University of Alberta

Dispersal of the biocontrol agent, *Cyphocleonus achates* (Coleoptera: Curculionidae), on the invasive plant, diffuse knapweed (*Centaurea diffusa*) (Asteraceae)

By

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Abstract

Dispersal is an often overlooked, but important aspect of a biocontrol agent's ability to control weeds in a new environment. I used individual mark-recapture (IMR) techniques in field and laboratory studies to determine if beetle or plant traits affected movement of *Cyphocleonus achates* on *Centaurea diffusa* (diffuse knapweed). I also assessed the effect of release strategies such as beetle release density and date of release on *C. achates* dispersal. *Cyphocleonus achates* was found to be sedentary, individuals moving a mean distance of 0.27m/day in a natural patch. Beetle sex, size and diffuse knapweed traits did not affect movement in these experiments. However, increasing the density of beetles and releasing beetles later in the season increased movement distances and rates of dispersal. Land managers can use this information to determine the number, density and date of releases to facilitate establishment and impact of *C. achates* on knapweed.

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Chapter 1

General Introduction

Diffuse knapweed (*Centaurea diffusa,* Lamarck) (Asteraceae) is an aggressive semelparous perennial plant (Thompson and Stout, 1990) that threatens rangeland, agriculture, biodiversity and natural forage for wildlife (Watson and Renney, 1974; Tyser and Key, 1988; Lacey, 1989). This weed is characterized by prolific seed production, a long-lived seed bank, allelopathic exudates, drought tolerance and formation of monocultures (Watson and Renney, 1974). It was first recorded in British Columbia in 1936 (Groh, 1943) and is now found principally in the dry interior of British Columbia and more recently in Southern Alberta (Lacey, 1989; Sheley *et al.*, 1998; Bourchier *et al.*, 2002). Knapweed is also listed as a noxious weed in Manitoba, Saskatchewan, Ontario and Quebec. Diffuse knapweed and the similar invasive species, spotted knapweed (*C. maculosa* (Lamarck), infest over 100,000 acres of land in British Columbia and cost approximately $400,000 annually in lost hay production alone (Pidwirny *et al.*, 2002). Diffuse knapweed is often found in disturbed areas (gravel beds, farmland, and roadsides) but can invade rangeland when knapweed plants are spread by recreational vehicles, wildlife, wind or water (Myers and Berube, 1983; Watson and Renney, 1974).

Weed managers currently employ several methods to control knapweed infestations, including mechanical methods (tillage, mowing, pulling and grazing), herbicides and biological control. Mechanical methods alone cannot control infestations because they are labour intensive and not feasible for large areas requiring treatment (Sheley *et al.*, 1998; Watson and Renney, 1974). Herbicides have been unsuccessful because of the economic and environmental cost of continuous applications to large areas for successful eradication (Harris and Cranston, 1979). These two methods are also inadequate because knapweed can persist on steep terrain where access for application of herbicides is difficult or impossible and because of the plant's prolific seed production and long-lived seed bank. Biological control, the introduction of specialized herbivores from the weed's native range, has the advantages of being renewable, relatively cheap and effective over the long term, making it the preferred method of control for this weed (Jacobs et. al, 2000).

There are two schools of thought as to how to successfully reduce knapweed densities with natural enemies: the first involves finding a single key biocontrol agent to suppress knapweed growth (silver bullet hypothesis: Myers, 1985; Denoth *et al.*, 2002) and the second requires multiple agents to attack different parts of the plant (cumulative stress approach: Harris and Cranston, 1979; Müller-Schärer and Schroeder, 1993). Regardless of the approach, the common goal is that the agent(s) will stress or kill the plant and reduce its density and spread. Both hypotheses have been difficult to test empirically, although separate studies of *Larinus* spp. and *Cyphocleonus achates* Fahraeus (Coleoptera: Curculionidae) have been associated with the disappearance of diffuse knapweed in the Okanagan Valley region of British Columbia and spotted knapweed in Montana, respectively (Myers, unpublished data; Story *et al.*, 2006).

Thirteen biocontrol agents have been released to control knapweeds in North America, and nine are considered established (Bourchier *et al.*, 2002). Currently, seven established agents are considered to have had an impact on diffuse knapweed. The two seedhead flies (*Urophora affinis* Frauenfeld and *U. quadrifasciata* Meigen (Diptera: Tephritidae) can cause up to 95% reduction in seed set (Harris, 1980). A single larva of the seedhead weevils *Larinus obtusus* Gyllenhal or *L. minutus* Gyllenhal can cause up to 100% destruction of seeds in attacked seedheads (Kashefi and Sobhian, 1998; Lejeune *et al.*, 2005). To date, this reduction in seed set by both *Urophora* spp. and *Larinus* spp. has not been experimentally demonstrated to reduce the spread or density of knapweed. The root moth, *Agapeta zoegana* L. (Lepidoptera: Tortricidae) reduces the number of capitula and above ground biomass of knapweed, but this does not translate into a reduction in plant densities (Story *et al.*, 2000). The bronze knapweed root borer, *Sphenoptera jugoslavica* Obenberger (Coleoptera: Buprestidae) can reduce seed output and survivorship of both diffuse knapweed seedlings and rosettes. However, fluctuating local population densities of *S. jugoslavica* result in unattacked patches of knapweed and therefore have only a limited impact on populations (Powell and Myers, 1988). The root-feeding weevil, *C. achates*, has a significant impact on spotted knapweed. *Cyphocleonus achates* causes extensive damage in the root which results in reduced plant densities in high beetle density sites (Story *et al.*, 2006) and kills individual plants in laboratory settings (Corn *et al.*, 2006).

