of egg removal to 75.3% compared to 94.6% removal for *B. quadrimaculatum* alone ($F_{3, 143} = 129.77$, $p < 0.0001$) (Figure 4.4). Egg removal in dishes with *B. quadrimaculatum* and *Pt. melanarius* was significantly greater than in dishes with *Pt. melanarius* only (17.4%). Egg removal was significantly greater in all dishes with beetles than in control dishes.

After 48 h, both the large and small beetles were recovered in 53.2% of trial dishes. Only one large beetle was not recovered, and was likely not added to the dish. In dishes with only the large beetle recovered, the number of missing eggs ranged from 5 to 100%; in 36% of these dishes, all *S. lineatus* eggs were removed (Table 4.2).

4.4. Discussion

No parasitoids of *S. lineatus* eggs or adults were observed in 2009. The absence of parasitoids may reflect the instability of annual agricultural systems, which can delay the arrival of natural enemies (Van Driesche and Bellows 1996; Kogan *et al.* 1999). The relatively recent establishment of *S. lineatus* in southern

Alberta (Vankosky *et al*. 2009) may also explain the absence of parasitoids. For instance, Dosdall *et al.* (2006, 2009) found no parasitism of another invasive species in southern Alberta, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae), the cabbage seedpod weevil, for several years after the species was first discovered, but later parasitoids were found to exploit both adult and larval weevils. Alternatively, the lack of evidence of parasitism may result from the rather small sample sizes used here. Future inquiries may therefore reveal indigenous parasitoids that attack *S. lineatus* in its newest geographical ranges.

In plots of *P. sativum*, *B. quadrimaculatum* and *Pt. melanarius* were the most abundant ground beetle species. *Poecilus lucublandus*, *P. scitulus*, *M. linearis*, *B. timidum*, *Agonum placidum* and *Amara littoralis* were collected in low to moderate numbers. A few staphylinid beetles of the genus *Philonthus* were collected in dry pitfall traps. Trap catch results for this research are similar to those reported for this region. For example, in his work to determine the activity density of ground beetles in agroecosystems near Lethbridge and Vauxhall, Bourassa (2006) reported that *B. quadrimaculatum* accounted for 28% of the trap catches in the summer of 2004, with their peak activity occurring in late June. *Pterostichus melanarius* was the dominant large carabid beetle, accounting for 43.1% of trap catches with its peak activity occurring in late summer (Bourassa 2006). Both *B. quadrimaculatum* and *Pt. melanarius* were among the six most abundant species in agricultural fields near Vauxhall (Bourassa *et al.* 2008) and among the four most abundant species near Lethbridge (Floate *et al.* 2007). Floate *et al.* (2007) collected 128 beetles on average per sample over four years, with *Pt.*

melanarius accounting for 27.5% of trap catches while *B. quadrimaculatum* accounted for 9.9% of trap catches.

In Petri dish trials, *P. scitulus* and staphylinids consumed 36 and 55% of eggs respectively. Members of the genus *Philonthus*, the taxa of Staphylinidae most commonly collected, are known to be predators of the eggs, larvae, pupae and adults of Lepidoptera, Coleoptera and Diptera (Good and Giller 1991) and have been extensively investigated as predators of the eggs and larvae of the horn fly, *Haematobia irritans* (L.) (Diptera: Muscidae) (Roth 1982; Fincher 1995). Neither *P. scitulus* nor the staphylinid beetles were collected in sufficient numbers to be included in the rearing dish and egg card trials. Further investigation of the potential of *Philonthus* beetles as predators of *S. lineatus* in southern Alberta should be undertaken, as this group consumed significant numbers of weevil eggs.

Of the most abundant carabid species, the fewest *S. lineatus* eggs were missing from dishes with *Pt. melanarius*. When exposed to *Pt. melanarius* in Petri dishes, 11.8% of eggs were missing after 48 h and no egg debris was observed. In the rearing dish trials, 17.4% of eggs were missing after 48 h; however, *Pt. melanarius* in rearing dishes burrowed actively, scattering soil over the filter paper and eggs, and as a result, eggs were often found on the soil surface rather than on the filter paper. Indirect disturbance may account for missing eggs in rearing dishes, as again, no egg debris was observed, providing no conclusive evidence of consumption. In the egg card test, only 3.4% of *S. lineatus* eggs were removed. These results were not unexpected, as *Pt. melanarius* has been investigated as an egg predator in the past with similar results (Baines *et al.* 1990;

Finch 1996; Prasad and Snyder 2004; Prasad and Snyder 2006). Finch (1996) suggested that *Pt. melanarius* adults were unable to manipulate insect eggs as prey due to their size, as in a survey of ground beetles, species larger than 10 mm in length only erratically consumed *Delia radicum* (L.) (Diptera: Anthomyiidae) eggs. Investigations regarding the use of *Pt. melanarius* as a biological control agent seem limited to their efficacy against larger prey, including slugs (Symondson *et al.* 2002; McKemey *et al.* 2003), other mollusks and annelid worms (Symondson *et al.* 2002) and adult insects (Baines *et al.* 1990; Holopainen and Helenius 1992).

