# Dogbane Beetle (*Chrysochus auratus* Fab.) as a Biological Control Agent of Spreading Dogbane (*Apocynum androsaemifolium* L.)

by

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## Abstract

Dogbane beetle, *Chrysochus auratus*, was studied for its biological control potential against spreading dogbane, *Apocynum androsaemifolium*, a native perennial weed in lowbush blueberry (*Vaccinium angustifolium*). No-choice host range experiments were conducted with common milkweed (*Asclepias syriaca*), periwinkle (*Vinca minor*), wild raisin (*Viburnum cassenoides)*, and lowbush blueberry. There was no significant feeding on these plant species by adult dogbane beetles. Significant decreases in foliar dry weight were achieved with 16 beetles per ramet. In Nova Scotia, beetles were present in the field for 8-12 weeks beginning in late June or early July (225-335 growing degree days). Peak beetle abundance occured at 357-577 growing degree days and varied from 4-7 beetles/$m^2$. The fecundity and fertility, timing of pupation, and number of instars were also examined. Females deposited approximately 100 eggs over a 20 day period, with an egg viability of over 90%. Pupae were found on June 1$^{st}$ and June 15$^{th}$.

## List of Abbreviations and Symbols Used

| | |
|---|---|
| mL | milliliter |
| cm | centimeter |
| °C | degrees Celsius |
| SD | standard deviation |
| g | gram |
| NA | not applicable |
| GDD | growing degree day |
| µm | micrometer |

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# Chapter 1.0: Introduction

## 1.1 Introduction

Weed management is one of the most laborious aspects of crop production (McFadyen 1998). Though there are several management options, including mechanical, cultural, biological, and chemical, the most successful long-term management plans integrate several different methods (DiTomaso 1997). An integrated system of weed management is more suited to the complexity of agricultural situations than any one approach. Hess (1994) opined that future weed management research must focus both on reducing herbicide inputs, and enhancing use of biological controls, natural products, and understanding the biology of weeds.

## 1.2 Biological Control

Biological control (syn. 'biocontrol') is defined as the use of natural enemies, such as predators, parasites, and pathogens, to manage the population of a pest (Harley 1985). There are several types of biological control, which are generally classified as classical (release of an exotic natural enemy to control an exotic pest), conservation (involving protection and promotion of existing populations of natural enemies of a pest), and augmentation (the release of large numbers of native control agents, or increasing their population by environmental manipulation) (McFadyen 1998). The majority of attention is paid to classical biological control, which carries inherent risks such as establishment failure or non-target impacts (McFadyen 1998). It is possible that the introduced agent could become a pest itself, though it is arguable that the potential benefit of a classical biological control program outweighs the risks (McFadyen 1998). Simberloff and Stiling

(1996) point out several documented cases of severe non-target impacts and suggest protocols for introducing exotic control agents were not stringent enough. Though Wapshere et al (1989) cites several successful biological control programs, Harris (1991) points out that about a third of agent releases in Canada for biological control of weeds have not established and another third have only become established at low densities. This results in little or no economic damage of the target weed in two thirds of releases. Canada's success rate is similar to the international success rate of about 65% agent establishment and 25% agent effectiveness (Harris 1991). Biological weed control research is ongoing in Canada, with projects targeting plants such as toadflax (*Linaria* spp.), dog-strangling vine (*Vincetoxicum* spp.), and Japanese knotweed (*Polygonum* spp.), though more stringent regulations and decreases in funding have led to far fewer agent releases than in earlier decades (Boyetchko et al 2009).

Much biological control literature focuses on instances of classical control, including how to find appropriate agents, protocol for host specificity testing and post-introduction studies (such as Wapshere 1975; Wapshere et al 1989; McFadyen 1998; Harris 1991). There has been some work involving introduction of exotic species to control native weeds, such as the prickly pear cactus, but this is risky. The exotic agent could form a new association with a valuable non-target plant, thus tarnishing biological control's reputation as a viable pest management strategy. This is why Pemberton (1985) suggested that such enterprises should be curtailed. Wapshere et al (1989) considers conservation biological control as a mostly theoretical concept, though it has been successfully employed (usually against insect pests).

There is some literature suggesting the potential for using native agents for management of native weed species. For example, Campbell et al (1994) documented successful use of native scale insects to control native *Cassinia* species. Valenti et al (1999) suggests that with certain insects it may be possible to purposely trigger a population outbreak for control of a native weed. While biological control is often more successful in less disturbed systems (such as forests or orchards, as opposed to annual crops), it can be employed in frequently disturbed systems if the right agent is selected (Rauwald and Ives 2001). The inundation technique can be done using native agents, even if they appear ineffective under normal conditions, and can be done in areas with short cropping cycles (Wapshere et al 1989), as it is often not intended to be a self-sustaining management tool. Pathogens and nematodes often work best for this type of approach because they are easily reared and stored. So-called 'inundative' or 'augmentation' biological control of weeds has been attempted with arthropods with varying degrees of success (Wapshere et al 1989; Valenti et al 1999). The risk of non-target damage is not thought to be as high when using a native agent, as their occurrence as a pest on other plants would likely have been recorded (Wapshere et al 1989).

The increasing public demand for certified organic and reduced pesticide use in agricultural production should encourage scientists to study biological control as part of an integrated pest management program in more areas of agricultural production, such as production of lowbush blueberries.

## 1.3 Blueberry Production

The lowbush (or wild) blueberry (*Vaccinium angustifolium* Ait., *V. myrtilloides* Michx.) is a rhizomatous plant that thrives in acidic, coarse soil (Jensen and Yarborough 2004).

Well known for its health benefits (Krzewińska 2004), the lowbush blueberry is produced mostly in Maine, Atlantic Canada, and Quebec (Yarborough 2004). In 2011, over 70 thousand hectares of blueberries were recorded in Canada, with a farm gate value of over $200 million (Statistics Canada 2012). In Nova Scotia alone, the farm gate value of blueberry is approximately $22 million (Statistics Canada, 2012).

Commercial blueberry fields are developed from naturally occurring stands within forested areas or abandoned agricultural fields, and are usually managed over a two year cycle. The first year in the cycle is the vegetative year, where previously pruned blueberry plants produce new shoots (Penney and McRae 2000). Flowers and berries are produced in the second year, which is also known as the "crop" year (Penney and McRae 2000). Productivity gains are largely attributed to advances in weed control, though fertility management and pollination also contribute (Jensen and Yarborough 2004). Weed control within lowbush blueberry fields is important for a number of reasons. Weed species may out-compete blueberry for nutrients, water, light and pollinators. Taller weeds that shade the blueberries not only lead to yield reduction (Jensen and Yarborough 2004), but make mechanical harvesting problematic.

Various weed management methods are employed. Preventative techniques such as cleaning equipment prior to entering a field can be effective. Boyd and White (2009) demonstrated that harvest equipment can be a significant factor in weed seed dispersal in blueberry fields. Mechanical control, such as hand-pulling of individual weeds, or plant patches, is often not economically viable (Jensen and Yarborough 2004), but pruning methods can help to control some species (Penney et al. 2008). However, the dominant form of weed management in the past several decades has been chemical (Jensen and

Yarborough 2004). Herbicides such as hexazinone and terbacil are commonly used in blueberry fields (Yarborough 2004), as well as glyphosate (Ismail and Yarborough 1981). Herbicides have been effective at managing weed species, but with consumers increasingly demanding healthier, or more ecologically sustainable production, other management strategies need to be developed to ensure a competitive blueberry industry.

Biological control within blueberry production is not yet common, but it has been examined and used by growers. Conservation biological control can be employed to protect the populations of natural enemies of various insect pests to lower the frequency and severity of a pest outbreak (Drummond et al 2009). The fungal pathogen *Beauveria bassiana* can also be used on many insect pests in lowbush blueberries (Drummond et al 2009). There have been no biological control programs against weeds unique to blueberry production, although agents against various weeds have been released in Atlantic Canada, and some of these plants can be an issue within lowbush blueberry (McCully and Jensen 2005). McCully and Jensen (2005) are of the opinion that the potential for biological control in lowbush blueberry is limited, due to the use of insecticides and fungicides. However, it is possible to use biological control in agricultural situations if susceptible stages of the insect are not present during application of pesticides and a lack of soil disturbance is an asset (Volenburg et al 1999). The reliance on pesticides within the industry can make biological control appear as the last resort for weed control, but given the difficulty in managing many weed species with current practices, biological control in blueberry fields is a concept worth exploring.

## 1.4 Spreading Dogbane

Spreading dogbane (*Apocynum androsaemifolium*) is a member of Apocynaceae (Dogwood) family (Sampson et al 1990). It is an herbaceous perennial normally found in light or sandy soils, reproduces by seed and rhizome, and tends to spread aggressively through a field (Sampson et al 1990). Spreading dogbane can grow up to 100 cm tall and has a somewhat variable growth habit. It is usually upright with spreading branches, but may be unbranched and somewhat prostrate (personal observation). Leaves are opposite, broadly elliptic or ovate, and spread out or droop from the stem (DiTommaso et al 2009). Flowers are terminal, with a tubular corolla and petals curving outward with interior pink markings (Fig 1.1). McCully et al (1991) reported that dogbane was present in 3.6% of the 115 surveyed fields in 1984-1985. A second survey of 128 fields between 2000-2001 showed a frequency of 7.6% (Jensen and Sampson, unpublished data). According to Agriculture and Agri-Food Canada (2005) this species is established in Quebec, Prince Edward Island, and New Brunswick, where it exerts moderate to high pest pressure, and is becoming established in Nova Scotia. Spreading dogbane is one of the more frequent species in Quebec blueberry fields (Lapointe and Rochefort 2001) as well as fields located in Maine, USA.

Spreading dogbane is problematic for several reasons. The above ground portion of the plant interferes with harvest, and sufficient densities can shade blueberry plants to the point that berries are not produced. There are no registered herbicides that effectively control dogbane without damaging the blueberries, and even if an efficacious product is identified the extensive root system makes long term control following a single herbicide application unlikely. Yarborough and Marra (1997) modeled yield loss of blueberries

due to spreading dogbane, and estimated that a 1% increase in dogbane cover led to a yield reduction of 41kg/ha. Additionally, glycosides in the latex-like sap of spreading dogbane have poisoning potential (Moore 1909).


Figure 1.1 Newly emerged dogbane ramet (a), fully grown spreading dogbane ramet (b), and spreading dogbane flowers (c).

Spreading dogbane can quickly establish within a field, making it important to detect invasions as quickly as possible. The closely related hemp dogbane (*A. cannabinum*) was observed to grow roots that penetrated soil 4.2 m deep and 5.9 m radially after two years of growth, when started from seed in a non-competitive environment (Frazier 1944). Webster et al (2000) studied patch expansion of *A. cannabinum* and found that patches could more than double in area in one year.

Control of spreading dogbane is difficult, and there is little literature on the subject. Yarborough and Bhowmik (1989) found that hexazinone use correlates with increases in cover and frequency of spreading dogbane. Yarborough and Marra (1997) wiped plants with glyphosate, which gave some control but did not address regrowth from the root system. Physical control alone does not effectively control spreading dogbane, and mowing may increase rhizome growth (Sampson et al 1990). Similar problems occur when attempting to control hemp dogbane (Welch and Ross, 1997). The difficulty in controlling spreading dogbane makes it necessary to explore other management options (Wu 2010).

## 1.5 Dogbane Beetle

*Chrysochus auratus*, first described by Fabricus in 1775 (Wilson 1934), is a leaf beetle (Coleoptera: Chrysomelidae) found throughout the midwest and the eastern United States (Williams 1992). It is considered common, though distribution is apparently uneven (Dobler and Farrell 1999). Bousquet (1991) lists dogbane beetle as occurring in the Canadian provinces from Quebec to British Columbia. There are no previously published records of the dogbane beetle in Nova Scotia, though I have found it in several areas. Williams (1992) noted that the dogbane beetle appears to occur in small populations without a large amount of movement between host patches. The literature on host specificity is variable, with some papers referring to it as feeding exclusively on the members of Apocynaceae (Peterson et al 2005, Dobler and Farrell 1999), and others either including Asclepiadaceae in its host range (Dobler et al 1998) or stating its occurrence (but not necessarily feeding) on milkweeds (Weiss and West 1921). Wilson

(1934) provided a detailed description of both external and internal anatomy of the adult beetle, while Weiss and West (1921) have detailed the anatomy of larvae.