Cyphocleonus achates is a univoltine weevil, which was first released in Canada in 1987 (Müller *et al.*, 1989; Stinson *et al.*, 1994) to control spotted knapweed. Adults are large (approximately 14 mm), long lived (8-15 weeks) and are not known to fly (Stinson *et al.*, 1994). Adults emerge from roots from mid-July to mid-September, bask on bolted plants and feed on both leaf and stem tissue (Stinson *et al.*, 1994). *Cyphocleonus achates* mate several times and lay eggs singly in the root crown or just below the soil on the root (Stinson *et al.*, 1994). Eggs hatch within 10-23 days and larvae mine through the root cortex (Story *et al.*, 1996; Goodman *et al.*, 2006). After larvae reach the center of the root, a conspicuous root gall approximately 2-4 cm long and 1 cm in diameter is produced in the central vascular tissue and larvae feed within the gall until eclosion (Stinson *et al.*, 1994; Muller, 1989). Larvae overwinter as second instars and complete development the following spring (Stinson *et al.*, 1994). Despite the damage *C. achates* can inflict on individual knapweed plants, widespread impact is difficult to achieve because this agent has been described as flightless and is thought to disperse poorly between knapweed patches (Story *et al.*, 1996).

Initial dispersal away from the release site is the most important stage when looking at spread and establishment of a biocontrol agent (Puth and Post, 2005). At least 36% of weed biocontrol releases fail to establish (Julien, 1989) and understanding factors that affect post-release dispersal may increase establishment success. Surprisingly few empirical studies describe initial post-release dispersal for any biocontrol agent (Lima and Zollner, 1996), which is likely due to the difficulty in empirically assessing movement for many biocontrol agents. Likewise, the dispersal ability of *C. achates* once released into patches is still poorly understood. To improve the distribution of *C.*

achates, mass-rearing and distribution programs have been established in Montana (Story *et al.*, 1996) and British Columbia (Val Miller, personal communication).

The overall objective of this thesis was to experimentally examine dispersal behaviour of *C. achates* after release, with the goal of determining the extent beetles can disperse, and to assess the factors that may affect beetle dispersal. In Chapter 2 of this thesis, I present empirical data testing mark-recapture methodologies and test factors that may affect dispersal of the beetles in field and lab experiments. In Chapter 3, I use the methodology tested in Chapter 2 to determine if release strategies affect dispersal of *C. achates* in knapweed patches. The final chapter, Chapter 4, describes the implications of C. *achates* dispersal to both population biology and biological control of knapweed.

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Chapter 2

Testing individual mark-recapture methods and the effects of beetle and plant traits on dispersal of *Cyphocleonus achates* (Coleoptera: Curculionidae)

2.1 Introduction

Movement is a crucial factor in population dynamics, and can give insight into genetics, spatial spread and persistence of insects (Turchin, 1998; Bohonak, 1999). Despite the importance of dispersal in population dynamics, it is often overlooked because of the labour-intensive nature of fieldwork and low recapture rates of the insect when performing quantitative field studies.

Empirical measurement of dispersal is difficult and care must be taken to reduce the effect of the testing methods on dispersal behaviour. Individual mark-recapture (IMR) in insect systems has an advantage over other methods of assessing dispersal because intrinsic factors such as insect sex and size as well as extrinsic factors such as the response to plant traits can be investigated with respect to movement. IMR has advantages over the more detailed path analysis method and the mass-mark recapture method because individuals can be followed over longer time spans (Turchin, 1998). Specifically, in path analysis, individuals are followed for short periods and every movement is analyzed in detail. With this level of detailed measurement, autocorrelated moves must be accounted for, whereas in IMR these complicated analyses can be ignored. Mass-mark recapture allows large numbers of insects to be marked at once, but because individuals cannot be followed, factors such as the effect of sex and size on movement cannot be assessed. A problem with the IMR method is the potential effect of excessive handling and storage due to marking the insects. Individuals may behave differently immediately after release than after acclimation to the new habitat. This differential dispersal is referred to as agitation dispersal (Turchin, 1998) and must be assessed when examining initial dispersal from biocontrol releases.