After 48 h of exposure to *B. quadrimaculatum*, significantly higher numbers of *S. lineatus* eggs were absent from Petri dishes, rearing dishes, and egg cards, relative to numbers remaining after exposure to the other beetle species. Across all test conditions, there was no evidence of burrowing by *B. quadrimaculatum* beetles and all individuals were found on the soil surface at the conclusion of the exposure period. Egg debris was observed in Petri dishes, rearing dishes and egg card tests, providing strong evidence of egg consumption. Overall, these results were expected, as previous research has shown that small ground beetles (<10 mm) readily preyed upon *Delia* spp. eggs (Finch 1996; Prasad and Snyder 2004) and *Drosophila* pupae (Cárcamo 1992). In similar studies, *B. quadrimaculatum* consumed the eggs of *Delia antiqua* (Meigen) (Diptera: Anthomyiidae) (Grafius and Warner 1989), *Delia floralis* Fallén (Diptera: Anthomyiidae) (Andersen *et al.* 1983), and *Listronotus oregonensis* LeConte (Coleoptera: Curculionidae) (Baines *et al.* 1990). Based on the results of this study and the abundance and widespread distribution of *B. quadrimaculatum* in agroecoystems (Grafius and Warner 1989; Holopainen and Helenius 1992; Cárcamo 1995; Ellsbury *et al.* 1998; Melnychuk *et al.* 2003; Fox *et al.* 2005; Bourassa 2006; Floate *et al.* 2007), this carabid species has great potential for use in a conservation biological control program against *S. lineatus*. Furthermore, its habit of remaining on or near the soil surface rather than burrowing deep belowground implies that it is likely an efficient predator of *S. lineatus* eggs, as *S. lineatus* eggs are found in the same section of the soil profile.

Conservation biological control initiatives aim to increase populations of predators or parasitoids through agronomic practices that maintain habitats for natural enemies of pests (Dent 1995; Van Driesche and Bellows 1996; Eilenberg *et al.* 2001). Conservation biological control of insect pests by indigenous species such as *B. quadrimaculatum* may become complicated by intraguild predation as conservation biological control practices often promote populations of a variety of species in addition to the target species (Polis *et al.* 1989; Prasad and Snyder 2004; Prasad and Snyder 2006). Intraguild predation and competition for prey is expected to reduce the efficiency of prey removal in assemblages of generalist predators, such as *B. quadrimaculatum* (Clark *et al.* 1994), especially where there are species of different sizes present in the community. Prasad and Snyder (2004, 2006), for example, observed that *Pt. melanarius* rarely consumed insect eggs, but did consume small *Bembidion* ground beetles which subsequently reduced the number of fly eggs preyed upon by *Bembidion* beetles.

When *S. lineatus* eggs were exposed to both *B. quadrimacualum* and *Pt.*

melanarius simultaneously, *B. quadrimacultum* continued to consume significant numbers of *S. lineatus* eggs, although at a reduced rate relative to tests with *B. quadrimaculatum* alone. In 46% of the rearing dishes, the *B. quadrimaculatum* specimens were not recovered, but in 36% of those dishes, the *B. quadrimaculatum* had consumed all of the *S. lineatus* eggs before being killed. The effects of intraguild predation were tested in relatively small arenas, which likely increased the chances of the beetles encountering one another and in turn the chances of intraguild predation. However, these results indicate that in field pea crops intraguild predation has the potential to reduce both the population of *B. quadrimaculatum* and its effectiveness as a predator of *S. lineatus* eggs. Further work to investigate the relationships between predatory carabid beetles should be done in the field, and should consider the effects of prey availability and the timing of peak beetle activity on the extent of intraguild predation. In the presence of the large *Pt. melanarius*, however, *B. quadrimaculatum* did consume a significant number of pea leaf weevil eggs relative to control, suggesting that if conservation biological control efforts increase *Pt. melanarius* and *B. quadrimaculatum* populations simultaneously, *S. lineatus* eggs will continue to be preyed upon to a significant degree.

In addition to being a victim of intraguild predation, *B. quadrimaculatum* are classified as generalists that prey upon various life stages of insects from several orders, including Coleoptera, Diptera, Homoptera and Lepidoptera (Sunderland 2002; Fox *et al.* 2005). The role of generalists in biological control is under debate (see review by Symondson *et al.* 2002). Generally, specialists are considered to be most effective against exotic pests, whereas generalists or generalist assemblages are most effective against native pests (Symondson *et al.* 2002). However, as Symondson *et al.* (2002) observed in their review of biological control research, in 75% of cases, generalists significantly reduced both exotic and indigenous pests. New pest-enemy associations are also effective for controlling exotic pests, as Hokkanen and Pimentel (1984) found that generalist enemies had significant suppressive effects in 45% of new associations for exotic pests. In the case of *S. lineatus*, an exotic pest, the indigenous ground beetle species, *B. quadrimaculatum*, appears to have significant potential for conservation biological control, despite its generalist habit.

In the field, pea leaf weevil egg consumption by *B. quadrimaculatum* is expected to be lower than in the laboratory, due to the presence of alternative prey, competition and intraguild predation. However, a conservation biological control program to promote *B. quadrimaculatum*, as well as *P. scitulus* and staphylinid beetles, which also preyed upon *S. lineatus* eggs, albeit in lower numbers, should be beneficial in agroecosystems. Conservation biological control is compatible with integrated pest management programs (Kogan and Lattin 1993) and can be promoted as an organic control tactic (Jonsson *et al.* 2008). Further investigation in the field is needed to better quantify the ability of indigenous ground beetle predators to suppress *S. lineatus* populations, as well as to understand the effects of alternative prey, competition and intraguild predation on *B. quadrimaculatum*. Research should also be undertaken in southern Alberta to determine the most effective conservation biological control practices for ground beetles in agroecosystems. Beetle banks as described by Thomas *et al.* (1991), for example, have been shown to provide carabid beetles with a suitable overwintering habitat and to contribute to the overall biological diversity of agroecosystems (Collins *et al.* 2003; MacLeod *et al.* 2004).

Table 4.1. Sample sizes for each beetle species for the Petri dish, rearing dish, egg card and competition tests.

Beetle Species	Petri Dish	Rearing Dish	Egg Card	Beetle Competition
Control	20	35	20	35
Bembidion quadrimaculatum	18	35	29	35
Pterostichus melanarius	20	29	31	29
Microlestes linearis	9	NA	NA	NA
Poecilus scitulus	4	NA	NA	NA
Poecilus lucublandus	7	NA	NA	NA
Staphylinidae	11	NA	NA	NA

NA indicates that beetles of a given species were not available for testing in specific predation tests.