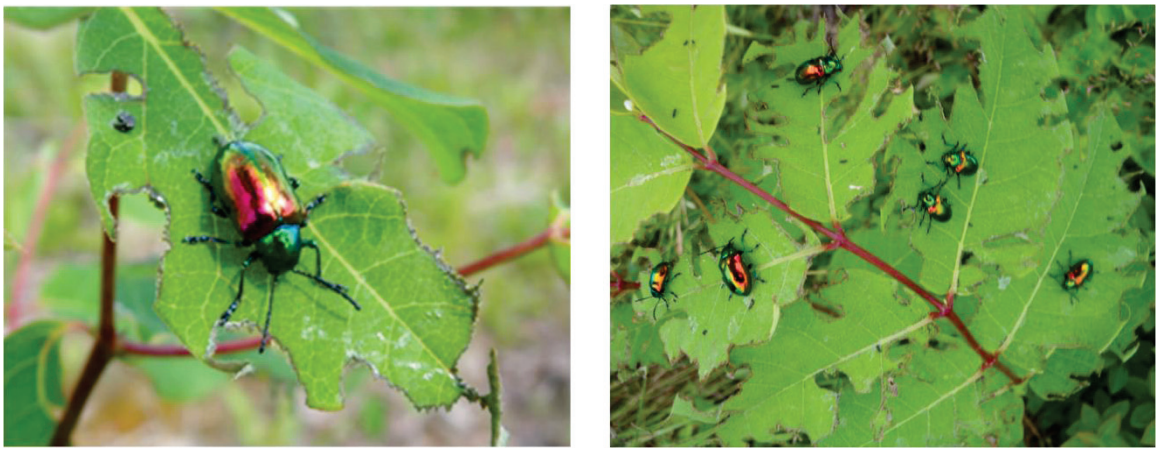

Figure 1.2 The dogbane beetle, *C. auratus*, on spreading dogbane.

Dogbane beetle is univoltine and polyandrous. Adults emerge in summer, feeding on foliage of host plants, while larvae feed on the roots of host plants (Peterson et al 2001) before eventually pupating in a 'chamber' in the soil (Peterson et al 2005). Weiss and West (1921) speculate that pupation occurs in the spring or early summer, as they observed larvae as late as November. Larval feeding usually occurs on the underside of roots (Weiss and West 1921). Wilson (1934) observed that eggs are covered in a black substance, with the appearance of frass, and first instar larvae remain beneath it for a short time after hatching. *Chrysochus auratus* adults usually feed on leaf margins, and exhibit the unique behaviour of cleaning their mouthparts of latex by dragging them backwards across the leaf (Williams 1991). During feeding, the beetle ingests cardenolides from the host plant, which are secreted from glands in the elytra and the pronotum when the beetle is disturbed (Dobler et al 1998). It is likely that this is a mechanism to fend off predators (Dobler et al 1998).

## 1.6 Summary

The importance of weed management to the lowbush blueberry industry is very clear. Thus far management has been reliant on cultural methods (such as mowing and burning) and chemical methods. There is an opportunity and need to study biological control as an additional management tool for spreading dogbane. This thesis research examines the biology and behavior of the native leaf beetle, *C. auratus*, on spreading dogbane in commercial lowbush blueberry fields. This is done through investigation of the beetle's life history in Nova Scotia, host specificity, and the extent of defoliation caused by *C. auratus*.

# Chapter 2.0: Host Specificity and Potential Impact of Dogbane Beetle, *Chrysochus auratus* Fab.

## 2.1 ABSTRACT

Dogbane beetle, *Chrysochus auratus*, is being studied for its biological control potential. The target plant is spreading dogbane, *Apocynum androsaemifolium*, a native perennial weed in lowbush blueberry (*Vaccinium angustifolium*) fields. In no-choice host experiments common milkweed (*Asclepias syriaca*), periwinkle (*Vinca minor*), wild raisin (*Viburnum cassenoides*), and lowbush blueberry were not eaten by adult dogbane beetles, whereas significant feeding occurred on spreading dogbane foliage. In one experimental unit, less than 5% defoliation to two blueberry leaves was observed. Results indicate host choice of the beetle is limited, and suggest that beetles will not consume all plants within Apocynaceae, not lowbush blueberry. Cage tests in the field determined that 16 beetles per ramet could decrease foliage dry weight by 65% within 12 days, compared to ramets protected from beetles.

## 2.2 Introduction

Zwölfer and Harris (1971) authored a definitive review on host specificity of insects in regards to biological weed control. They stated that there is no guarantee that an insect will not cause non-target damage. However, following the multi-dimensional approach described in the review, and refined by subsequent authors (ex: Heard 1999, Heard and Van Klinken 2004, Briese 2005), can allow a more educated determination of the risk of a proposed biological control agent. This approach includes searching for records of the insect's plant use within its native range, host records of closely related insect species, and knowledge of the biological and ecological restrictions that affect the host range of the insect. Insect diversity and behavior makes it impossible to completely standardize

testing methods (Heard 1999). Nevertheless, host specificity testing is still necessary in order to determine the likelihood of a biological control agent inflicting damage on non-target plants. Especially worrisome is the potential for economic damage by feeding on crop plants.

Another step in the investigation of a biological control agent is to evaluate the effectiveness of the agent. In the case of defoliating insects, it is important to determine how much damage they can inflict on the target plant. This is a complex undertaking because herbivory affects a plant in numerous ways. Decreased flower number and delayed flowering can occur from direct feeding or through decreased energy availability for floral production (Crawley 1989). This may have an effect on seed set and weight (Proveda et al 2003, but see Stephenson 1981), or the ability of the plant to attract pollinators (Mothershead and Marquis 2000). The asexual reproduction of a plant can also be altered, by releasing apical dominance or compromising a plant's ability to manufacture sufficient energy to produce new ramets or tillers. Defoliation decreases photosynthetic capacity which affects energy allocation within the plant by direct loss of foliage and by decreasing leaf longevity (Crawley 1989). Factors such as nutrient availability, competing plants, interactions between root and shoot herbivores, and even visitation of plants by humans, affect how a plant responds to herbivory (Wise and Abrahamson 2005, Hambäck and Beckerman 2003, Blossey and Hunt-Joshi 2003, Cahill et al 2001, respectively). A plant may tolerate herbivory and exhibit compensatory growth, or may have a chemical or physical resistance mechanism that lowers plant attractiveness to herbivores (Strauss and Agrawal 1999).

Spreading dogbane, *Apocynum androsaemifolium* L. (Apocynaceae), is a perennial plant native to North America. This species reproduces by seed and rhizome, and can be a problematic weed in lowbush blueberry (*Vaccinium angustifolium* Ait. Ericaceae) fields of Canada and the USA. The constraints of this cropping system have led to producers relying heavily on herbicides for management of spreading dogbane. Mowing the plant provides limited control, but an application of glyphosate is much more effective (Yarborough and Marra (1997). Wu (2010) provided several additional options for control of established spreading dogbane in blueberry fields, including spot sprays of dicamba for small populations, and broadcast sprays with nicosulfuron for large established populations. However, non-chemical weed management options are desirable to help ensure the ecological sustainability of the lowbush blueberry agro-ecosystem, as well as to conform to governmental regulations and consumer demands that are increasingly health and environmentally focused.

The native dogbane beetle, *Chrysochus auratus* Fabricus (Coleoptera: Chrysomelidae), has been observed in a number of lowbush blueberry fields. The beetle is known to feed on members of Apocynaceae, such as hemp dogbane (*Apocynum cannabinum*) (Peterson et al 2005, Dobler and Farrell 1999) and occurs throughout Canada (Bousquet 1991) and much of the United States (Williams 1992), but little is known of its host range. This chapter describes host specificity experiments on the dogbane beetle, as well as relevant observations of *C. auratus* within lowbush blueberry fields. I tested the following null hypotheses: i) starved dogbane beetles will not consume lowbush blueberry foliage; and ii) dogbane beetle will not consume plants closely related to spreading dogbane which are available in and around lowbush blueberry fields of Nova Scotia. Additionally, this

chapter describes the results of a cage test of defoliation of spreading dogbane by dogbane beetle.

## 2.3 Materials and Methods

### 2.3.1 Host Specificity of Dogbane Beetle

Two no-choice feeding experiments were conducted. Plastic jars (200 mL, 11 cm high), with the top covered with organza were each used to contain two beetles during the experiments. Each container held a small stem of one plant species in a 15 mL vial of water. All stems except milkweed were approximately 9 cm long. Milkweed was cut so that each vial contained a single 9 cm leaf. When plant stems were completely defoliated or appeared visibly wilted they were replaced with fresh stems of a comparable size. Jars in which beetles died within two days of placement in the containers were replaced with new beetles and plant material.

*2010 Experiment*

In 2010, beetles were collected by hand from Oxford, Nova Scotia (45°45'17" N 36°49'35" W). No regard was given to whether the beetles were male or female, or the plant species from which they were collected. Beetles and dogbane foliage were placed in a plastic container with a ventilated lid. They were then held in the lab (21°C) in a large glass beaker containing fresh dogbane foliage for two days prior to starting the experiment. Plant species selected for the experiment were spreading dogbane, lowbush blueberry, and common milkweed (*Aslepias syriaca* L. Asclepiadaceae). Spreading dogbane and lowbush blueberry were collected from the site of beetle collection in Oxford. Common milkweed was collected in Debert, Nova Scotia. Common milkweed was chosen because Asclepiadaceae is the closest family to that of dogbane, and they

have similar chemical characteristics (Dobler and Farrell 1999). Lowbush blueberry was tested because it is the crop plant within the proposed biological control system. The jars were placed in a growth chamber (16:8 hours light:dark, 20°C:10°C) and organized as a completely randomized design with three replicates, with spreading dogbane as the control. Plant stems that reached defoliation ratings of 7 or higher were replaced to ensure plant material retained sufficient turgor pressure.

*2011 Experiment*

Beetles were collected from Westbrook, Nova Scotia (45°32'57" N 64°17'0.81" W). The plant species included were spreading dogbane, lowbush blueberry, periwinkle (*Vinca minor* L. Apocynaceae), and wild raisin (*Viburnum cassenoides* L. Adoxaceae). The first two species were collected in Westbrook, periwinkle from the campus of the Nova Scotia Agricultural College, Bible Hill, Nova Scotia, and wild raisin from Oxford. Periwinkle was selected because it belongs to the same family as spreading dogbane and has value as an ornamental. Wild raisin was included because dogbane beetles were frequently observed on it in at the Oxford blueberry field. I was unable to obtain milkweed for this experiment. Jars were placed on a lighted shelf in a room at 21°C and 16:8 light: dark as a completely randomized design with five replicates. Beetles were starved for two days prior to introducing them to the plant specimens.

*Data Collection and Analysis*

Beetles were monitored daily for survival. Defoliation was recorded when both beetles were dead, or if the stem had to be replaced. A visual assessment rating on a 0-10 scale was used, with 0 representing no defoliation and 10 equaling complete defoliation. Ratings were averaged for jars with multiple stems. It should be noted that only

spreading dogbane stems in the 2010 experiment reached a defoliation rating of 7 and needed to be replaced. Data analysis was conducted in SAS® 9.1 (SAS Institute Cary, NC). A one-way ANOVA was conducted on beetle survivorship data. A Kruskal-Wallis test was used to analyze effect of beetles on defoliation ratings for each experiment. All analyses were done at $\alpha = 0.05$.

### 2.3.2 Potential Impact of Dogbane Beetle on Spreading Dogbane

A cage experiment was conducted in a lowbush blueberry field in Oxford, Nova Scotia (45°45'17"N 36°49'35"W) from 18 August 2011 to 30 August 2011. Rectangular cages were constructed using four survey stakes, and window screen. Cage dimensions were 40 x 40 x 81 cm tall. Screening was securely fastened to the stakes with staples. Cages were placed over individual spreading dogbane ramets. Care was taken to choose ramets of consistent size and with minimal evidence of damage and senescence. Ramet height, number of branches, and the presence/absence of flowers were recorded. Beetles were collected from within dogbane patches at two different sites, Westbrook (45°32'57"N 64°17'0.81"W) and Halfway River NS (45°31'12"N 64°20'37"W). Beetles from both locations were randomly assigned to the cages in Oxford at densities of 0, 2, 4, 8, and 16 beetles per cage. This experiment was conducted as a completely randomized design with five replicates.

At the end of the experiment, cages were removed and the number of beetles, both dead and alive, was recorded. The ground under each ramet was examined for dead beetles. The height of each ramet, number of branches on each, and the presence/absence of flowers/seed pods was also recorded. A defoliation rating on a scale of 0 to 10 assigned, with zero representing no defoliation and 10 complete defoliation. Ramets were cut at

ground level, placed in labeled paper bags, and transported to the lab in a cooler. The area of a leaf on the third branch from the bottom of each ramet was measured using CompuEye, Leaf & Symptom Area software (Bakr 2005) from digital scans. As the leaves of dogbane are arranged in pairs, the right leaf of the second pair was generally selected, unless it was no longer on the plant. The right leaf was identified by holding the base of the branch and selecting the leaf when the branch was right-side up. The total fresh weight, and the total fresh leaf weight, of each ramet were recorded. Total leaf weight was obtained by cutting off all leaves and weighing separately. Ramets were then dried for a week in an oven (70°C) and total try weight, and total dry leaf weight was measured.

*Data Analysis*

The differences in height and branch number for each ramet were calculated by subtracting initial measurements from those recorded at the end of the experiment. The changes in height and branch number were analyzed, along with fresh and dry weights, leaf area, and defoliation ratings in SAS 9.1 using PROC GLM to conduct a one-way ANOVA with Tukey's mean comparison when significant treatment effects were found. A square root transformation was applied to the difference in height after adding a constant of 5 to make the values positive. Defoliation ratings were square root transformed. Back transformed results are presented as required. Orthogonal contrasts were performed to determine if there were linear or quadratic relationships between beetle number and the dogbane physical parameters. All analyses were done at $\alpha = 0.05$.