The movement behaviour of insects in the field is often a response to intrinsic (insect sex and size) and extrinsic factors (plant size, number of oviposition sites). Sex-biased dispersal can be found when there is competition for resources such as mates or oviposition sites (female-biased: Kuusaari *et al.*, 1996; Albrectsen and Nachman, 2001; Petit *et al.*, 2001 male-biased: Lawrence, 1988) or if there is mortality associated with dispersal (Waser *et al.*, 1994). For example, competition for oviposition sites in the tephritid fly, *Parpxyna plantaginis*, results in female dispersal in response to insect density, whereas males stake out territories and do not disperse (Albrectsen and Nachman, 2001). The size of individuals can also influence their propensity to disperse. Larger individuals may have more reserves and are therefore more able to exploit resources further away from competitors without sacrificing fecundity (Lawrence, 1987; Anholt, 1990). Some insects may even need to reach a reserve threshold before dispersal can occur (Lawrence, 1987), whereas smaller individuals, in highly competitive situations, may disperse to unoccupied areas where there is less competition (Lawrence, 1987). Plant preference can also play a role in dispersal behaviour. When oviposition and feeding sites are competed for, insects that can recognize and disperse to high quality hosts in the field have the advantage over insects that do not disperse.

Cyphocleonus achates was released in Canada in 1987 to control the Eurasian invasive knapweed (Stinson *et al.*, 1994). *Centaurea diffusa* (diffuse knapweed) is a semelparous perennial, which can persist as a rosette for several years until bolting. *Cyphocleonus achates* feed on bolted and rosette lifestages of diffuse knapweed and lay eggs in the root crown of rosettes where the larvae then overwinter. *Cyphocleonus achates* is not a strong disperser and land managers in British Columbia and Montana have set up programs to manually redistribute this beetle to new patches (Story *et al.*, 1996). To date, the extent to which *C. achates* can disperse has not been quantified. Estimation of this beetle's dispersal ability can lead to improved release strategies in the field.

This chapter focuses on the individual mark-recapture (IMR) methods used to collect dispersal data and identifies factors that may influence *C. achates* movement after release in both the field and laboratory. Bioassays were used to test assumptions about the marking methods including 1) the lifespan of marked beetles, 2) the best time during the day to observe beetles 3) storage and frass effects on beetle movement and 4) predation on marked beetles.

This chapter also reports on the factors that may influence movement in the field experiments. To do this, data from the field experiments featured in Chapter 3 were used to assess beetle movement. Specifically, studies were undertaken to determine if beetles exhibited agitation dispersal, the effect of sex and size on beetle movement and the response of beetles to the size of diffuse knapweed plants and number of rosettes around bolted plants.

2.2 Methods

Individual beetles were hand picked or sweep-netted from established populations in British Columbia and transported in containers of 50 beetles to the Agriculture and Agri-Food Canada Research Centre in Lethbridge, Alberta. All beetles were held in a controlled environment chamber at 5°C night, 10°C day and at a photoperiod of 14light:10dark prior to use in field experiments or bioassays.

The sex of the beetles was determined by examining lateral abdominal curvature. Females have a rounded abdomen whereas males have a flattened abdomen. For individuals that were difficult to separate by sex, the last segment of abdomen under the elytra was gently pulled down with forceps and examined dorsally to determine if an extra segment was present (found in male *C. achates*) (see Goodman *et al.*, 2006). Beetles were weighed using an analytical balance (Mettler Toledo AB54-S) and marked with a two-colour combination of enamel model paint (described below in marking techniques and beetle lifespan).

Beetles were released at an approximate 1:1 sex ratio in the 2003, 2004 and 2005 field experiments. Beetle location was recorded daily for each individual by monitoring labeled knapweed plants in each experiment. To reduce the influence of continuous daily monitoring on beetle behaviour, care was taken not to disturb the plants and beetles.

Bioassays to test methodology

Marking techniques and beetle lifespan

Individual marking may affect lifespan and dispersal ability of insects (Turchin, 1998). Specifically, marking techniques and material used to mark the beetles had the potential to be toxic to *C. achates*. To determine which marking technique was appropriate for *C. achates,* 30 individuals per treatment were marked with enamel model paints, liquid paper or Day-Glo™ powder and tested against unmarked controls ($N_{\text{total beetles}}$=120). A two-colour combination of Testors™ enamel model paint was applied to the beetles in either a four or six-dot pattern on the elytra. The enamel paint and White Liquid Paper™ were applied to the elytra with a head of an insect pin, taking care to avoid joints, pronotum, head parts and sealing the elytra. Individual beetles were also marked with Day-Glo™ powder, by placing them in a plastic bag with the powder, and shaking it to coat the beetles. Unmarked control beetles were handled but not marked. These commonly used methods of marking were chosen for their ease of application and visibility of marks in the field (Hagler and Jackson, 2001).

Individuals were randomly assigned one of four cages, each containing a potted bolting diffuse knapweed plant. Plants were replaced with new potted plants as required when feeding damage was observed. Fluorescent bulbs were used for overhead lighting and the temperature was kept at 25 °C day and 18°C night for a 15 hour day length. Marking treatment and time of death in days after marking for each beetle were recorded. Analysis of Variance (ANOVA) was used to determine if beetle LIFESPAN[1] after marking was dependent on the marking method (TREATMENT), SEX of the beetle or the blocking factor of CAGE. The differences among treatments were analyzed with Tukey's Honest Significant Difference (HSD) test.