Table 4.2. Summary of beetle survival, given in number of rearing dishes, and the range of egg consumption for dishes in which both beetles or only the large beetle was recovered.

	Number of dishes within each range of missing *S. lineatus* eggs					
	0%	1 to 20%	21 to 75%	76 to 99%	100%	Total Dishes
B. quadrimaculatum recovered	1	1	2	6	15	25
B. quadrimaculatum not recovered	0	5	8	1	8	22
Total Test Dishes	1	6	10	7	23	47

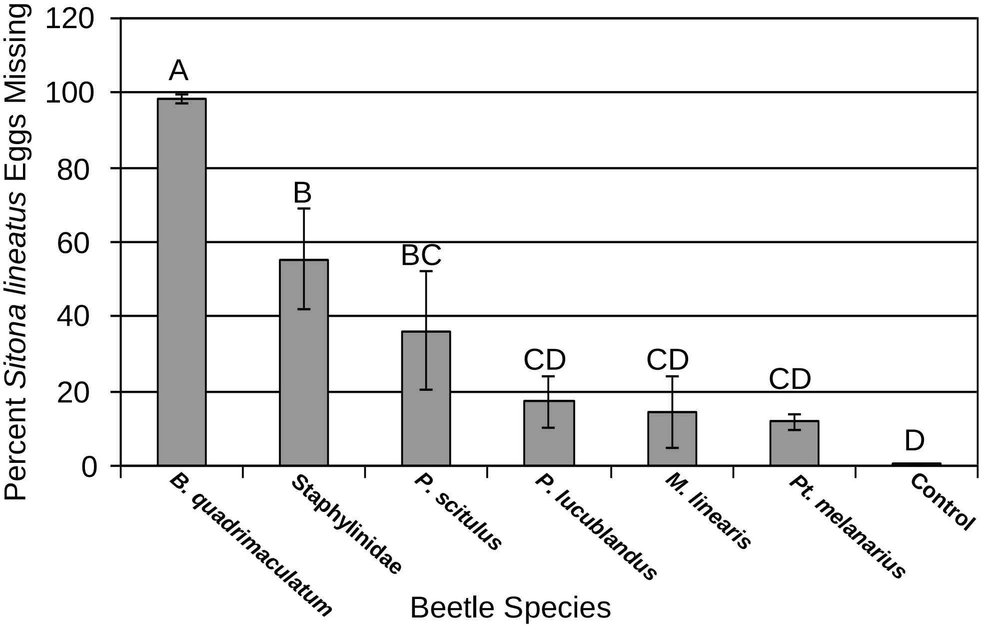

Figure 4.1. Mean percentage of 20 *Sitona lineatus* eggs destroyed or missing (± S.E.) from Petri dishes after 48 h of exposure to various beetle species. Means with the same letter are not significantly different ($p > 0.05$).

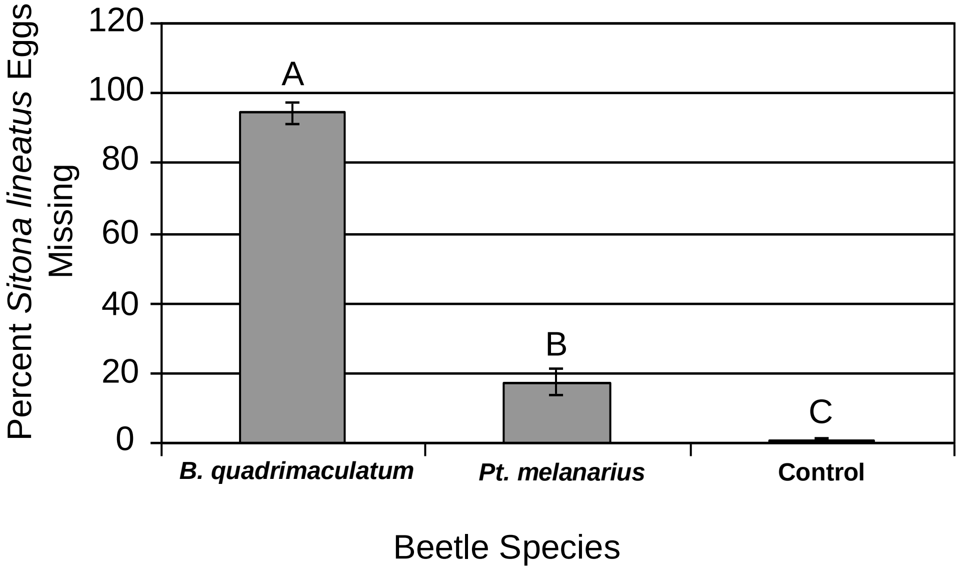

Figure 4.2. Mean percentage of 20 *Sitona lineatus* eggs destroyed or missing (± S.E.) from rearing dishes after 48 h of exposure to adult carabid beetles. Means with different letters are significantly different ($p < 0.05$).