## 2.4 Results

### 2.4.1 Host Specificity of Dogbane Beetle

Beetle longevity did not differ on the different plant species in 2010 ($F = 0.92$, $P = 0.449$, $df = 2$ trt, 6 error) or 2011 ($F = 0.66$, $P = 0.591$, $df = 3$ trt, 16 error) (Table 2.1). Beetles tended to live longer in 2010 than 2011.

Table 2.1: Longevity of dogbane beetle, *C. auratus*, on different plant species in 2010 & 2011.

| Year | Plant Species | Longevity (days) ± SD |
|---|---|---|
| 2010 | Spreading dogbane | 17.17±5.51 |
| | Common milkweed | 12.50±3.28 |
| | Lowbush blueberry | 12.83±5.03 |
| 2011 | Spreading dogbane | 7.00±1.37 |
| | Periwinkle | 6.90±0.82 |
| | Lowbush blueberry | 7.70±1.51 |
| | Wild raisin | 6.90±0.82 |

Dogbane beetle consumed dogbane. Common milkweed, periwinkle, and wild raisin were not consumed in the 2010 ($H = 6.72$, $P = 0.035$, $df = 2$) or 2011 ($H = 18.53$, $P < 0.001$, $df = 3$) experiments (Table 2.2). There was a slight amount of defoliation to blueberry in 2010, but blueberry was not consumed by dogbane beetles in 2011.

Table 2.2: Defoliation of plant species by dogbane beetle, *C. auratus,* in no-choice experiments.

| Year | Plant species | Defoliation rating± SD[1] | Median |
|---|---|---|---|
| 2010 | Spreading dogbane | 5.7± 1 | 6 |
| | Common milkweed | 0 | 0 |
| | Lowbush blueberry | 0.33±0.58 | 0 |
| 2011 | Spreading dogbane | 5.8±2.2 | 6 |
| | Periwinkle | 0 | 0 |
| | Lowbush blueberry | 0 | 0 |
| | Wild raisin | 0 | 0 |

[1] defoliation rating on a 0-10 scale, where 0 represents no defoliation and 10 represents complete defoliation.

### 2.4.2 Potential Impact of Dogbane Beetle on Spreading Dogbane

In the field cage experiment, the presence of dogbane beetles did not reduce the height of spreading dogbane ramets ($F = 0.15$, $P = 0.959$, $df = 4$ trt, 20 error). Some ramets continue vertical growth and others were shorter at the conclusion of the experiment (Table 2.3). The presence of dogbane beetles did not result in changes in branch number ($F = 2.03$, $P = 0.129$, $df = 4$ trt, 20 error). Although the fresh weight of ramets treated with 16 beetles weighed approximately 45% less than the treatment with no beetles, ramet fresh weight did not differ significantly ($F = 1.96$, $P = 0.503$, $df = 4$ trt, 20 error). However, total dry weight of with ramets treated with 16 beetles weighed significantly less than the control ($F = 2.86$, $P = 0.050$, $df = 4$ trt, 20 error). Both fresh and dry weight of leaves was affected by the number of beetles (fresh: $F = 3.37$, $P = 0.029$, $df = 4$ trt, 20 error; dry: $F = 3.69$, $P = 0.021$, $df = 4$ trt, 20 error), with decreased weight of leaves found with increased numbers of beetles, but mean leaf area did not differ among treatments ($F = 1.04$, $P = 0.413$, $df = 4$ trt, 20 error). Beetle numbers had a significant effect on defoliation ratings ($F = 6.18$, $P = 0.0021$, $df = 4$) (Table 2.4). Orthogonal contrasts showed a linear relationship between beetle number and dogbane physical parameters, with the exception of leaf area. There was also a linear relationship between beetle number and the defoliation ratings. The total number of live beetles at the end of the experimental period was 20, which is only a fraction of the original number. There is no accurate way to account for beetle escapes and deaths, as other insects could have consumed or removed them. It is not possible to tell precisely when the beetle numbers declined, but the majority of beetles were in the cages for the first week of the experiment.

Table 2.3: Effect of dogbane beetle, *C. auratus*, density on dogbane physical parameters (mean ± SD) in a field cage experiment, Oxford Nova Scotia, 2011, with results of orthogonal contrasts testing for linear and quadratic relationships.

| Treatment (#beetles) | height difference (cm)[1] | branch # difference | fresh weight (g) | dry weight (g) | fresh leaf weight (g) | dry leaf weight (g) | leaf area ($cm^2$) |
|---|---|---|---|---|---|---|---|
| 0 | 2.4 | 0.6 ± 0.894 | 36.00 ± 14.10 | 13.4 ± 4.48*a* | 16.86 ± 7.27*a* | 7.56 ± 2.95*a* | 16.173 ± 6.05 |
| 2 | 3.0 | 1.2 ± 1.304 | 31.84 ± 12.86 | 10.64 ± 3.91*ab* | 11.22 ± 4.31*ab* | 5.06 ± 1.911*ab* | 14.567 ± 3.92 |
| 4 | 2.4 | 0.8 ± 0.837 | 25.32 ± 11.91 | 9.66 ± 4.32*ab* | 9.70 ± 5.51*ab* | 5.54 ± 2.45*ab* | 12.593 ± 2.82 |
| 8 | 2.7 | -0.2 ± 0.447 | 21.50 ± 7.78 | 7.74 ± 2.31*ab* | 8.20 ± 2.47*ab* | 3.72 ± 0.835*ab* | 13.295 ± 1.894 |
| 16 | 0.8 | 0.2 ± 0.447 | 19.80 ± 8.21 | 6.04 ± 3.09*b* | 6.10 ± 3.83*b* | 2.66 ± 1.922*b* | 11.916 ± 2.35 |
| *Contrasts* | | | | | | | |
| *linear* | | | *0.0120* | *0.0032* | *0.0022* | *0.0014* | *0.0773* |
| *quadratic* | | | *0.7384* | *0.8513* | *0.4018* | *0.4783* | *0.6201* |

[1] Differences were calculated by subtracting initial measurements from final measurements. Back-transformed means are displayed, thus lack of SD

Table 2.4: The effect of dogbane beetle, *C. auratus*, density on dogbane defoliation (range, mean and 95% CI) in a field cage experiment, Oxford Nova Scotia, 2011.

| | Defoliation Ratings[1] | | |
|---|---|---|---|
| Treatment (# of beetles) | Range | Mean | 95% CI |
| 0 | 0-1 | 0.16*b* | 0.22, 0.90 |
| 2 | 1 | 1.0*ab* | 1 |
| 4 | 0-1 | 0.78*b* | 0.13, 1.98 |
| 8 | 1-2 | 1.2*ab* | 0.81, 1.61 |
| 16 | 1-5 | 2.8*a* | 1.42, 4.68 |
| *Contrasts* | | | |
| *linear* | | *0.0002* | |
| *quadratic* | | *0.6632* | |

[1] Back-transformed data shown, original data were square root transformed.

## 2.5 Discussion

Initial investigations into weed biological control using insects require insight into both the host specificity and the potential impact of the insect. The experiments described in this chapter, coupled with previous studies, suggest dogbane beetle has a narrow host range, a desirable trait in a biological control agent. My experiments also demonstrated that dogbane beetle can have a significant negative impact on the target plant.

### 2.5.1 Host Specificity of Dogbane Beetle

Beetles readily consumed spreading dogbane, but did not eat common milkweed, periwinkle, wild raisin, or lowbush blueberry (in most cases). In the host specificity experiment I did not find any difference in beetle longevity between plant species despite observing significant differences in leaf consumption. Though the longevity of the beetles was consistent with starved leaf beetle longevity in literature (DeLoach et al 2003; Heard and Van Klinken 2004), this lack of significance suggests that starvation did not cause beetle death in this experiment. It is possible that beetles consumed sufficient

spreading dogbane prior to collection to sustain themselves during the experiment. The lack of difference in longevity due to feeding could also be attributed to the beetles reaching the end of their lifespan. Longer survival in 2010 lends some support to this explanation, as the beetles were collected approximately one week earlier in 2010 versus 2011.

I often observed dogbane beetles on wild raisin at the Oxford, Nova Scotia site. However, the lack of consumption of wild raisin in laboratory experiments suggests that dogbane beetle does not use this plant as a food source. The beetle may simply use the plant as substrate or for reproductive purposes. Dogbane beetle egg masses were found on this plant species, but the beetle tends to oviposit on many different surfaces within dogbane patches. I have observed egg masses on blades of grass, several different shrubs, marking flags, and the screen used in the experiment. Beetles were also observed mating on wild raisin. My results indicate lowbush blueberry is not a suitable nutritional host for the dogbane beetle. A small amount of feeding occurred over 24 hours in one replicate in the 2010 experiment midway through the experimental period, after which the beetles survived a number of days without further consumption. The amount of leaf area removed on each of the two leaves that were attacked was estimated to be about 5%. This small amount of feeding may have been due to contamination as blueberry stems were harvested in the field using the same clippers used to harvest dogbane ramets. If there was transfer of dogbane sap onto blueberry leaves, this may have stimulated beetle feeding upon the blueberry foliage. Beetles were never observed feeding on lowbush blueberry in any the fields used throughout the summers of 2010 and 2011.

A limited number of past studies have reported on the host range of dogbane beetle and at least two other species in genus *Chrysochus*: *C. cobaltinus* LeConte, which is also native to North America, and *C.* (*Eumpolpus*) *asclepiadeus* Pallas, which is found in Eurasia. Results of a limited number of surveys, field observation, and experiments indicate that *C. auratus* has a limited host range (Table 2.5). Plant host studies focused on members of Asclepiadaceae (*Asclepias* spp*,* and *Vincetoxicum* spp.) and Apocynaceae (*Apocynum* spp.).

Table 2.5: Reported host plants of *C. auratus*, *C. cobaltinus*, and *C. asclepiadeus*.

| Insect species | Plant species[1] | Feeding recorded | Source |
|---|---|---|---|
| *C. auratus* | *Asclepias syriaca*$^{n}$ | NA[2] | Dailey et al 1978 |
| *C. auratus* | *Asclepias* sp.$^{n}$ | NA | Weiss and West 1921 |
| *C. auratus* | *Apocynum cannabinum*$^{n}$ | Yes | Dobler and Farrell 1999 |
| *C. auratus* | *Apocynum cannabinum*$^{t}$ | Yes | Dobler and Farrell 1999 |
| | *Asclepias speciosa*$^{t}$*, Asclepias syriaca*$^{t}$ | No | |
| *C. cobaltinus* | *Asclepias speciosa*$^{t}$*, Asclepias eriocarpa*$^{t}$*,* *Apocynum cannabinum*$^{t}$ | Yes | Dobler and Farrell 1999 |
| *C. asclepiadeus* | *Vincetoxicum hirundiaria*$^{n}$ | Yes | Dobler and Farrell 1999 |
| *C. asclepiadeus* | *V. hirundinaria, V. nigrum, V. rossicum* | Yes | Weed et al 2011 |

[1]: *n*- indicates author noted presence or feeding in the field; *t*-indicates a host choice experiment.
[2]: only presence on the plant was noted, not feeding behavior

Dobler and Farrell (1999) considered three different *C. auratus* populations from different states. Consistent with the results of this study, they found that dogbane beetle would not consume two different milkweed species in both choice and no-choice experiments. Other authors have recorded the presence, but not feeding, of *C. auratus* on milkweeds (Dailey et al 1978, Weiss and West 1921). I often observed dogbane beetles

on non-host plants in addition to wild raisin, including bracken fern (*Pteridium aquilinum*, Dennistaedtiaceae), sweet fern (*Comptonia peregrine*, Myriaceae), Northern bush honeysuckle (*Diervilla lonicera*, Caprifoliaceae), and tickle grass (*Agrostis hyemalis*, Poaceae). Dogbane beetle was often observed on many of the other taller species including ferns, grasses, and shrubs. *C. cobaltinus* seems to have a broader host range, but is still limited to closely related species (Dobler and Farrell 1999). There was one report of extensive defoliation of pecan trees by the dogbane beetle, but this was discredited by Williams (1988), who believed it highly unlikely and suggested the account could have been due to misidentification or a typographical error. I qualitatively observed that *C. auratus* shows no preference in oviposition sites, often making use of non-host plants and other surfaces. However, there is no information available on the host specificity of larvae. It has also been suggested that both North American *Chrysochus* species have evolved to feed on plants in the Asclepiadaceae and Apocynaceae that produce cardenolides, which are toxic (Labeyrie and Dobler 2004). Insects can have host ranges limited by physiological and behavioral adaptations (Zwölfer and Harris 1971), and this could be one such case.