Time of Day

Cyphocleonus achates adults sit on the tops of knapweed plants, basking during the hottest times of the day (Stinson *et al.*, 1994). This behaviour can be used to observe the maximum number of beetles on each plant during monitoring. To determine the time of day that the maximum number of beetles could be observed on a plant, I recorded the number of beetles on each plant at ninety-minute intervals on 14 August 2003. Thirty knapweed plants in a 67m by 110m plot (N:49.7331674 W:112.9760360) were labeled and twenty marked beetles per plant were released on 13 August 2003. The top branches and seedheads of each knapweed plant were surveyed for thirty seconds during each interval. Beetle identification and air temperature were also recorded. To reduce the effect of multiple observations on beetle behaviour, care was taken not to disturb plants and beetles while observing.

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[1] Factors included in statistical models are presented in SMALL CAPS font

Beetle movement in response to storage and frass

In order to maintain beetles at a similar degree-day age for use in the field release experiments (see Natural Patch Experiment in Chapter 3) beetles were kept cool (5°C night and 10°C day) in environment controlled chambers before release. The photoperiod was maintained at 14light:10dark using fluorescent lights or a combination of fluorescent and incandescent light sources.

Initial field observations indicated an increased rate of disappearance of beetles from release plants later in the season when a second release occurred on the plants (data not shown). Beetle response to frass density was tested to determine if the increased frass density later in the season contributed to the disappearance of beetles. Trials were run in 44 litre (50cm by 32cm) Rubbermaid™ tubs and the total distance traveled by individual beetles during the first 30 minutes after release was calculated. Replicates occurred each week from 19 Aug – 30 Sept 2004. During the 30-minute trial, the beetle's x and y coordinates were recorded every minute by noting the beetle's location on a grid towel placed on the bottom of the tub. Beetle movement was recorded in treatments of no frass, low frass (5 pellets), and high frass (30 pellets) simultaneously in individual tubs. The frass was placed in the centre of the grid (x=17.5cm, y=25cm) and the beetles were placed at x=15cm and y=15cm (Figure 2.1). Differences in the TOTAL DISTANCEs (cm) moved by the beetles among each frass treatment (DENSITY) and over time (DATE) were tested with ANOVA.

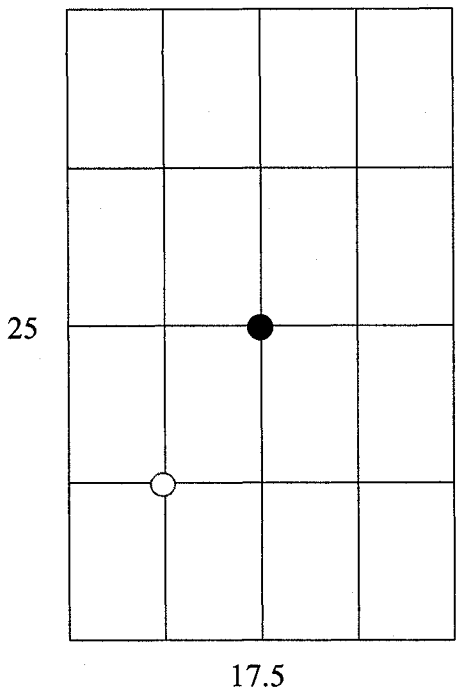

Figure 2.1: Plot design for the 2004 laboratory experiment. Frass (filled circle) was placed at x=17.5cm y=25cm and an individual *Cyphocleonus achates* (open circle) was placed at x=15cm y=15cm of the 32cm by 50cm rectangular arena.

Predation on marked beetles

Some predation of released beetles was observed during the field experiments in 2003 to 2005. To determine whether enamel paint markings on *C.. achates* caused an increase in predation, a field experiment was carried out on 28 September 2005 at Agriculture and Agri-food Canada Research Centre in Lethbridge, Alberta (N:49.69727 W:112.76255). Ten marked or unmarked beetles were released at a 1:1 sex ratio into a black plastic arena measuring 30 cm in diameter. Arenas were arranged in groups consisting of two arenas (n=11 groups), one with marked beetles and one with unmarked beetles. The two arenas within a group were buried in the field, 10m from each other. Each replicate group was placed 25m from each other. The number of beetles remaining in the arenas was counted on 30 September and 4 October 2005. The number of marked and unmarked BEETLES remaining in the ARENAs on each DATE was tested using a log linear model with a quasipoisson error distribution for overdispersed data.

Testing factors affecting movement using field experiments

Study Areas

Mark-release-resight studies were conducted in two locations: on a pre-existing patch of knapweed (2003 and Natural Patch) and on artificial plots (Potted Patches: Summer 2004, Summer 2005 and Fall 2005 trials).