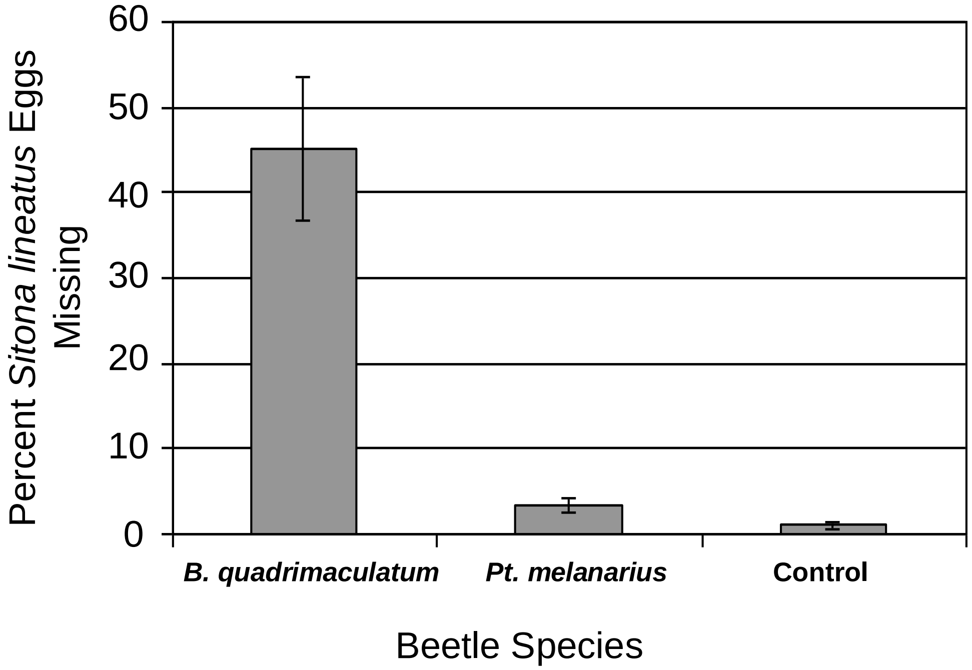

Figure 4.3. The mean percentage of 25 *Sitona lineatus* eggs destroyed or missing from rearing dishes with egg cards after 48 h of exposure to adult carabid beetles. Significant differences in egg removal were observed between beetle species where error bars (± S.E.) do not overlap ($p < 0.05$).

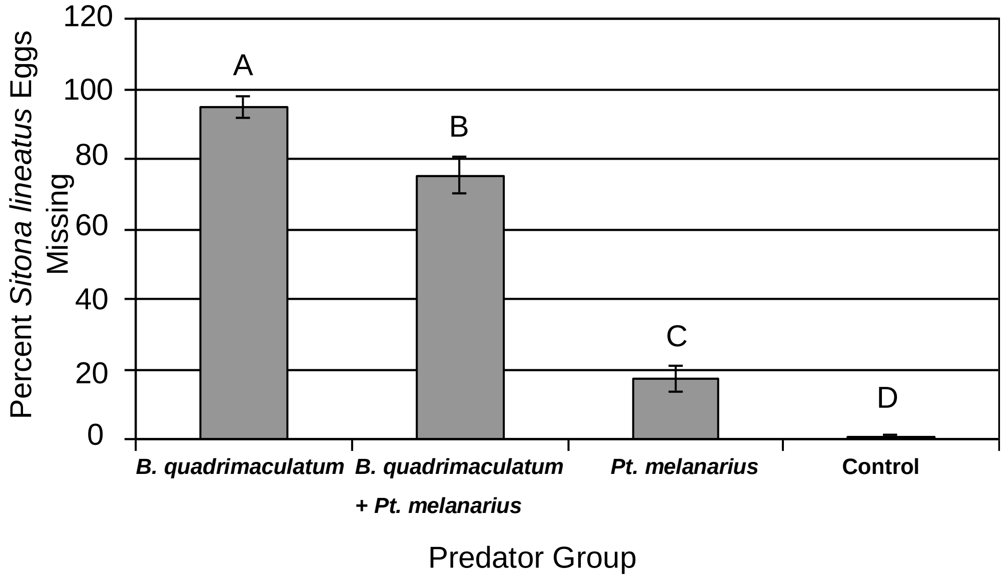

Figure 4.4. Representation of the effects of beetle competition, between species of different sizes, on the mean rate of *Sitona lineatus* egg removal (± S.E.) from rearing dishes over 48 h. Twenty eggs were exposed to ground beetle predation in rearing dishes. Means with the same letter are not significantly different ($p < 0.05$).

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5. Chapter Five: General Discussion

5.1. The *Sitona lineatus* Research Initiative

Sitona lineatus L. (Coleoptera: Curculionidae) has only recently become established in the Canadian prairies, but it has already reached pest status and has generated considerable concern for producers and agronomists. The 2009 pea leaf weevil survey found weevils as far north in Alberta as Red Deer, but the highest population densities remain limited to the southern regions of the province, especially in the region surrounding Lethbridge and east of Medicine Hat (Alberta Agriculture and Rural Development 2010). *Sitona lineatus* is also established in southern Saskatchewan (Hartley 2009; Dosdall *et al.* 2010).

In 2005 and 2006, populations of *S. lineatus* reached alarming levels (Dosdall *et al.* 2010) and the economic and environmental importance of field pea as a rotational crop (Verkleij *et al.* 1992; Corre-Hellou and Crozat 2005) fostered a sense of urgency within the agricultural community to find a way to efficiently manage *S. lineatus*. The agricultural industry sponsored a comprehensive research program led by Dr. Cárcamo, to investigate several aspects of *S. lineatus* biology and strategies for its control. The program involved investigations of the biology of *S. lineatus* in Alberta, foliar and systemic insecticide products, winter pea trap crops, the effect of seeding dates, integrated pest management, economic thresholds and an investigation to identify potential natural enemies of *S. lineatus* in Alberta.

However, managing *S. lineatus* has not been simple and there are a number of reasons for this, as discussed in earlier portions of my thesis. To briefly summarize, both adults and larvae can damage field pea and faba bean plants and both life stages contribute to yield loss (El-Dessouki 1971; Nielsen 1990). Adult weevils migrate to field pea, *Pisum sativum* L. (Fabales: Fabaceae), over a period of several weeks in the spring, so foliar insecticides must have extensive residual activity for one application to prevent adult weevils from becoming established (Hamon *et al.* 1987). Overwintered adults are also quite long lived, and continue feeding until late July to mid-August (Hoebeke and Wheeler 1985). Larvae develop within root nodules where they consume nitrogen-fixing bacteria (Jackson 1920; Johnson and O'Keeffe 1981; Hoebeke and Wheeler 1985), and where they are protected from predation and above-ground insecticide applications (King 1981; Bardner *et al.* 1983; Ester and Jeuring 1992). In addition, the incredible reproductive potential of *S. lineatus* enables females to deposit massive numbers of eggs after a short pre-oviposition period (Jackson 1920; Williams *et al.* 1995), and adds a further complication in developing an adequate population control strategy.