### 2.5.2 Potential Impact of Dogbane Beetle

The data was analyzed as if the original number of beetles remained present in the cages throughout the duration of the experiment. However, many beetles either died or escaped from cages. A larger amount of feeding would be expected in a situation where the assigned number of beetles was present throughout the entire experimental period. Although no reductions in leaf area were detected, defoliating ratings and dry weight data suggested that the treatment with 16 beetles caused significant injury to plants. A 65%

reduction in the dry leaf weight, compared to the control, was achieved with this treatment. The results contradict each other, but as only one leaf/ramet was used for leaf area, I believe more credence should be given to the defoliation ratings and dry weight data. The linearity of the weight data demonstrates that increasing numbers of beetles decreases the weight of ramets. Little change in ramet height or branch number was expected as ramets reach maximum growth prior to the flowering stage, which was the growth stage of most ramets at the beginning of this experiment. It would have been ideal to start the experiment at least a week earlier, before the beetle population began to decline. The later start may have resulted in less vigorous beetles, and a less palatable food source due to older foliage. Any repetition of this experiment should include a larger number of replications, as there were high levels of variability in the results.

Responses of Apocynaceae and Asclepidaceae to herbivory have been studied in several papers. Delaney and Higley (2006) demonstrated that the photosynthetic rate of *Apocynum cannabinum* leaves was unaffected at 25% defoliation, but significantly reduced with 75% defoliation. There were no treatments with mid-range defoliation levels so it is not possible to tell if, for example, 50% defoliation would have a significant effect. Severing the lateral veins of *Asclepias syriaca* (which has a leaf structure similar to dogbane) can reduce photosynthesis by 50% (Delaney and Higley 2006), and midrib severing of *Nerium oleander* (Apocynaceae) had a similar effect (Delaney 2008). The timing of defoliation has been shown to have a role in determining how the plant responds. Defoliation of *A. cannabinum* in early June tended to have less of an effect than subsequent injury (Delaney and Higley 2006) and *A. syriaca* showed greater decreases in photosynthesis when defoliated by insects during early flowering and early

seed formation stages compared to the pre-flowering and seed maturing stages (Delaney et al 2009).

Based on the results of this experiment, 16 beetles per ramet would be required to achieve a statistically significant level of damage. The visual assessment of defoliation indicated that 16 beetles can remove about half of the foliage of an average sized ramet (in this experiment, about 80 cm tall) within two weeks.

A reduction in photosynthetic rates would reduce the flow of carbohydrates to underground structures, and over several seasons could affect dogbane's ability to produce new ramets. The dogbane beetle does not emerge until late June or early July in Nova Scotia, at which point many ramets have formed flower buds, or reached the early flowering stage (personal observation, Wu 2010). If the goal is to interrupt the flow of carbohydrates to underground structures, and thus interfere with the production of new ramets, then the beetle is present during the ideal time period. However, further experimentation is required in order to determine if the dogbane beetle has a long-term negative impact on the dogbane population. Short-term defoliation may be useful in reduction of blueberry shading, but such benefits do not necessarily justify the potential cost of a biological control program.

## 2.6 Conclusion

My experiments and previously published reports suggest *C. auratus* is specific to the genus *Apocynum* and does not accept all members of Apocynaceae. However, more extensive host testing would be needed to confirm this hypothesis. Further testing should include as many members of Apocynaceae and other cardenolide producing plant species

as can be located within the province of Nova Scotia. More important for pest management, the negligible amount of lowbush blueberry feeding observed here was probably due to contamination from spreading dogbane, and it is unlikely that the dogbane beetle would inflict damage on blueberry in a field situation. However, cage tests found that high numbers of beetles (16) were required to cause significant defoliation. This number of beetles on a single ramet was rarely observed in the field, so augmentation of natural populations would likely be needed to achieve effective control. Experiments spanning multiple growing seasons are necessary to ascertain if the beetle could reduce the spread or ramet density of spreading dogbane.

## Chapter 3.0 Life History of Dogbane Beetle, *Chrysochus auratus* Fab. (Coleoptera: Chrysomelidae) in Nova Scotia.

### 3.1 ABSTRACT

The abundance, distribution, and development of dogbane beetle, *Chrysochus auratus*, was studied in several lowbush blueberry fields in Nova Scotia. The fecundity, fertility, timing of pupation, and number of instars were also examined. Beetles were present in the field for 8-12 weeks beginning in late June or early July, or 225-335 growing degree days. Peak beetle abundance varied from 4-7 beetles/$m^2$, with the peak occurring at 357-577 growing degree days. Beetle density at individual sites was well fitted with a five parameter Weibull model, as predicted by growing degree days, with $R^2$ values ranging from 0.8591 to 0.9752. However, model fit across sites was poor, indicating that other factors need to be incorporated to develop a model that could be used predict emergence across multiple sites. Females deposited approximately 100 eggs over a 20 day period, with an egg viability of over 90%. Pupae were found on June 1$^{st}$ and June 15$^{th}$. Cluster analysis on head capsule widths of field collected beetles could not ascertain the number of dogbane beetle instars.

### 3.2 Introduction

Knowledge of a prospective biological control agent's life history is fundamental to predicting potential to succeed in controlling a pest, or to induce harm in an agricultural system. A study of the life history of a potential agent can inform us of its compatibility with its new climate, the target weed or insect pest, and the production system in which it could be utilized. In a broader context, these studies provide insights into relations within and between taxonomic groups through comparisons of morphology and behavior

(Michener 1953). They also provide insights into how plasticity in life history traits can influence fitness in terms of growth rate, development time and reproductive success (Nylin and Gotthard 1998).

The dogbane beetle, *Chrysochus auratus* Fab. (Coleoptera: Chrysomelidae), is a phytophagous beetle native to North America, found throughout the Midwestern and Eastern United States (Williams 1992). Bousquet (1991) recorded it in Canada, from British Columbia to Quebec. Weiss and West (1921) observed dogbane beetle in New Jersey, where the adults were present from late May to mid-August. Capsules containing multiple eggs were deposited over a long period of time, with most hatching in July. Larvae dropped to the ground and fed on the roots of spreading dogbane, and pupation was suspected to take place in spring or early summer (Weiss and West 1921). Wilson (1934) primarily described adult morphology, with limited observations on behavior. There have been accounts of feeding behavior (Williams 1991), host use (Dobler and Farrell 1999), and some information on longevity, fecundity, and fertility (Peterson et al 2005). Williams (1992) conducted a small study on short distance movements of the beetle. The majority of movement was over distances less than 5 meters, suggesting the beetle could be a poor colonizer in a situation where host plants are patchy. However, a larger scale experiment by St Pierre et al (2005) showed that small numbers of beetles will move several kilometers and that beetles remained for longer periods of time in large patches.

Information on the life history, movement, and population sizes of dogbane beetle is fragmented throughout literature and there is no information relating to emergence timing, or population sizes of dogbane beetles in Eastern Canada. The life history of the

dogbane beetle is also incomplete, with no documentation on the number of instars. The use of calendar days in existing literature makes it difficult to extrapolate the timing of life history events of the beetle in different locations. This type of information is necessary to evaluate the potential of dogbane beetle for biological control. This chapter describes the life cycle of dogbane beetle in Nova Scotia at several sites, with emphasis on adult population dynamics but includes information on the fecundity and fertility of the beetle. Non-linear regression was employed to discover if a model based on growing degree days could be used to describe growth of the adult dogbane beetle population over thermal time. The relationship between beetle density and dogbane density and growth stage was determined. The spatial pattern of adult dogbane beetle in several experimental fields is also discussed.

## 3.3 Materials and Methods

### 3.3.1 Adult Distribution and Abundance in Blueberry Fields

Intra-field distribution of adult dogbane beetle was studied in fields known to have a population of the insect. All sites had an orthic humo-ferric podzol soil (Nowland and MacDougall 1973). Data was collected in 2010 from a blueberry field in Oxford, NS (45°45'17" N, 36°49'35" W) three times a week between 22 June 2010 and 2 September 2010. This site had a diverse plant population, including grasses, several shrub species, hardwood saplings, and various herbaceous perennials. Beetle count data were collected within twelve randomly placed one meter square quadrats. Quadrat placement varied with each counting date, but was patterned over the field in a 'w' pattern within the dogbane area. The number of beetles on each dogbane ramet, number of beetles on other plants, and the number of mating pairs was recorded. A visual assessment of defoliation

was made at each quadrat, using a 0-10 scale (0=no defoliation, 10=complete defoliation). Soil and air temperature was monitored hourly using a Hobo® Pro V2 logger (Onset Computer Corporation) starting on 22 June 2010, with the soil sensor 5 cm in the ground. Air temperature data prior to this date was obtained from Environment Canada data recorded at the Nappan weather station, which is approximately 30 km from Oxford, NS. The station is located at 45°45'34.400" N and 64°14'29.200" W, at an elevation of 19.8 m and is the Environment Canada weather station closest to the Oxford site.

Data were collected in 2011 from three lowbush blueberry fields, with permanent quadrats arranged in a grid pattern. Grid dimensions differed among sites due to the differences in the size and shape of dogbane patches. All quadrats were one meter square. The same Oxford, NS site was used as in 2010. Four transects were set up five meters apart. Each transect extended into the central part of the field, starting at the roadside, and contained six quadrats, spaced ten meters apart. The second blueberry field in Westbrook NS (45°32'57" N 64°17'0.81" W) had five transects, each five meters apart, extending from the roadside into the central part of the field with four quadrats set five meters apart. The flora at this site was predominantly lowbush blueberry, spreading dogbane, and vetch. The third blueberry field in Halfway River, NS (45°31'12.18" N 64°20'37.52" W) contained four transects set five meters apart, starting at the forest edge. Quadrats within transects were placed five meters apart, with six quadrats in each transect (Appendix I). This site's plant community consisted mostly of lowbush blueberry, spreading dogbane, and a couple of other tall herbaceous plant species. Soil and air temperatures were measured hourly in Oxford with a Hobo® Pro V2 logger (Onset

Computer Corporation) beginning 18 May 2011. Air temperatures prior to this date were obtained from the Nappan weather station. Westbrook and Halfway River air temperatures were monitored hourly using Hobo Pro Series loggers (Onset Computer Corporation) starting 1 June 2011. Air temperatures prior to this date were obtained from the Environment Canada weather station in Parrsborro, NS (45°24'48.00" N 64°20'49.00" W, elevation 30.90m) located approximately 20 km from Westbrook and 15 km from Halfway River.

Data were collected three times a week at each site. Number of beetles per ramet, beetles on other plants, mating pairs/ramet, mating pairs on other plants, and number of spreading dogbane ramets were recorded. A visual assessment of defoliation was done as in 2010. In addition, the growth stage of the ramets in each quadrat was recorded. The scale used for this was based on the extended BBCH scale (an acronym for the developers of the scale: Bleiholder, Van den Boom, Langeluddeke & Stauss), as originally described by Lancashire et al (1991) and then adapted slightly for weed species by Hess et al (1997) (Appendix II). This scale is not applied to individual plants, but is used to describe groups or stands of the plants.

### 3.3.2 Fecundity and Fertility

This experiment evaluated the fecundity and fertility of dogbane beetle and attempted to establish whether female beetles mating with multiple males (polyandry) would yield more eggs and larvae than monogamous mating pairs.

The experiment was established at the Westbrook, NS site on 22 July 2011. A pair of beetles was isolated on a dogbane branch by enclosing it with a white organza bag (20 x

27 cm). Each dogbane branch had 6-8 leaves and was free of egg clusters. Two treatments were used: monogamous mating pairs (the same pair was isolated for the entire period), and a polyandry treatment (where the same females were used, but the males were replaced). Every second or third day beetles were moved to different dogbane branches, enclosed with a fresh bag, and male beetles in the polyandry treatment were replaced with new males collected from the field. The used bags and the dogbane branches were transported back to the lab. Over the experimental period, the male beetles were replaced six times. If beetles escaped during the first five days they were replaced and those replicates were restarted. Any subsequent escapes resulted in discarding the replicate. Each female was isolated for a period of 20 days. The experiment was conducted as a completely randomized design with five replicates.

After each bag switch the number of egg clusters on the bag and plant material was counted and recorded in the lab. Egg clusters were then gently removed and placed on a double layer of moist filter paper in Petri dishes. Petri dishes were kept a 21°C with a 16:8 light: dark regimen. Dishes were monitored each day and emerging larvae were recorded and removed. Several larvae from each dish were stored in 70% ethanol, and the remainder were brought back to the field and placed at the base of dogbane ramets.

### 3.3.3 Larval Head Width

Larvae were sampled in the field weekly from 23 September 2010 to 12 November 2010 and 18 May 2011 to 5 December 2011. This was done by removing a 20 cm wide and 10 cm deep section of soil from five randomly selected locations within the dogbane patch at the Oxford, NS site. The Halfway River, NS site was also sampled in 2011. Each sample was sifted using 5.6 mm and 3.35 mm brass sieves. The sample portion larger than 3.35

mm was checked visually for larvae, and then discarded. The remaining sample was then placed in Berlese funnels for one week to extract soil organisms into 70% ethanol. Extracted specimens were examined under a microscope, and dogbane beetle larvae were identified as described by the diagram of Jolivet and Verma (2008), and Weiss and West (1921). The head capsule width of each dogbane beetle larvae was measured using an ocular micrometer.