Experiments in 2004 (Natural Patch and Potted Patch: Summer 2004) took place on the Blood Tribe Indian Reserve approximately 20km west of Lethbridge, AB. The Natural Patch experiment was located in a gravel bed 65m by 50m (N:49.7400460 W:112.9888450) approximately 100m from the Old Man River. Diffuse knapweed, open bare spaces, mullein, shrubs and trees characterized this plot. Two biocontrol agents were already present at this site: the seedhead flies *Urophora affinis* and *U. quadrifasciata*.

The 2004 Summer Potted Patch experiment was located on rangeland approximately 2.5km east of the Natural Patch (N:49.7332392 W:112.9753424) in a 50m by 90m plot. The primary vegetation at this site was grasses, wild rose, and sedges. The biocontrol agents present were the seedhead gallflies *U. affinis*, *U. quadrifasciata* and second-generation *C. achates* from a nearby 2003 release.

The 2005 Summer and Fall Potted Patch trials were located on land near the Agriculture and Agri-food Canada Research Centre in Lethbridge, Alberta (N:49.69727 W:112.76255). Knapweed in this plot was planted in two trials during August and September respectively, in field plots measuring 50m by 150m with no knapweed biocontrol agents present.

Plot Designs

Potted Patch Experiment - Summer 2004, 2005 and Fall 2005 Trials

Centaurea diffusa plants were either collected in the field and potted as bolting plants (2004 trial) or grown from seed in the greenhouse and used at the rosette stage (2005 trials). Bolted field plants could not be used in 2005 because the field-collected plants were unavailable due to flooding. Pots were buried in the ground in replicates of four rows (2004 trial) or six rows (2005 trials) (Figure 2.2). Each row consisted of three groups of pots 10m from each other. Each group contained three plants including a central release plant with two target plants 5m on either side. For each group of plants, one of three release densities of beetles (low=6 individuals, medium=24 individuals and high=96 individuals) was assigned to the centre release plant. Release plants were assigned densities in a modified Latin Square design. Release dates were 24 August 2004 (Summer 2004 trial), 19 August 2005 (Summer 2005 trial) and 9 September 2005 (Fall 2005 trial). All plants in each plot were monitored daily with the exception of days with inclement weather (one day for Summer 2005 and two days for Fall 2005).

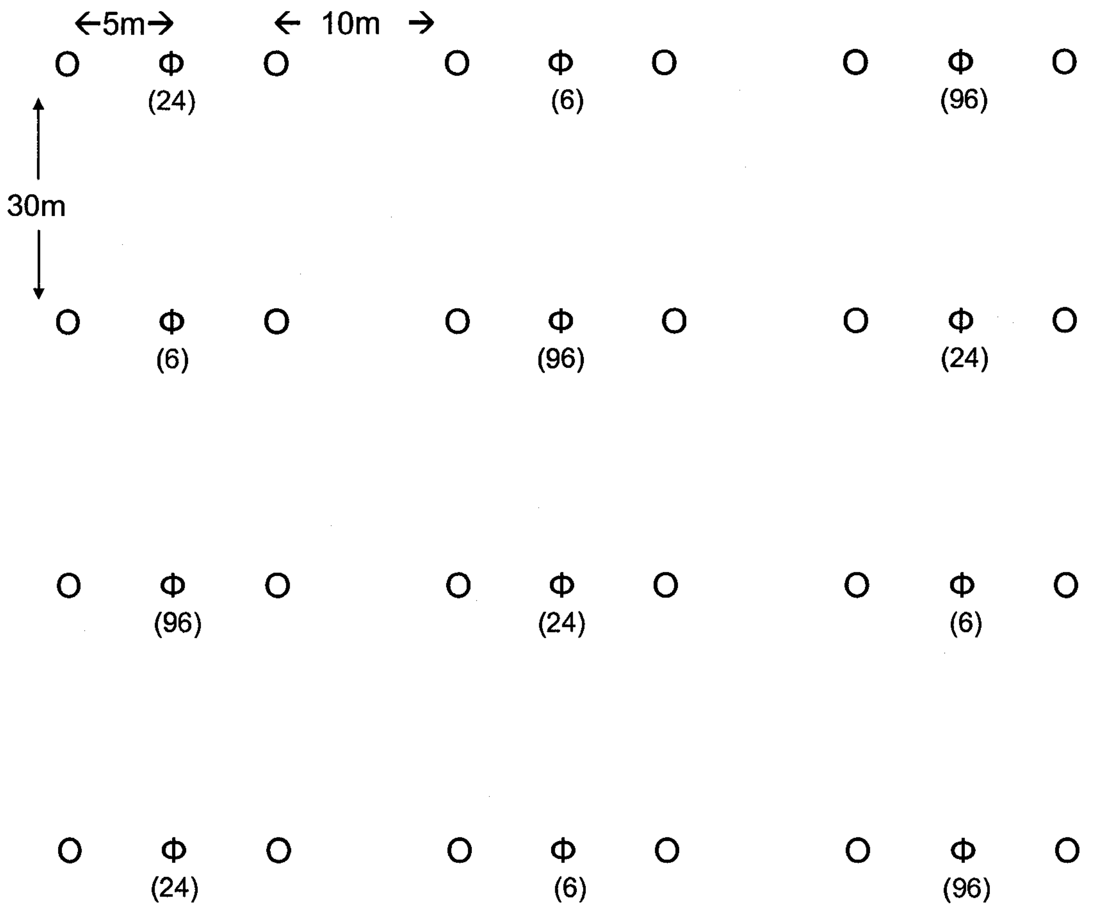

Figure 2.2: Plot design of the Potted Patch trial in 2004 with n=4 rows and three groups of pots per row (n=6 rows in both 2005 trials). The number of beetles released for each group is in parentheses below the central release plant. The symbols O = target plants and Φ = release plants.