Several noteworthy researchers have tackled the problem of developing management options for *S. lineatus*, but in spite of their efforts, a number of problems have remained unsolved. For instance, monitoring systems are unable to predict local *S. lineatus* population densities and are limited to identifying the presence or absence of weevils in field pea and faba bean fields. Economic threshold estimates should also be considered a shortcoming in *S. lineatus* research, as sweep net sampling (Quisenberry *et al.* 2000), egg-counting (Doré and Meynard 1995) and the counting of feeding notches (Cantot 1986) are either impractical or fail to consider the complexity of the relationship between the host plant and the pest population (El-Dessouki 1971; Nielsen 1990; Landon *et al.* 1995). Biological control, a key element of integrated pest management (Dent 2000), has been inadequately studied in the context of integrated management, although a number of potential biological control agents have been identified. The need for improved understanding of these and other aspects relating to management of the pea leaf weevil prompted my studies on this system.

Overall, I believe that the results of my thesis research will be of value to producers and agronomists, as will the information regarding trap crops, foliar insecticides and *S. lineatus* biology gained from the pea leaf weevil research project. However, there are some aspects of this research that I would change if the experiments were to be repeated. I would like to have included a bio-pesticide or antifeedant treatment in the open plot study. For example, Smart *et al.* (1994) observed that plants in plots treated with neem extract, *Azadirachta indica* A. Juss. (Sapindales: Meliaceae), had less foliar damage, fewer larvae and fewer damaged nodules than control plants. Neem oil has a shorter residence time than many foliar products, but it can be applied using chemical sprayers (Smart *et al.* 1994). As part of an integrated pest management program, neem oil and other biological products may be effective against *S. lineatus* populations and we need to learn more about these options when they are applied alone and in combination with other management options such as thiamethoxam. In light of the synergistic interaction between thiamethoxam and *Rhizobium* inoculant, I would also like to have an opportunity to investigate the relationship between adult density and larval populations in cages with thiamethoxam plus inoculant-treated plants. This is discussed in more detail below.

5.2. *Pisum sativum* Response to *Sitona lineatus* Attack

The objective of the study described in Chapter Three was to identify a relationship between adult weevil density and above- and below-ground plant damage and yield parameters that could be used as the basis for an economic threshold estimate in western North America. Adult weevil density had a significant effect on the number of *S. lineatus* feeding notches on the entire plant and on the terminal leaves, with the number of feeding notches increasing as density increased. This means that the extent of feeding damage can be used to predict the population density of adult *S. lineatus*. However, strong linear relationships were not observed between adult weevil density and larval density, between adult density and root nodule damage or between adult density and yield loss in the cage study. Similar results were obtained by Nielsen (1990), who suggested that root nodules are a limiting resource for *S. lineatus* larvae, and as a result, plants exert a carrying capacity effect on larval populations. Nielsen (1990) predicted that the carrying capacity of *Vicia faba* L. (Fabales: Fabaceae) plants for *S. lineatus* was 13 larvae per plant, and my cage study results suggest that the carrying capacity of *P. sativum* is in the range of nine larvae per plant based on the average number of larvae collected per plant at Lethbridge.

In Chapter Three, I reported that the yield of caged plants was greater when plants were treated with *Rhizobium* inoculant plus nitrogen (N) fertilizer than with inoculant alone, and yield was significantly lower in the uncaged controls than in the cage treatments. Within the cages, no significant differences in yield were observed. However, yields were 8.86 and 9.96% lower in cages with densities of 0.50 and 1.00 weevils per plant, respectively, relative to control cages. Yield losses of this magnitude would cost a producer $75 to $85 per hectare with an even greater economic impact when one considers that destruction of root nodules by larvae would decrease the soil N availability for crops planted in the same field in subsequent years. As yield was not significantly affected by adult weevil density, as shown by the lack of a relationship between yield and adult density, yield losses should be attributed to larval damage or a combination of larval and adult damage. But larval populations cannot be predicted by above-ground feeding damage, or by the size of adult weevil populations, as observed in this study and that of Nielsen (1990), and even at the lowest adult weevil densities, enough larvae were present to cause yield losses.

As both adult and larval *S. lineatus* were present in the cages, the effect of adult feeding could not be considered independently, nor could the effect of larval feeding. However, although considerable differences in the amount of above-ground feeding were observed between the adult weevil densities, similar differences were not observed below-ground or in terms of yield. Therefore, the results discussed in Chapter Three suggest that the presence of larvae and damage to the root nodules will result in yield loss, even if levels of foliar consumption remain below the economic threshold set by El-Lafi (1977). As levels of foliar damage do not predict the size of the larval population, thresholds based on above-ground feeding damage may not be appropriate. This is because small populations of adult weevils, which have the potential to lay thousands of eggs, may not be considered a threat based on El-Lafi's (1977) economic threshold. Preventing female *S. lineatus* from laying eggs is vital for ensuring that yield losses do not occur. Historically, this has been a considerable challenge. Although the proportion of plants with terminal leaf damage may be used as a guide to determine the extent of yield loss in field pea, a number of other important questions remain to be answered.