### 3.3.4 Data Analysis

*Adult Distribution and Abundance in Blueberry Fields*

Growing degree days were calculated, using a Biofix date of 1 April, and a base development temperature of 10°C with the following formula:

$$\text{Cumulative GDD} = \sum (T_{average} - T_{base})$$

Where $T_{average}$ is the average daily air temperature and $T_{base}$ is the lower development threshold temperature. The base temperature was chosen using the Insect Development Database compiled by Nietschke et al (2007). The average of base development temperature of the 55 coleopterans included in the database by Nietschke et al (2007) was 10.61°C, with the 11 base temperatures for chrysomelids giving an average of 10.13°C.

Non linear regression was performed using SigmaPlot (Systat Software Inc) to fit a model to the data, using growing degree days as the independent variable. Several different peak models were evaluated using the r-square value as an estimate of the goodness of fit of the data, and the root mean squared error. Models included 4 and 5- parameter Weibull, 5- parameter Pseudo Voight, 4- parameter Lorentzian, and the 4- parameter

Gaussian. The data from all sites was combined and the result was compared to the individual models. A Weibull 5 parameter model was selected for this data:

$$y = y_o + a\left(\frac{c-1}{c}\right)^{\frac{1-c}{c}}\left[\frac{x-x_0}{b}+\left(\frac{c-1}{c}\right)^{\frac{1}{c}}\right]^{c-1} e^{\left[\frac{x-x_0}{b}+\left(\frac{c-1}{c}\right)^{\frac{1}{c}}\right]^{c}} + \frac{c-1}{c}$$

Where $y$ is the number of dogbane beetles/m$^2$, $x$ is cumulative GDD, $y_o$ is the asymptote, $x_o$ is cumulative GDD at the midpoint, $a$, $b$, and $c$ are scale, location, and shape parameters, respectively. The scale parameter corresponds with the x value at which point approximately 63% of the values are below it. The location parameter is the minimum value of x, and the shape parameter indicates how symmetrical the curve is.

Contour plots were constructed for each site in SigmaPlot, using three different dates (early, peak population, and late-season), and examined for spatial patterns. Spearman correlations between the beetle population and growth stage, as well as beetle population and dogbane ramet density were computed using PROC CORR in SAS® 9.1 (SAS Institute Cary, NC) for each individual site. Neither the assumptions of normality or independence could be met for parametric or non-parametric tests, thus the effect of field edges could not be statistically analyzed. Analysis was conducted using a confidence level of $\alpha = 0.05$.

*Fecundity and Fertility*

Egg viability was calculated as the percentage of eggs from which larvae emerged. PROC MIXED was used in SAS 9.1 to perform a repeated measure ANOVA, with the LSMEANS statement used to make multiple Tukey adjusted comparisons, in order to

check for the effects of treatment and day on total eggs deposited and egg viability. Due to escapes, the repetitive mating treatment was analyzed with 4 replicates, and the polyandry treatment with 3. It was necessary to remove three outliers from the total number of eggs in order to satisfy the assumption of normality.

*Larval Head Width*

Single linkage cluster analysis was done on larval data from each site using PROC Cluster in SAS 9.1 to determine if the head capsule width data could be separated into clusters representing different instars. A histogram of the larvae head capsule widths was constructed for each site in an attempt to identify clusters.

## 3.4 Results

### 3.4.1 Adult Distribution and Abundance in Blueberry Fields

Dogbane beetles were first observed in 2010 at Oxford at 225 GDD (30 June), and were first observed mating at 357 GDD ( Fig 3.1). The population peaked at approximately 4 beetles/$m^2$ (mean 0.5 beetles/ramet) and then rapidly declined. Dogbane beetles were found in the field up to, and including, 725 GDD (19 August). Each day, an average of 18% of the dogbane beetle population was found on plants other than dogbane, though it ranged between 0 and 40%. There was a weak correlation between the number of dogbane beetles/$m^2$ and dogbane ramet density ($r_s$=0.267, $P < 0.001$). Defoliation rating ranged from 0-6 over the season, with a mean of 1.4. The five parameter Weibull was chosen to model beetle population, as it had the highest $R^2$ value, and the second lowest root mean square error of the tested models (Table 3.1).

Beetles emerged later in Oxford in 2011 than 2010, and were first observed at 318 GDD (11 July), and were first observed mating at 474 GDD. A population peak of approximately 4 beetles/$m^2$ (mean 0.6 beetles/ramet) was reached at 577 GDD, and dropped to zero by 27 September (940 GDD) (Fig 3.1). Each day, an average of 5% of the beetles were found on plants other than dogbane, though this ranged from 0 to 25%. There was a weak correlation between dogbane beetles/$m^2$ and dogbane ramet density ($r_s$ =0.250, $P < 0.001$), as well as between dogbane beetles/$m^2$ and dogbane growth stage ($r_s$ =-0.126, $P = 0.009$). A Weibull 5 parameter model was also fitted to this data as it had the highest $R^2$ and lowest root mean square error (Table 3.1). Defoliation ratings ranged from 0-4 over the season, with a mean of 0.7.

Dogbane beetles were first observed on 11 July at the Halfway River, NS site, corresponding to 290 GDD (Fig 3.1). Mating was first recorded at 350 GDD. A peak population of 4 beetles/$m^2$ (mean 0.5 beetles/ramet) was reached at 431 GDD, after which it declined until the last beetle was observed on 4 October (914 GDD). Each day, an average of 5% of dogbane beetles could be found on plants other than dogbane, ranging from 0 to 23% over the season. There was a positive correlation between beetle and dogbane density ($r_s$ =0.432, $P < 0.001$), and a weak negative correlation between beetle density and dogbane growth stage ($r_s$ =-0.186, $P = 0.007$). A five parameter Weibull model was fitted to the data at this site, as it had the highest $R^2$ value, and a low root mean square error (Table 3.1). The seasonal defoliation rating average was 0.6, and ranged from 0-2.

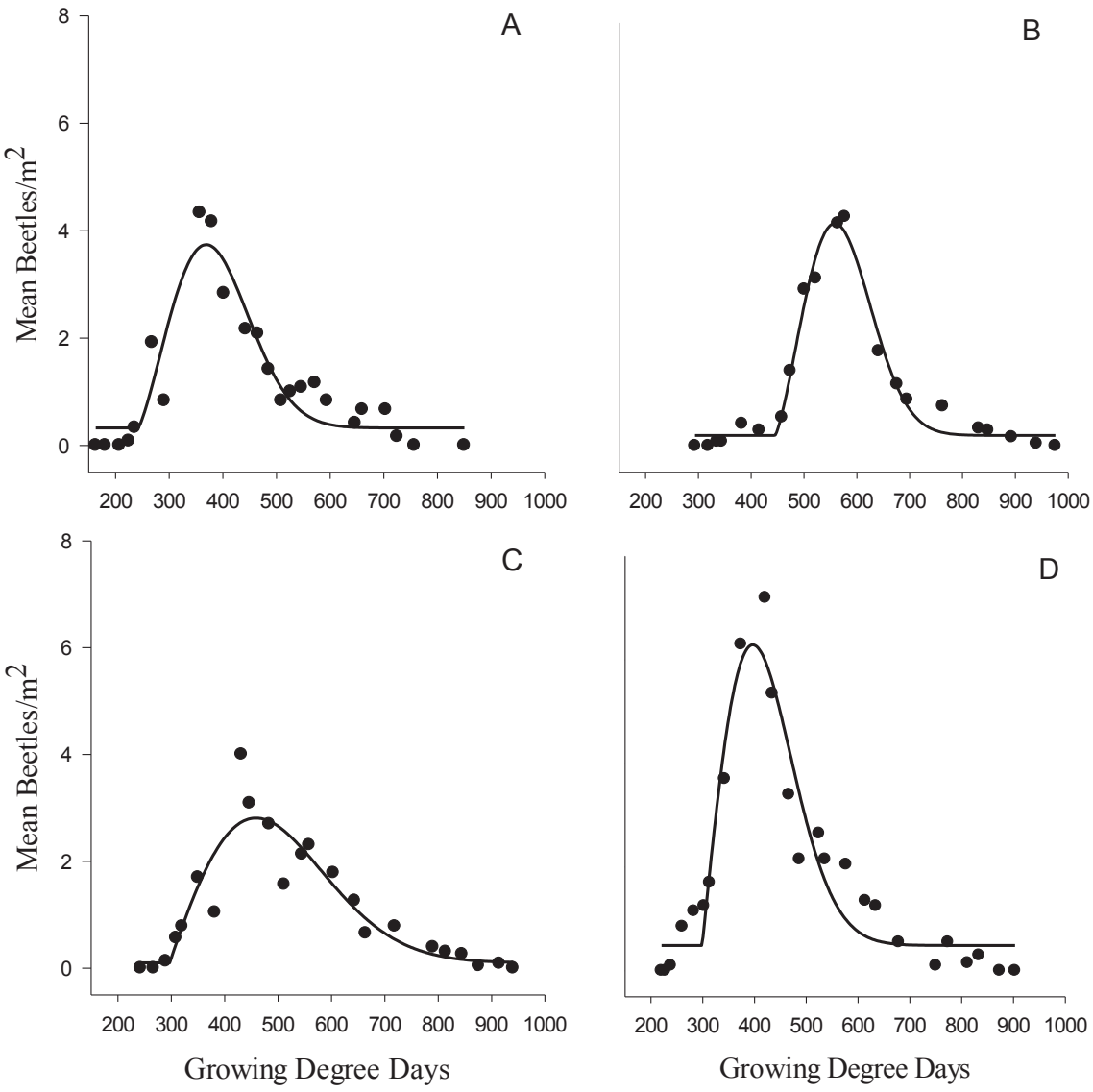


Figure 3.1: Dogbane beetle (*C. auratus*) density in Oxford NS in 2010 (A), Oxford NS 2011 (B), Halfway River NS 2011 (C), Westbrook NS (D) as a function of growing degree days.

Beetles were first observed on 6 July at the Westbrook, NS site, which corresponded to 238 GDD (Fig 3.1). The first mating pair was observed at 260 GDD. A peak population was reached at 420 GDD with 7 beetles/$m^2$ (mean 1.5 beetles/ramet), after which the

population declined. The last beetle was observed on 27 September, or 833 GDD. Fewer beetles were found on plants other than dogbane at this site, averaging just 2% of the population on a given day (range 0-6.5%). There was a larger positive correlation between beetle and dogbane ramet density at this site ($r_s$=0.449, $P < 0.001$). There was a weak correlation between beetle density and dogbane growth stage ($r_s$ =-0.213, $P$ = 0.004). A four parameter Lorentzian model would have worked well for this site, having the highest $R^2$, and the lowest root mean square error, but a five parameter Weibull model had slightly lower $R^2$ value and was chosen for consistency, and because the level of fit was almost as high (Table 3.1). Over the entire season, defoliation ratings had an average of 1.3, but ranged between 0 and 9.

The contour maps of the Oxford site in 2011 show that beetles tend to cluster, but this clustering does not necessarily correspond with the areas of highest dogbane density (Fig 3.2), though areas with very few dogbane ramets tended to have very few beetles. The clustering of the beetles is most obvious at the peak of the season (Fig 3.3), but as the season reaches its end the population is restricted to a couple of isolated pockets despite the existence of ramets throughout the rest of the patch (Fig 3.4.). Beetle density does not appear to correspond with field edges, or spreading dogbane developmental stages. A similar trend was exhibited at Westbrook and Halfway River.

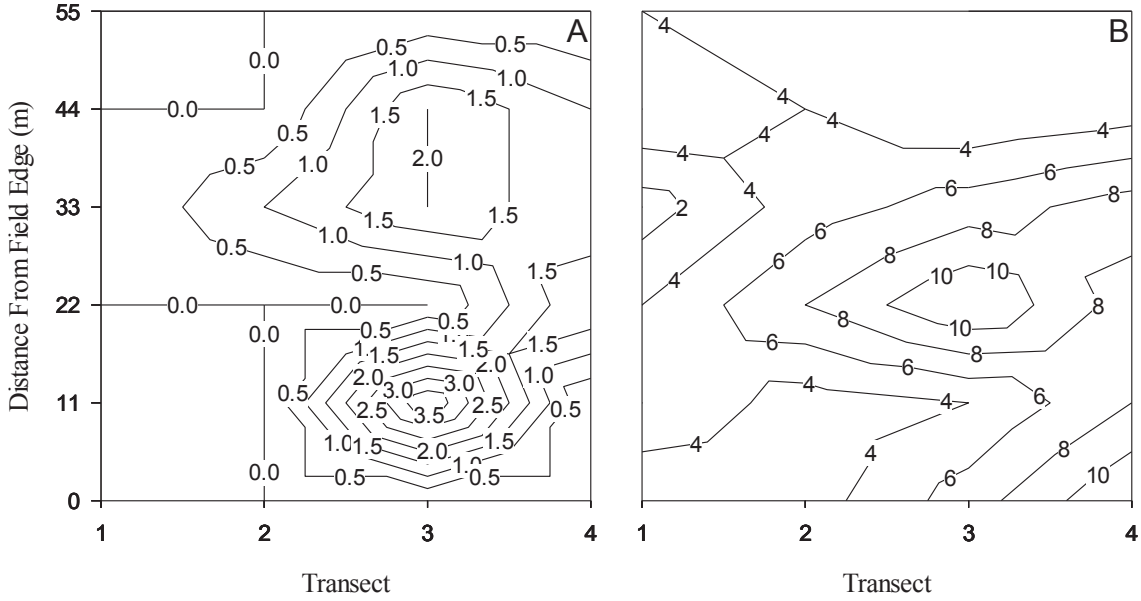


Figure 3.2: Contour map of dogbane beetle (*C. auratus*) (A) and spreading dogbane ramets (units per $m^2$) (B) at 208 growing degree days at Oxford, NS, 2011.