Natural Patch Experiment 2004

To record beetle movements between plants in the Natural Patch, each plant was assigned a number and tagged with a metal label at the base of the plant. GPS coordinates for all 324 bolting *C. diffusa* plants as well as height, width of the crown, and number of rosettes within a 1m^2 area around each bolted plant were recorded before beetle release. The number of rosettes and bolted plants did not change during the monitoring period. The plot was divided into six quadrats and one release plant was chosen randomly per quadrat for each week (Figure 2.3). One hundred *C. achates* were released on a bolting plant in a randomly chosen quadrat each week from 27 July – 31 August 2004 ($N_{\text{total beetles}} = 600$). One of the pre-determined quadrats could not be used because beetles had already been found in that quadrat. As a result, beetles for the 24 August 2004 release were released on a group of isolated plants without *C. achates*, closest to the unusable quadrat.

Daily beetle monitoring took place between 1300hrs and 1500hrs from 28 July 2004 – 21 September 2004 by scanning the leaves and stalk of each of the bolting plants and the surrounding rosettes for 10 seconds per plant to standardize sampling effort. The markings of each observed *C. achates* were recorded. Elytra from predated beetles were collected and their markings were recorded as an estimate of mortality from predation.

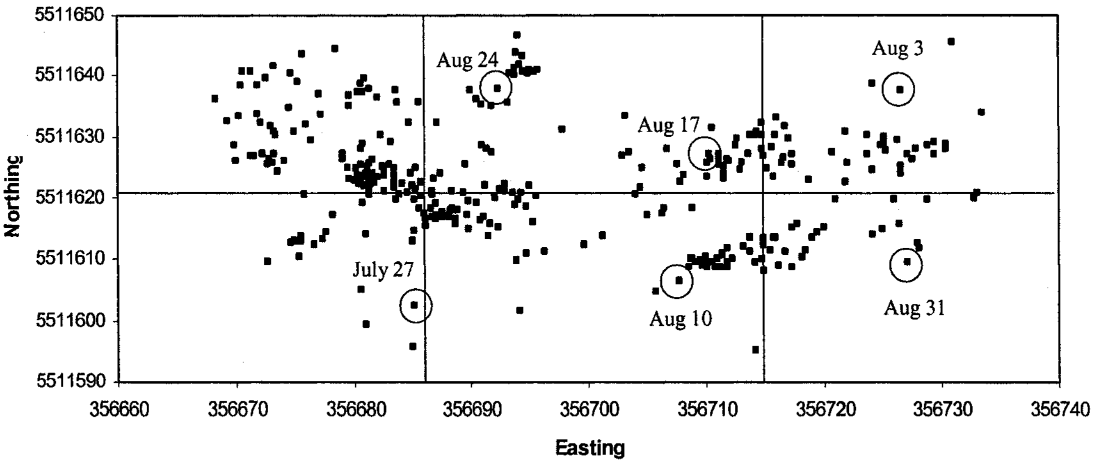

Figure 2.3: GPS coordinates of bolting diffuse knapweed plants (n=324 *Centaurea diffusa*) in the Natural Patch experiment. The plot was divided into six quadrats (solid lines) in which a randomly chosen release plant (circle) was picked for the release of 100 *Cyphocleonus achates* each week (n=6 releases).

Statistical analyses of mark-recapture field experiments (Natural and Potted Patches)

For each observed beetle, I calculated the distance it had moved, if any, from the last position by using grid coordinates or the distance matrices function in R-statistical package (R_{gui} 2.3.1, 2006). In the Potted Trials, the distance (d) between plants were calculated using x, y grid coordinates in the equation $d = \mathrm{sqrt}\,((x_2-x_1)^2 + (y_2-y_1)^2)$. In the Natural Patch, distances were calculated from collected GPS coordinates of the bolted knapweed plants. Because data were collected daily for two months in the Natural Patch, beetle movement histories were truncated to ten days after release to enable comparisons among releases.

Agitation dispersal

Agitation dispersal due to handling and releasing beetles has the potential to bias our estimate of beetle movement (Turchin, 1998). To test for agitation dispersal, movement data were truncated for consecutive moves over a 24hr time interval (MOVELENGTH) and compared among DATEs released, and among treatments in ANOVA for both the Potted Patch (TRIAL) and the Natural Patch (RELEASE DATE). If agitation dispersal was occurring, then more movement would be found on the day immediately after release compared to subsequent days.