What method should be used to prevent the presence of larvae or reduce their abundance? The window of opportunity available in which to take action to prevent weevil reproduction is extremely short, 10 days at most (Williams *et al.* 1995). So fields must be monitored frequently in the spring, and producers must be prepared for late invasions of weevils. Once weevils are detected, action must be taken within seven to 10 days. Although adult weevil density can be estimated by counting feeding notches, this knowledge will not really be useful until we have a better understanding of how many pairs of weevils can be present before economic losses become inevitable. Ester and Jeuring (1992) observed that only two larvae per plant were required to cause economic losses in faba bean crops. Evidence from the cages at Vauxhall suggests that a similar number of larvae will cause yield losses in field pea, as no difference in yield or larval population density was observed between adult density treatments. A single female weevil can lay approximately 24 eggs per day at peak oviposition (Jackson 1920). If every egg hatches and every larva finds a host plant, then one female weevil can produce enough larvae to cause economic damage to 12 plants in one day. According to this logic, and the results of the cage study, even small populations of weevils can be economically damaging.

A great deal remains to be learned about the relationship between *S. lineatus* and its field pea host plants. An objective of future studies should be to conduct a detailed life table analysis of *S. lineatus* in field pea, in both western Canada and in Europe, the Mediterranean and the Middle East, where *Sitona* weevils are naturally distributed (Aeschlimann 1980). I believe that such an analysis will lead to a better understanding of the factors associated with its population outbreaks in North America. Perhaps such an undertaking will also identify important mortality factors. The period between oviposition and pupation is most likely when the greatest mortality occurs, due to factors such as egg dessication and extreme temperatures (Jackson 1920; Lerin 2004), and studies to confirm this assumption in North America would be particularly useful. Determining where adult weevils overwinter and the degree of pupal mortality could also benefit producers and agronomists, especially in terms of predicting population densities in particular regions. Nevertheless, *S. lineatus* currently poses a significant threat to the profitability and sustainability of field pea production in many regions of the world and solutions are needed to limit its impact. Field pea crops should be carefully monitored for *S. lineatus* and effective management strategies need to be developed so that producers have viable management options. To this end, chemical, cultural and biological approaches must all be explored further.

Finally, data from Chapters Two and Three were used to determine the validity of the economic threshold estimate of 30% of plants with terminal leaf damage for *S. lineatus* proposed by El-Lafi (1977). Data from the cage study indicated that the proportion of *P. sativum* plants with terminal leaf damage was predictive of the adult population density and the overall degree of foliar damage done to plants, but did not predict larval populations or yield loss. Yield did decrease slightly as the proportion of plants with terminal leaf damage increased in the cage study, but the relationship between the two parameters was extremely weak. Data from Chapter Two (excluding plots treated with thiamethoxam) showed that yield decreased as the proportion of plants with terminal leaf damage increased. This relationship was significant and considerably stronger than observed in the cage study, suggesting that yield loss may be predicted by determining the proportion of plants with terminal leaf damage when plants are at the three- or five-node growth stages. A possible explanation for the difference in results between the cage and open plot studies in terms of the relationship between yield and the proportion of plants with terminal leaf damage could be the result of a microclimate effect, as discussed in Chapter Three. Inside the cages, plants may have been better able to compensate for *S. lineatus* feeding damage, for example, because of the more favorable growing conditions inside the cages.

Several methods can be used in research regarding the determination of economic thresholds, including cage studies, using artificial damage to mimic pest damage, and open plot studies where pest densities are controlled using insecticides or not controlled at all. Each method has positive and negative characteristics and the results may not always be applicable to large scale production systems as a result. In Chapters Two and Three, different methods of investigating economic thresholds were used, and very different results were observed. The results from Chapter Two indicate that terminal leaf damage does predict yield loss, whereas the opposite was true of the cage study. I believe that the results of both studies are useful in understanding the impact of *S. lineatus* on field pea, and that further investigation, perhaps using larger cages or large scale field trials, will be necessary to arrive at a useful economic threshold. In the meantime, producers should monitor their fields for terminal leaf damage at the three-node growth stage and if more than 30% of seedlings are damaged, producers should consider taking action to manage *S. lineatus* populations.

5.3. Biological Control

A number of biological control options for *S. lineatus* have been explored. These include entomopathogenic fungi (Poprawski *et al.* 1985; Verkleij *et al.* 1992), the insertion of *cryIII* genes from *Bacillus thuringiensis* subsp. *tenebrionis* Berliner (Bacillales: Bacillaceae) into *P. sativum* plants (Bezdicek *et al.* 1994; Skøt *et al.* 1994; Quinn and Bezdicek 1996), parasitoids (Aeschlimann 1980) and predators (Hamon *et al.* 1990; Ropek and Jaworska 1994). Most biological approaches have had only limited efficacy. However, biological control is a crucial component of integrated pest management programs (Burn *et al.* 1987), and even if the efficacy of biological control agents is limited, their inclusion could increase the effectiveness and sustainability of a management strategy.

Laboratory studies using carabid and staphylinid beetles collected from field peas in southern Alberta, as described in Chapter Four, indicated that the ubiquitous ground beetle, *Bembidion quadrimaculatum* L. (Coleoptera: Carabidae), can consume *S. lineatus* eggs. *Microlestes linearis* (LeConte), *Bembidion rupicola* (Kirby), *B. timidum* (LeConte), *Poecilus scitulus* LeConte (Coleoptera: Carabidae) and *Philonthus* sp. (Coleoptera: Staphylinidae) consumed *S. lineatus* eggs in laboratory conditions, but significantly fewer than *B. quadrimaculatum*. Although only observed in the laboratory, these results indicate that potential natural enemies of *S. lineatus* eggs occur in southern Alberta. *Sitona lineatus* eggs are a good target for biological control as they are extremely plentiful (Jackson 1920), and are more vulnerable to the elements (Jackson 1920; Lerin 2004) and to predation than larvae because larvae are concealed (Jackson 1920; Hamon *et al.* 1990).