Table 3.1: Model comparison for adult dogbane beetle (*C. auratus*) density as predicted by growing degree days at several lowbush blueberry fields in NS.

| Site | Oxford 2010 | | Oxford 2011 | | Halfway River 2011 | | Westbrook 2011 | | All Sites | |
|---|---|---|---|---|---|---|---|---|---|---|
| Model | R2 | √MSE | R2 | √MSE | R2 | √MSE | R2 | √MSE | R2 | √MSE |
| Weibull 4 Parameter | 0.8366 | 0.5373 | 0.9667 | 0.2646 | 0.8578 | 0.4475 | 0.8869 | 0.7211 | 0.4579 | 1.1260 |
| Weibull 5 Parameter | 0.8613 | 0.5078 | 0.9752 | 0.2343 | 0.8591 | 0.4562 | 0.9072 | 0.6685 | 0.4572 | 1.1331 |
| Pseudo Voight 5 Parameter | 0.8558 | 0.5180 | 0.9704 | 0.2559 | 0.8155 | 0.5218 | 0.9171 | 0.6320 | 0.4345 | 1.1566 |
| Lorentzian 4 Parameter | 0.8553 | 0.5056 | 0.9664 | 0.2659 | 0.8005 | 0.5302 | 0.9171 | 0.6175 | 0.4251 | 1.1595 |
| Gaussian 4 Parameter | 0.8464 | 0.5209 | 0.9685 | 0.2559 | 0.8155 | 0.5098 | 0.8909 | 0.7085 | 0.4345 | 1.1500 |

Table 3.2: Parameter values and 95% confidence intervals for adult dogbane beetle (*C. auratus*) population models, as predicted by growing degree days, for several lowbush blueberry fields in NS.

| Site | Oxford 2010 | | Oxford 2011 | | Halfway River 2011 | | Westbrook 2011 | |
|---|---|---|---|---|---|---|---|---|
| Parameter | Value | 95% CI | Value | 95% CI | Value | 95% CI | Value | 95% CI |
| $a$ | 3.413 | 2.452, 3.695 | 4.041 | 3.712, 4.370 | 2.734 | 2.226, 3.242 | 5.804 | 4.898, 6.709 |
| $b$ | 164.1 | 77.68, 250.5 | 145.3 | 124.7, 165.9 | 234.2 | 182.7, 285.7 | 140.2 | 132.1, 142.3 |
| $c$ | 2.271 | 0.735, 3.807 | 2.303 | 1.864, 2.742 | 1.944 | 1.264, 2.642 | 1.967 | 1.509, 2.425 |
| $x_0$ | 369.0 | 334.5, 403.5 | 558.3 | 549.2, 567.4 | 458.3 | 422.7, 493.9 | 396.6 | 380.5, 412.7 |
| $y_0$ | 0.3271 | 0.018, 0.6362 | 0.1581 | 0.0307, 0.2855 | 0.0668 | -0.2410, 0.3746 | 0.4443 | 0.0739, 0.8147 |

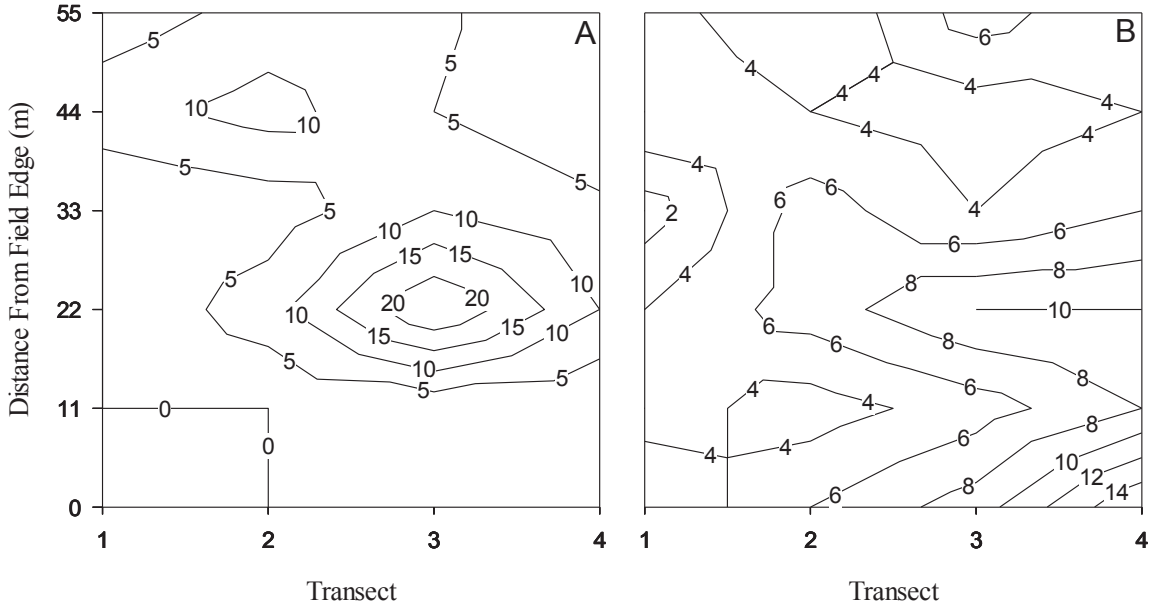


Figure 3.3: Contour map of dogbane beetle (*C. auratus*) (A) and spreading dogbane ramets (units per $m^2$) (B) at 224 growing degree days at Oxford, NS, 2011.

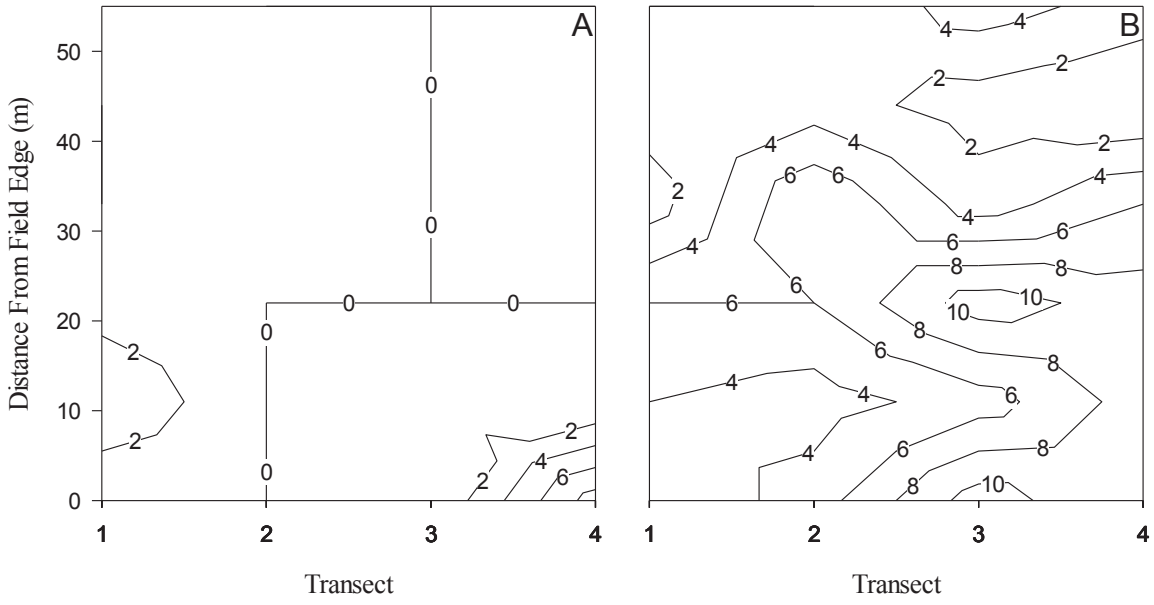


Figure 3.4: Contour map of dogbane beetle (*C. auratus*) (A) and spreading dogbane ramets (units per $m^2$) (B) at 244 growing degree days at Oxford, NS, 2011.

### 3.4.2 Fecundity and Fertility

Monogamous mating pairs produced an average of 109 eggs, and 103 larvae. The polyandry treatment resulted in an average of 129 eggs and 116 larvae. This led to a viability of 95% for the monogamous mating treatment and 90% for the polyandry treatment. Treatment did not have a significant effect on the number of eggs produced, or the viability of the eggs ($P$=0.1822, $F$=2.41, $df$=1; $P$=0.1660, $F$=4.53, $df$=1, respectively). Day, and the interaction of treatment with day, had a significant effect on the number of eggs ($P$<0.0001, $F$=17.79, $df$=6; $P$=0.0093, $F$=3.72, $df$=6, respectively) but did not have a significant effect on the viability of the eggs ($P$=0.8129, $F$=0.48, $df$=6; $P$=0.3511, $F$=1.18, $df$=6, respectively). There was a significant difference between the two treatments for days 3 and 11 (Table 3.3), and the polyandry treatment showed a more erratic rate of egg production.

Table 3.3: Comparison of mean eggs produced over time by dogbane beetle (*C. auratus*) after monogamous or polyandrous mating.

| Day after experiment initiation | Mean eggs produced/counting date | |
|---|---|---|
| | Monogamous | Polyandrous |
| 1 | 14*Ba* | 18*Ba* |
| 3 | 22*Aa* | 48*Ab* |
| 5 | 7*Ba* | 5*Ba* |
| 11 | 2*Ba* | 37*Ab* |
| 14 | 5*Ba* | 21*ABa* |
| 17 | 18*Ba* | 23*ABa* |
| 20 | 8*Ba* | 8*Ba* |

* Uppercase letters indicate differences within columns, lowercase indicate differences within rows.

### 3.4.3 Larval Head Width

There were 52 individuals collected from Oxford NS, and 29 individuals from Halfway River NS (Fig 3.5). The majority of larvae were found to have head widths between 450 and 1000 µm. The clustering procedure indicated only one cluster from the larvae at either site, presumably because of the small sample size and the presence of several large values that the program may have viewed as outliers. Thus, examination of the histogram gives a better picture of possible instar numbers, suggesting there were two instars among the individuals collected. The larvae from the fertility and fecundity experiment, which were preserved shortly after emergence, were found to have head capsules between 533 and 600 µm in length.

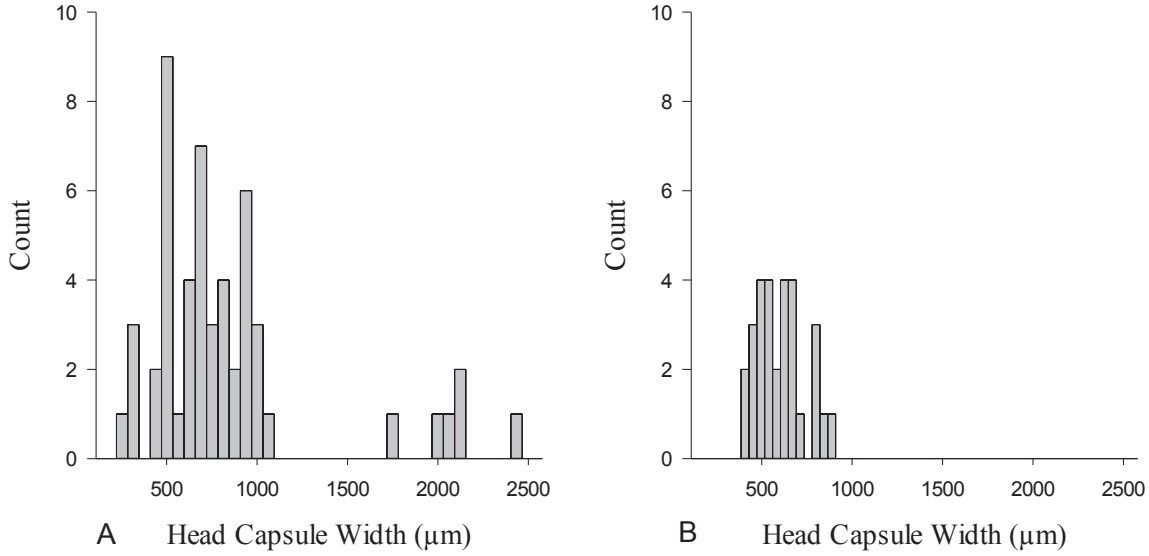


Figure 3.5 Frequency of head capsule width of dogbane beetle (*C. auratus*) larvae from Oxford NS (A) and Halfway River NS (B).