Effect of beetle sex and size on movement

To assess the effect of beetle size on movement, the WEIGHT of all beetles collected from British Columbia was recorded. Weight was compared between SEXes and among YEARs (2003-2005). To determine if the sex of the beetles influenced movement, each beetle in the Potted Patch experiments was scored as 'Never Found' 'No Moves' 'Move Once' or 'Multiple Moves'. Chi-squared tests for each trial were used to compare movement by males and females.

Effect of plant traits on movement

To determine if plant characteristics affected beetle host selection, beetle presence/ absence was scored for each plant in the Natural Patch experiment. Plant SIZE, ROSETTE number and DISTANCE from the nearest release was measured and/or calculated for each plant. Height (h) and radius (r) of each plant was measured and used to calculate plant size using volume (v) ($v = 3.14 * r^2 * h$). The distance to the nearest release plant was calculated from the GPS coordinates of the plants using the distance matrices in R_{gui} (release plants were excluded from the analysis). The effect of DISTANCE from the nearest release plant, knapweed SIZE and the number of ROSETTEs on the dependent variable beetle PRESENCE (Y/N) on bolted knapweed plants were tested using a generalized linear model with binomial error distributions. The non-linear effects of DISTANCE, knapweed SIZE and number of ROSETTEs on beetle PRESENCE were tested using a generalized additive model with link logit and binomial error distributions.

2.3 Results

Bioassays to test methodology

Marking techniques and beetle lifespan

There was no difference in survivorship among marking techniques nor between marked and unmarked beetles (Table 2.1, Figure 2.4). Enamel paints were selected for field experiments because they were easy to apply and more individual beetles could be marked using the two-colour, six dot coding system. CAGE had a significant effect on lifespan, with beetles in Cage #2 surviving for fewer days (Cage 2: mean=33.1days compared with Cage 1: 56.7days, Cage 3: 52.7days and Cage 4: 51.8days) (Figure 2.5). Male beetles survived for shorter periods after the start of the experiment, (mean=36 days), than did females (mean=62 days) (Figure 2.6). The difference in lifespan between sexes suggests that males either emerge earlier than females prior to collecting the beetles or have a shorter total lifespan.

Table 2.1: Analysis of deviance table for LIFESPAN (days after marking) of *Cyphocleonus achates* as a function of marking TREATMENT, beetle SEX, CAGE and a TREATMENT x SEX interaction in the 2004 marking experiment.

Term	**df**	**Deviance**	**F-value**	**p(F)**
Null	114	88860		
TREATMENT	3	1694	0.96	0.415
SEX	1	19302	32.76	**< 0.0001** ***
CAGE	3	5558	3.14	**0.028** *
TREATMENT x SEX	3	1022	0.58	0.631
Residual	104	61284		

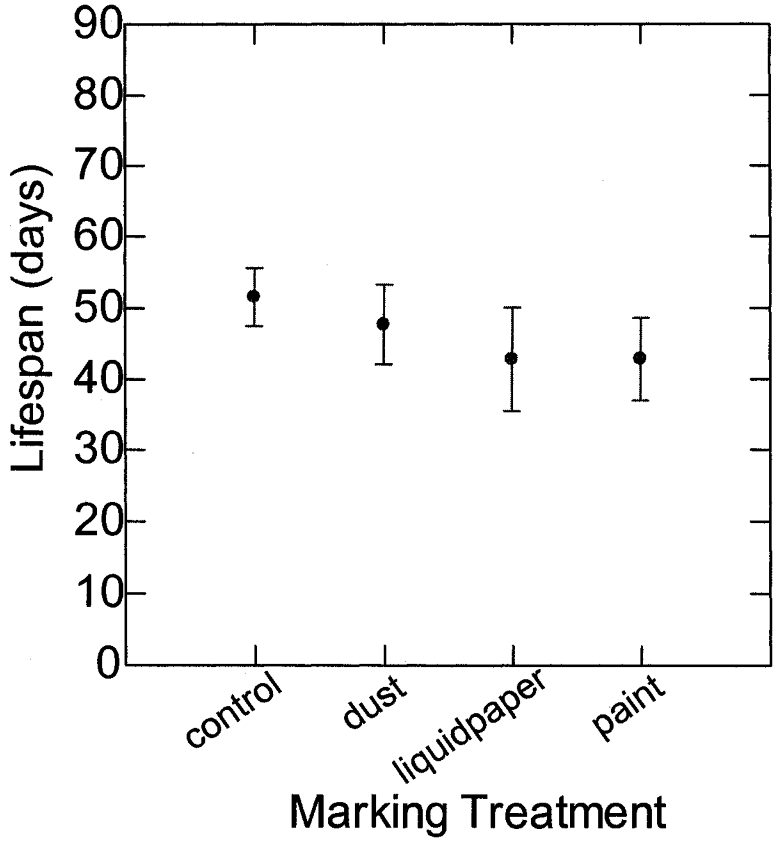

Figure 2.4: Lifespan (days after marking) of *Cyphocleonus achates* between treatments ($n_{beetles}$= 120) in the 2004 lifespan experiment. Error bars are standard errors.