While not tested directly, the results of my laboratory experiments suggest that *Pterostichus melanarius* (Illiger) (Coleoptera: Carabidae) may be an effective predator of adult *S. lineatus*. *Pterostichus melanarius* has been recorded as a predator of a number of insect groups (Baines *et al.* 1990; Holopainen and Helenius 1992), as well as slugs and other mollusks and annelid worms (Symondson *et al.* 2002; McKemey *et al.* 2003). As other *Pterostichus* species have been observed to prey upon *S. lineatus*, including *Pterostichus cupreus* L. (Ropek and Jaworska 1994), it is very likely that *Pt. melanarius* can also consume *S. lineatus* adults. Furthermore, *Pt. melanarius* is extremely abundant in the agricultural regions of southern Alberta (Floate *et al.* 2007; Bourassa *et al.* 2008;

Bourassa *et al.* 2010) and it reaches its peak activity density in late summer (Bourassa 2006), when new generation adult *S. lineatus* would be emerging from pupation cells in the soil.

The value of *B. quadrimaculatum* and *Pt. melanarius* as biological control agents of *S. lineatus* will be substantial if they are efficient predators of *S. lineatus* eggs and adults, respectively, in field conditions. Both ground beetle species studied in the laboratory occur commonly in southern Alberta where *S. lineatus* occurs at pest densities. Therefore, classical biological control approaches, which seek to find and release biological control agents for exotic pests from their region of origin, may not be necessary, saving a substantial amount of time, effort and funding. What may be necessary is a program for making *B. quadrimaculatum* an efficient predator of *S. lineatus* eggs or for making *Pt. melanarius* a more efficient predator of adult *S. lineatus*. A conservation biological control program for these ground beetles, for instance, with a significant focus on beetle banks (see: Thomas *et al.* 1991), will not only have implications on *S. lineatus* populations, but will also contribute to the biological diversity of agricultural systems (Collins *et al.* 2003; MacLeod *et al.* 2004). Many of the benefits of biological diversity in agricultural systems have been lost, and by practicing biological control as part of an integrated pest management program, the biodiversity of agroecosystems can be improved (Kogan and Lattin 1993). Therefore, even if *B. quadrimaculatum* and *Pt. melanarius* prove to be inefficient predators of *S. lineatus*, there are other benefits that can potentially arise in other cropping systems.

No evidence of *S. lineatus* parasitiods was observed in the summer of 2009, perhaps because of the small number of samples that were taken for this investigation, or because of the lag time between pest establishment and parasitoid establishment (Van Driesche and Bellows 1996; Kogan *et al.* 1999). Some promising parasitoid species have been described by Aeschlimann (1980) and these species should be considered for use in a classical biological control program.

5.4. Identifying Strategies to Use in an Integrated Pest Management Program

My thesis research has identified two potential components of an effective integrated pest management program: thiamethoxam and *Rhizobium* inoculant. The results of Chapter Two indicate that thiamethoxam, a systemic neonicotinoid insecticide (Maienfisch *et al.* 2001), significantly reduced the amount of adult *S. lineatus* feeding damage observed on *P. sativum* plants relative to plants not treated with thiamethoxam for 40 to 50 days after planting. Although integrated pest management advocates the limited use of insecticides (Burn *et al.* 1987; Kogan and Lattin 1993), as a systemic product, thiamethoxam is among the most sustainable insecticide options available. Systemic products are considered among the most environmentally friendly insecticides as they only target insects consuming plant tissues and rarely have a negative impact on natural enemy populations and pollinators (Ripper *et al.* 1949; Maienfisch *et al.* 2001; Elbert *et al.* 2008). The inclusion of an insecticidal seed treatment in an integrated pest management program for *S. lineatus* has significant advantages over the use of foliar sprays. Difficulties associated with properly timing foliar sprays, poor spray coverage, and negative impacts upon non-target and pollinator species are avoided by using seed treatments. Finally, conditions that interfere with the timely application of foliar products, such as adverse weather conditions, can be avoided with seed treatments. The cost of seed treatments is one factor that may limit their use in agricultural systems.

Rhizobium bacteria are required for the formation of the symbiotic relationship that develops nitrogen-fixing root nodules on pea plants (Allen and Allen 1981) and an inoculant of the bacteria is routinely applied to field pea seed in western Canada to ensure maximum rates of root nodulation (Rennie *et al.* 1993). *Rhizobium* inoculant is recommended for application to field pea seed, especially where soils are acidic or where natural populations of *Rhizobium* are small (Allen and Allen 1981; Fettell *et al.* 1997). Of the eight plot treatments considered in Chapter Two, those that included *Rhizobium* inoculant at Lethbridge in 2008 were the top four yielding treatments. The ability of plants to compensate for insect-induced damage varies depending on a number of factors including nutrient availability, environmental conditions and the type of damage inflicted (Trumble *et al.* 1993). By applying *Rhizobium* inoculant to field pea seed before planting, producers help ensure that their crops will be as vigorous and competitive as possible, and therefore, better equipped to tolerate or compensate for insect herbivory.

When applied together, *Rhizobium* inoculant and thiamethoxam appear to have had a synergistic effect on root nodules, promoting the development of more root nodules, and more tumescent root nodules, than plants treated with inoculant only. Significantly more root nodules developed on plants receiving inoculant plus thiamethoxam than on plants receiving inoculant plus nitrogen fertilizer. One possible explanation is that thiamethoxam improves the below-ground vigor of field peas, similar to the above-ground vigor effect observed in canola, *Brassica napus* L. (Brassicales: Brassicaceae) (Doyle *et al.* 2001). If this is the case, then I expect that thiamethoxam promotes the development of healthier root systems and provides *Rhizobium* bacteria with more root nodule initiation sites. Future work should investigate the relationship between *Rhizobium* inoculant and thiamethoxam further, in order to better explain this relationship.