## 3.5 Discussion

### 3.5.1 Adult Distribution and Abundance in Blueberry Fields

Beetles emerged earlier at Oxford in 2010 than 2011, and remained for a shorter period of time than the subsequent season. The lag of about 130 GDD between emergence and mating could be due to a period of feeding before mating begins, but is more likely from lack of opportunity caused by low population density. Peak population occurs swiftly, suggesting the majority of the population emerges in a short period of time, but is not sustained for more than a few days, resulting in low densities for the majority of the season. This is not consistent with all leaf beetles, as *Leptinotarsa decemlineata* may take several weeks to reach peak population, and sustain it for over a week (Blom et al 2002). Management does not appear to contribute to lack of a sustained peak population, as the Oxford site was unmanaged for several years and exhibited the same pattern as Westbrook and Halfway River, which were actively managed. The population density in 2011 was consistent with 2010, though fewer beetles were found on plants that were not dogbane. The Westbrook site showed marked differences: The beetle density was higher, mating was observed almost immediately, and fewer beetles were found on other plants. The latter two differences could be explained by the different vegetation. Oxford had numerous shrubs and other tall plants while Westbrook's tallest plant was dogbane. This would have left the beetle little choice but to remain mostly on dogbane. It could also have made it easier to locate potential mates and led to increased opportunities.

Emergence timing is typically impacted by air and soil temperature. While soil temperature was not monitored at every site, it tends to be highly correlated with air temperature. The Halfway River site had a slightly lower air temperature and thus

accumulated growing degree days at a slower rate than Westbrook, which could explain the later emergence date.

Beetles were present for 8 weeks at Oxford in 2010, and 11-12 weeks at all sites in 2011. The beetles emerged about a month later than the dates given for New Jersey (Weiss and West 1921), and Virginia (Williams 1992). Beetles were recorded in Virginia for approximately 6 or 7 weeks (Williams 1992), and beetles observed by Weiss and West (1921) may have persisted for as many as 12 weeks. *C. cobaltinus* emerges in late May in California, and remains for 6-8 weeks (Dickinson 1995). Climatic differences between these areas can explain the differences in dates of emergence and the temporal length of adult population presence. The average beetles/ramet at population peak was lower at all sites than the 4.5 or 6 beetles/ramet reported in Iowa by St. Pierre and Hendrix (2004) and St. Pierre et al (2005). All 2011 sites showed a weak negative correlation between beetle density and ramet growth stage, suggesting that beetles show a slight preference for younger ramets. Sites should be studied for longer than two years to gather a more comprehensive picture on population fluctuations between seasons.

A five parameter Weibull model was chosen to represent each site. It gave consistently high $R^2$ values, the lowest being 0.85. Much of the population of the individual sites is accounted for by the cumulative GDD. However, the combined model did not adequately describe beetle numbers across sites for unknown reasons and the parameter values varied widely between sites. The parameters $c$ and $y_0$ have narrow, overlapping ranges, and so could be accurately predicted across sites. Thus, it was not possible to model beetle population dynamics across sites. Future work would benefit from obtaining site specific temperatures earlier in the season, to more accurately calculate cumulative

growing degree days at individual sites. More research is needed to determine how site characteristics impact beetle population. It may also be possible to build a model that includes parameters such as vegetation type or dogbane density. The model parameters themselves do not have a practical, intuitive significance.

The contour plots displayed in this chapter, and in Appendix 3, do show a spatial pattern. As one might expect, the beetles have an area of concentration. This shifts around the field throughout the season, and there is no apparent edge effect, except when the dogbane ramet density is very low. An area of concentration is expected, as dogbane beetles spend a great deal of time mating. Their sister species *C. cobaltinus* mates an average of once a day, males guard mates for over an hour and will actively compete for females (Dickinson 1995), which would lead to some congregating. Dogbane beetles spent little time in small clusters of hemp dogbane ramets located away from larger patches, and did not deposit eggs in those small clusters (St Pierre and Hendrix 2004). This suggests that dogbane beetles may not be an effective control at sites with multiple, small, 'satellite' patches of dogbane.

### 3.5.2 Fertility and Fecundity

In terms of number of eggs, and egg viability, there appears to be no advantage to monogamy over polyandry (or vice versa). This does not rule out the possibility of offspring being more genetically diverse and having higher fitness manifest later in their lifecycle, or at the population level. The high viability of the eggs is a definite advantage to a potential rearing program. Peterson et al (2005) found lifetime (about 30 days) egg production of the dogbane beetle to be, on average, 90 eggs. This is very similar to the results of this experiment. However, in the study by Peterson et al (2005), only about

30% of eggs hatched, which is a stark contrast with the high viability found in this experiment. It is possible that the low viability was due to females mating with *C. cobaltinus*, or their hybrid, as the data was gathered from females collected from a hybrid zone. A similar experiment, with *C. cobaltinus*, found no difference in egg production, or female longevity, between repetitive mating and polyandry treatments (Schwartz and Peterson 2006).

### 3.5.3 Larval Head Width

The samples taken over a period of two seasons gave inconclusive results, as the number of instars cannot be stated definitively. The clustering of bars on the Oxford histogram (Fig 3.5A), indicates the presence of at least two instars. It was difficult to find specimens of the later instars. The width of fully gown larvae is 3500 μm (Wiess and West 1921), which indicates that none of the larvae measured in this experiment had reached maturity. Combined, this information suggests dogbane beetle has three instars. The lack of specimens with a head width of approximately 3500 μm could be due to the larvae burrowing below 10 cm (depth of sampling), or there could be a high mortality rate beyond the early instars. Several different sizes of larvae were found in the fall months, which is consistent with an insect that deposits eggs over a period as long as 9 weeks. The larval sampling did confirm that pupation occurs in the spring, rather than the fall, as early instar larvae were found as late as December 5$^{th}$ in 2011. A couple of pupae were found in late spring in 2011. A much larger sample of larvae would be necessary to confirm the number of instars. It may be best to attempt to rear them in a laboratory setting, where it would be possible to track the growth of individual larvae.

## 3.6 Conclusion

The abundance of dogbane beetle varied between sites, but overall population sizes were lower than in other regions reported in the literature. While the population abundance was consistent between two years at one site, a longer-term study is necessary to determine how consistent beetle abundance is between years. When modelling the beetle population, as predicted by growing degree days, the five-parameter Weibull was often the most accurate. Growing degree days explained much of the variability in beetle abundance, suggesting that such a model may be appropriate to use in predicting beetle emergence at individual sites. Females produce a large number of eggs, regardless of whether they mate with a single male or multiple males, and eggs displayed high viability when hatched in a laboratory setting. The number of larval instars is still unknown, but two pupae discovered in the field indicate that pupation occurs in the spring or early summer.

## Chapter 4.0 Overall Conclusions

Biological control of a native weed species with a native agent is rarely, if ever, practiced. However, it is occasionally studied. For example: two species of native scale insects were indicated as potential biological control agents of a native shrub in Australia (Campbell et al 1994), and *Gastrophysa viridula* (Coleoptera: Chrysomelidae) was reviewed as a potential agent for control of *Rumex* species in the Czech Republic (Martinkova and Honek 2004). This project was an opportunity to study the potential of such a program, within the unique production system of lowbush blueberry.

The key objectives of the project were designed to examine the beetle from several angles. The first key objective was to examine the beetle's host specificity. Will the beetle consume species closely related to spreading dogbane? Will the beetle consume lowbush blueberry? The second key objective was to investigate the severity of defoliation caused by increasing densities of dogbane beetle. The third key objective was to examine the lifecycle of the dogbane beetle in Nova Scotia as well as ascertain its natural abundance.

It is unlikely the beetle would consume lowbush blueberry in a field situation, though no biological control agent comes with a guarantee of complete safety. However, the natural abundance of dogbane beetle recorded during this project is not sufficient to inflict severe defoliation on spreading dogbane. Defoliation of small ramets was sometimes over 50%, but in most cases the defoliation of ramets was less than 20%. Dogbane beetle does have a window of 8-12 weeks in which to defoliate dogbane. Thus, the natural population of dogbane beetle would need to be augmented for a biological control program.

There are many questions still unanswered, especially if the beetle is to be reared for augmentative release. Can the beetle be easily reared? During this project, eggs were easily hatched in the laboratory, but rearing was not attempted beyond this point. Other questions include: How is spreading dogbane affected by herbivory on a larger scale? When should beetles be released, and at what density? If too many are released, will they emigrate from the field? Would augmentative releases be necessary every year? What are the long-term effects of defoliation of spreading dogbane on a field scale? Several of these questions require a research period beyond the short time span of this project.

Rearing beetles can be difficult and expensive. For example, the need for host plant material can require large amounts of money and labour. This could be alleviated by an artificial diet, containing smaller amounts of the host plant, as demonstrated for Colorado potato beetle (Gelman et al 2001). Other leaf beetles have been reared successfully in the laboratory: green dock leaf beetles (*Gastrophysa viridula,* Coleoptera: Chrysomelidae) (Voight et al 2011), red turnip beetle (*Entomoscelis americana,* Coleoptera: Chrysomelidae) (Lamb and Gerber 1985), and *Galerucella calmariensis* and *G. pusilla* (Coleoptera: Chrysomelidae) has been mass reared (Blossey and Hunt 1999). It should be possible to rear dogbane beetle, and learn much about its biology in the process. Ideally, beetles should be released when flower buds are formed, in order to impair the flow of carbohydrates to the roots, but at what densities and the frequency of the released will require further study. The effects of such releases will have to be studied, as each plant species responds differently to defoliation. Defoliation of 50% just prior to flowering has been found to significantly reduce allocation of resources to the roots of fireweed (*Epilobium angustifolium*) (Michaud 1991). A similar response is possible in

dogbane, though the number of seasons necessary to adequately deplete root resources is still unknown. It is also possible that dogbane beetle larvae may decrease the regrowth of spreading dogbane in subsequent seasons.

In the end, I believe dogbane beetle has the potential to be an effective biological control agent. However, biological control is not an exact science, and the true worth of an insect as a control agent can only be proven with time.

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## Appendix 1: Scale for the Description of Weed Growth Stages

The scale used in this research was based on the extended BBCH scale-general, as originally described by Lancashire et al (1991), and then adapted slightly for weed species by Hess et al (1997). Below is the scale used to describe the particular growth stages of spreading dogbane, with only the stages relevant to perennial weeds included.

Table A1.1: Adapted BBCH scale-general for describing growth stage of spreading dogbane.

| Principle growth stage | Secondary stage | Description |
|---|---|---|
| **0** | | **Germination/sprouting** |
| 0 | 0 | Winter dormancy/resting period |
| 0 | 1 | Beginning of bud swelling |
| 0 | 3 | End of bud swelling |
| 0 | 7 | Beginning of sprouting/bud break |
| 0 | 8 | Shoot growing towards soil surface |
| 0 | 9 | Shoot emerges through soil |
| **1** | | **Leaf development (main shoot)** |
| 1 | 0 | First leaves spread/separated |
| 1 | 1 | First leaves unfold |
| 1 | 2 | Two true leaves or whorls unfolded |
| 1 | 3 | Three true leaves or whorls unfolded (continues in this vein until:) |
| 1 | 9 | Nine or more leaves or whorls unfolded |
| **2** | | **Formation of side shoots** |
| 2 | 1 | First side shoot visible.... |
| 2 | 9 | Nine or more side shoots visible |
| **3** | | **Stem elongation/main shoot development** |
| 3 | 0 | Beginning of stem elongation |
| 3 | 1 | One visibly extended internode... |
| 3 | 9 | Nine or more visible extended internodes |
| 5 | | Inflorescence emerging (main shoot) |
| 5 | 1 | Inflorescence or flower buds visible |
| 5 | 5 | First individual flowers visible (still closed) |
| **6** | | **Flowering (main shoot)** |
| 6 | 0 | First flowers open sporadically |
| 6 | 1 | 10% of flowers open |
| 6 | 3 | 30% of flowers open |
| 6 | 5 | Full flowering |
| 6 | 7 | Flowering finishing- most petals falling or drying |
| 6 | 9 | End of flowering: fruit set visible |
| **7** | | **Development of fruit** |
| 7 | 1 | Fruit begin to develop |
| 7 | 9 | Nearly all fruit have reached final size |
| **8** | | **Ripening or maturity of fruit and seed** |
| 8 | 1 | Beginning of ripening or fruit coloration |
| 8 | 9 | Fully ripe |
| **9** | | **Senescence or beginning of dormancy** |
| 9 | 7 | Plant resting or dormant |

## Appendix 2: Additional Contour Plots

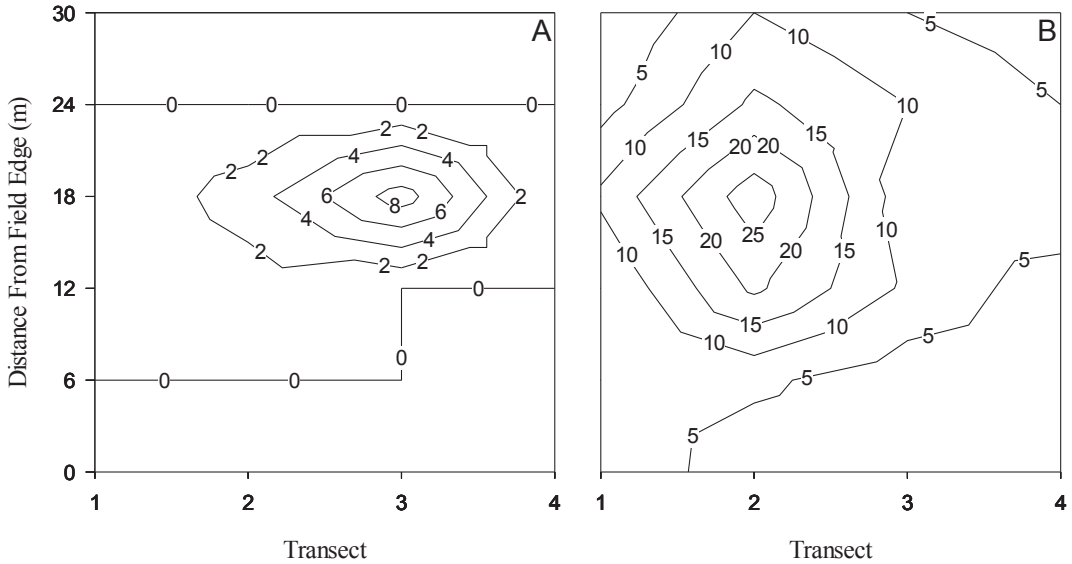


Figure A2.1 Contour map of dogbane beetle (*C. auratus*) (A) and spreading dogbane ramets (units per $m^2$) (B) at 309 growing degree days at Halfway River, NS, 2011.

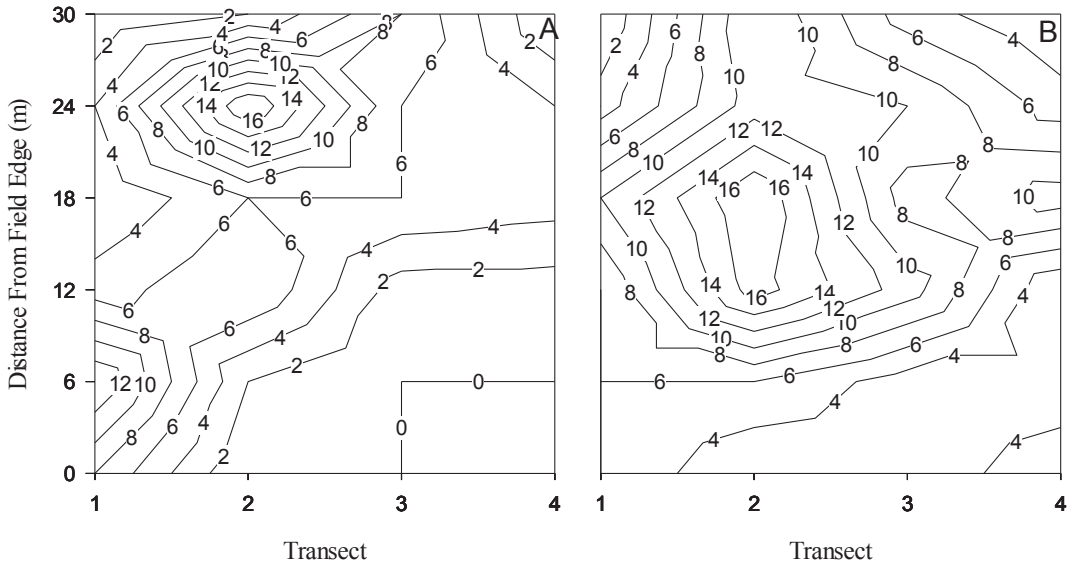


Figure A2.2 Contour map of dogbane beetle (*C. auratus*) (A) and spreading dogbane ramets (units per $m^2$) (B) at 432 growing degree days at Halfway River, NS, 2011.

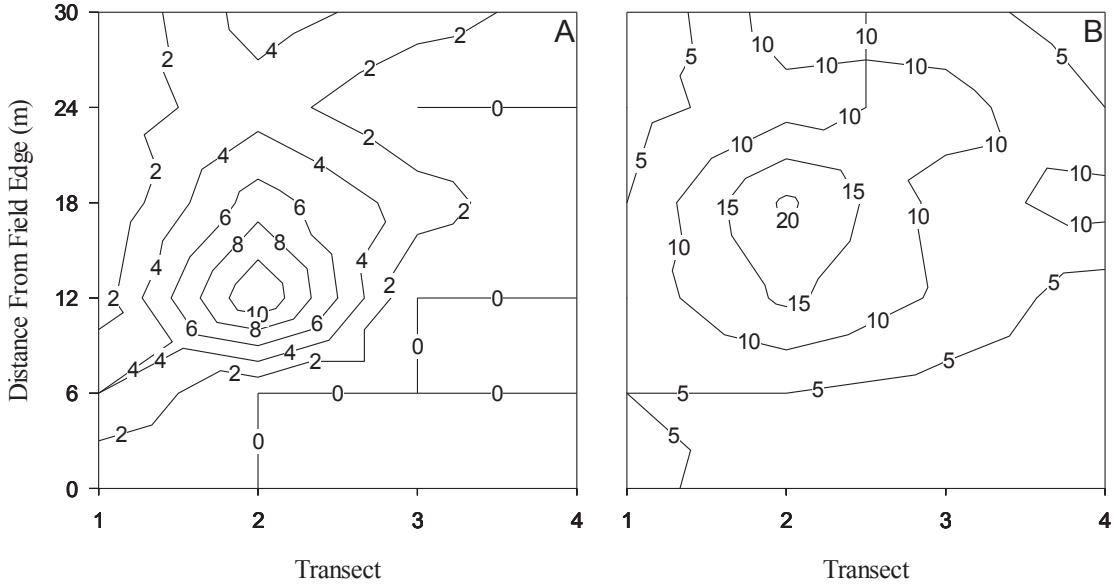


Figure A2.3 Contour map of dogbane beetle (*C. auratus*) (A) and spreading dogbane ramets (units per $m^2$) (B) at 604 growing degree days at Halfway River, NS, 2011.

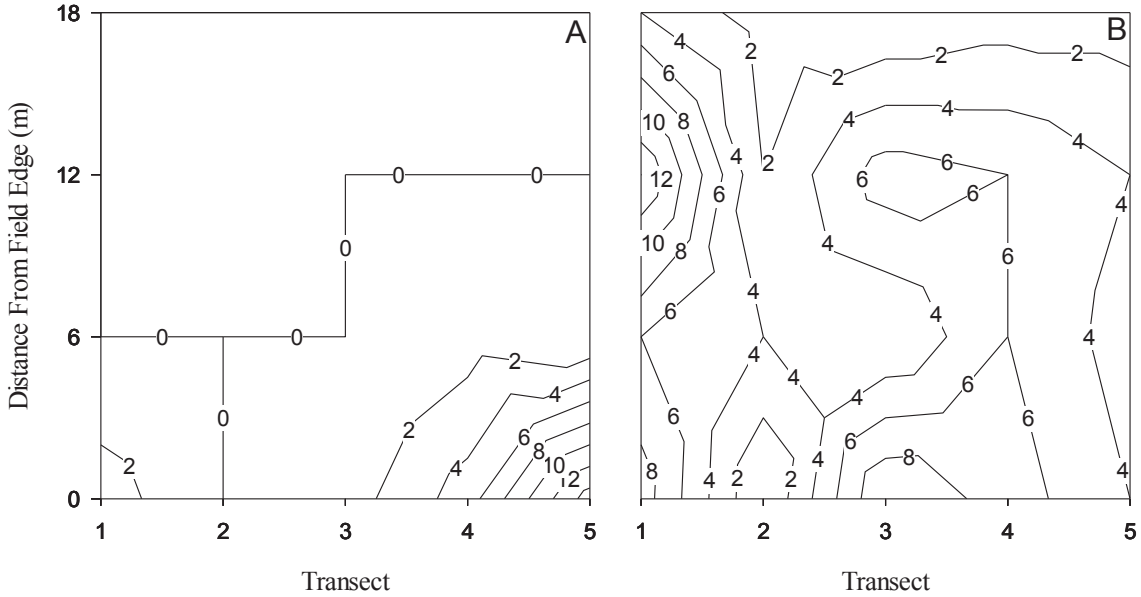


Figure A2.4 Contour map of dogbane beetle (*C. auratus*) (A) and spreading dogbane ramets (units per $m^2$) (B) at 194 growing degree days at Westbrook, NS, 2011.

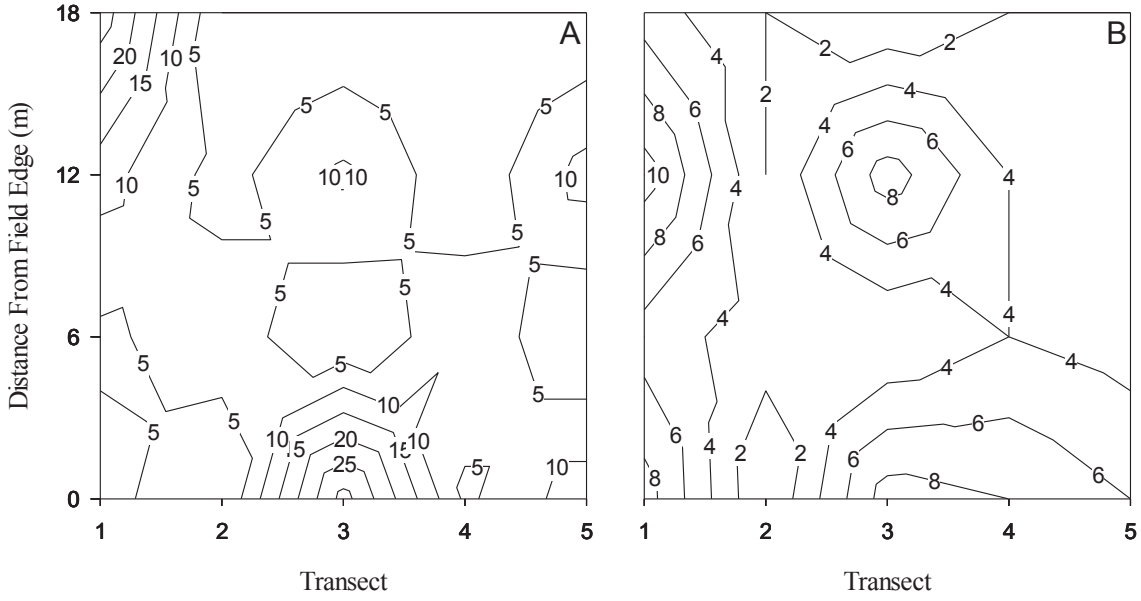


Figure A2.5 Contour map of dogbane beetle (*C. auratus*) (A) and spreading dogbane ramets (units per $m^2$) (B) at 208 growing degree days at Westbrook, NS, 2011.

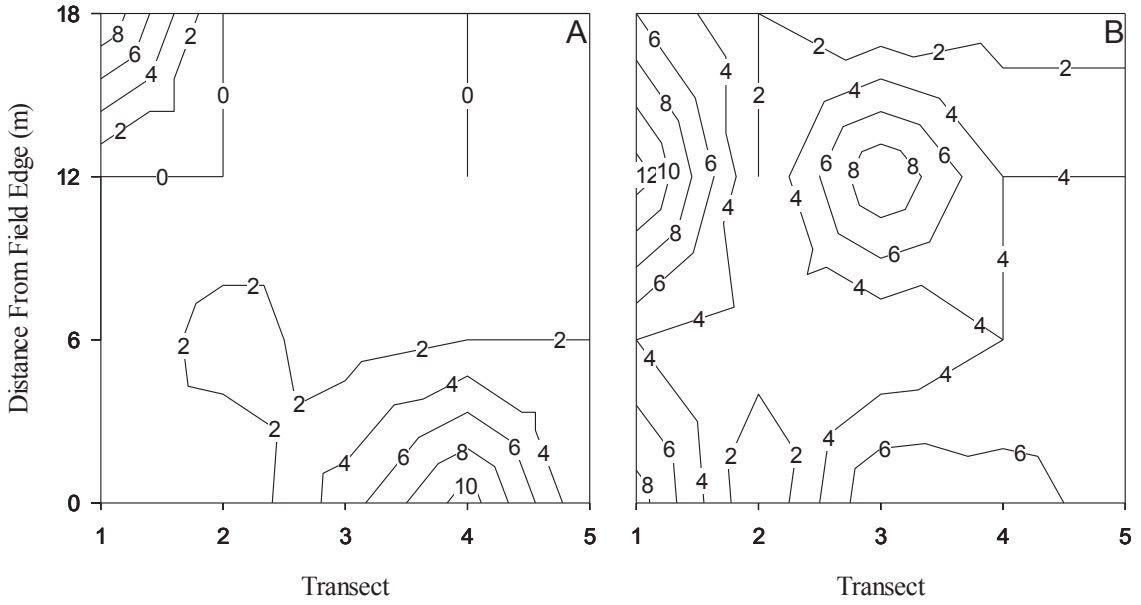


Figure A2.6 Contour map of dogbane beetle (*C. auratus*) (A) and spreading dogbane ramets (units per $m^2$) (B) at 229 growing degree days at Westbrook, NS, 2011.