It would be very interesting to re-run the cage study described in Chapter Three on plots where field pea seed was treated with inoculant plus thiamethoxam. In greenhouse studies, the root nodules of thiamethoxam-treated plants were toxic to *S. lineatus* larvae (H. Cárcamo, personal communication). In addition, in greenhouse experiments, oviposition by female weevils feeding on thiamethoxam-treated plants was delayed by seven days and once oviposition began, the number of eggs laid was significantly lower than the number of eggs laid by weevils feeding on untreated plant tissue for an additional 15 days (Cárcamo *et al.* 2010). I expect that the toxic effects of thiamethoxam on *S. lineatus* larvae and adults, including delayed oviposition, and the increased levels of root nodulation of thiamethoxam-treated field pea plants should enable field peas to tolerate larger populations of adult weevils than plants treated with inoculant only.

5.5. Implementing an Integrated Pest Management Program for *Sitona lineatus* in Western Canada

Several gaps still exist in the research that has been conducted to manage *S. lineatus* infestations. As already stated, accurate monitoring systems and comprehensive economic thresholds are two shortcomings, and a third deficiency is the lack of effort that has been invested in designing, testing and implementing an integrated pest management program. This is not unique to *S. lineatus*. The stimulo-deterrent diversionary strategy, or push-pull system, described by Smart *et al.* (1994) is the only multi-level management system described for *S. lineatus* in European production systems. In this push-pull system, an antifeedant is used to 'push' weevils onto a trap crop, while the male produced aggregation pheromone is used to 'pull' weevils to the trap crop and once in the trap crop, a foliar insecticide product is used to kill adult weevils (Smart *et al.* 1994). Any of these strategies, employed alone, would have limited effects. Employed together, they are hypothesized to be much more effective, and we need to develop similar programs in North America to ensure that field pea production remains profitable and sustainable.

The research described in my thesis has identified several potential management options that can be included in an integrated pest management program for *S. lineatus*. First, results from the cage study have revealed a strong linear relationship between feeding notches and adult population density, and this should be utilized in the development of a monitoring system for the pea leaf weevil. In open plots, the proportion of field pea plants with terminal leaf damage at the three- and five-node stages can predict yield loss to some extent. Counting notches on the terminal leaves is relatively easy and this type of monitoring should be utilized by producers and agronomists to determine how serious *S. lineatus* infestations are. This information can then be used as a decision-making tool. Most importantly, the cage study has provided further evidence that larval feeding is more damaging than adult feeding to *P. sativum* plants, as although levels of foliar damage varied between weevil densities, yield loss did not. Therefore, chemical, cultural and biological control efforts should focus on preventing larval populations from developing or should focus on finding methods of increasing the carrying capacity of field pea plants for larval populations.

Sitona lineatus already has natural enemies in southern Alberta, and these should be incorporated into an integrated pest management strategy. *Bembidion quadrimaculatim* and *Pt. melanarius* are both commonly found in agricultural systems and if their populations are protected from insecticide applications, then they have potential to prey upon *S. lineatus* eggs and adults, respectively. Both have the potential to help reduce larval populations indirectly. Moreover, *B. quadrimaculatum* were active in pea fields in early June, when field peas are emerging and adult weevils are migrating. Options for increasing ground beetle populations in agricultural systems include the provision of refugia, alternative tillage regimes, intercropping, corridors and selective insecticides, among others (Van Driesche and Bellows 1996; Eilenberg *et al.* 2001). All of these strategies should increase the carabid biodiversity of field pea production systems, and have the potential to encourage the establishment of parasitoid populations as well, which is important, because parasitoids of *S. lineatus* have not yet been observed in southern Alberta.

Systemic insecticides are relatively selective, targeting only insects feeding on the tissues of treated plants (Ripper *et al.* 1949). Thiamethoxam was shown to reduce levels of foliar damage and to prevent the proportion of plants with terminal leaf damage from exceeding the economic threshold recommended by El-Lafi (1977). However, the effects of thiamethoxam on yield were inconclusive and greenhouse studies have shown that thiamethoxam does not kill significant numbers of adult weevils, although it does delay oviposition and feeding activity (Cárcamo *et al.* 2010). If producers and agronomists agree that an insecticide must be applied to control *S. lineatus* populations, systemic products should be their first consideration. At present, thiamethoxam is one of a few products available for use against *S. lineatus*. Until a more efficacious product is available, thiamethoxam should be the first consideration of producers and agronomists. One shortcoming of employing a systemic product is that it must be applied at planting, before pests have appeared. In regions where *S. lineatus* has reached outbreak densities, the risk of unnecessary application should be considerably less. Monitoring the emergence of the new generation *S. lineatus* population in late July and August may also help to reduce the risk associated with using systemic products.

Finally, when thiamethoxam and *Rhizobium* inoculant were applied simultaneously, field peas had more root nodules, and more tumescent root nodules than observed on plants not treated with thiamethoxam plus inoculant. Because field pea inoculation with *Rhizobium* bacteria is a fairly standard practice (Rennie *et al.* 1993), and appears to help maintain yields under *S. lineatus* attack with no known negative effects on natural enemy populations, this production strategy should continue.

Developing an integrated management program for *S. lineatus* is necessary because of the difficulties inherent in managing *S. lineatus* populations and preventing larval populations from causing economic yield losses in field pea. The research conducted for my thesis has identified some important relationships between *S. lineatus* population densities and yield losses, as well as some potential management strategies for inclusion in an integrated program. It would be unfortunate if research initiatives do not continue. More work is needed to improve economic thresholds for *S. lineatus*, to determine the impact of natural enemies in the field and to develop an integrated management program that is inexpensive and effectively prevents yield losses. Research in any field is about answering questions and developing new questions based on experimental results. We need to continue to move towards an integrated management approach by considering these results, and the new questions that my research has uncovered.

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