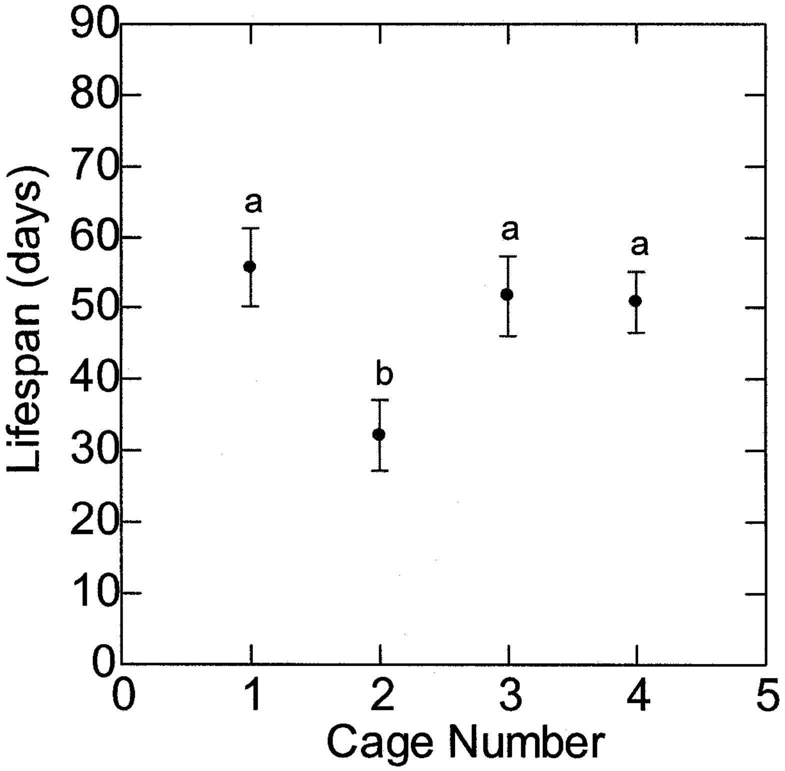

Figure 2.5: Lifespan (days after marking) of *Cyphocleonus achates* between cages ($n_{beetles}$= 30/cage) in the 2004 lifespan experiment. Error bars are standard errors and letters represent Tukey's HSD differences between means.

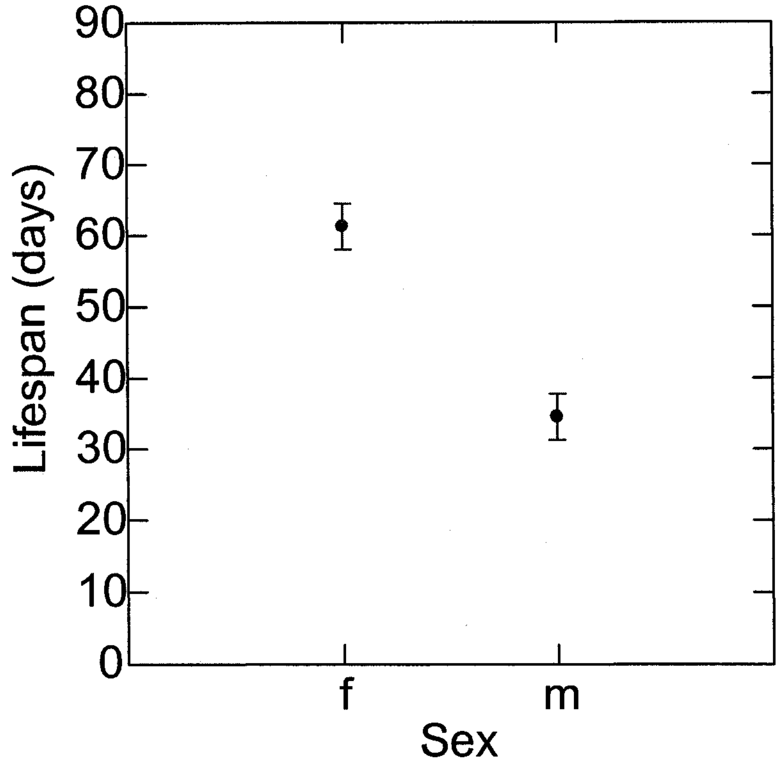

Figure 2.6: Lifespan (days after marking) of male and female *Cyphocleonus achates* ($n_{beetles}$=120) in the 2004 lifespan experiment. Error bars are standard errors.

Time of Day

I found the highest number of beetles were visible between 1200hrs and 1630hrs (Figure 2.7). These findings agree with observations that beetles move to the tops of knapweed plants during the hottest times of the day (Stinson *et al.*, 1994; Val Miller personal communication). Taking advantage of this behaviour helped minimize the disturbance to the beetles during daily monitoring. All subsequent experimental sampling was done during this interval.

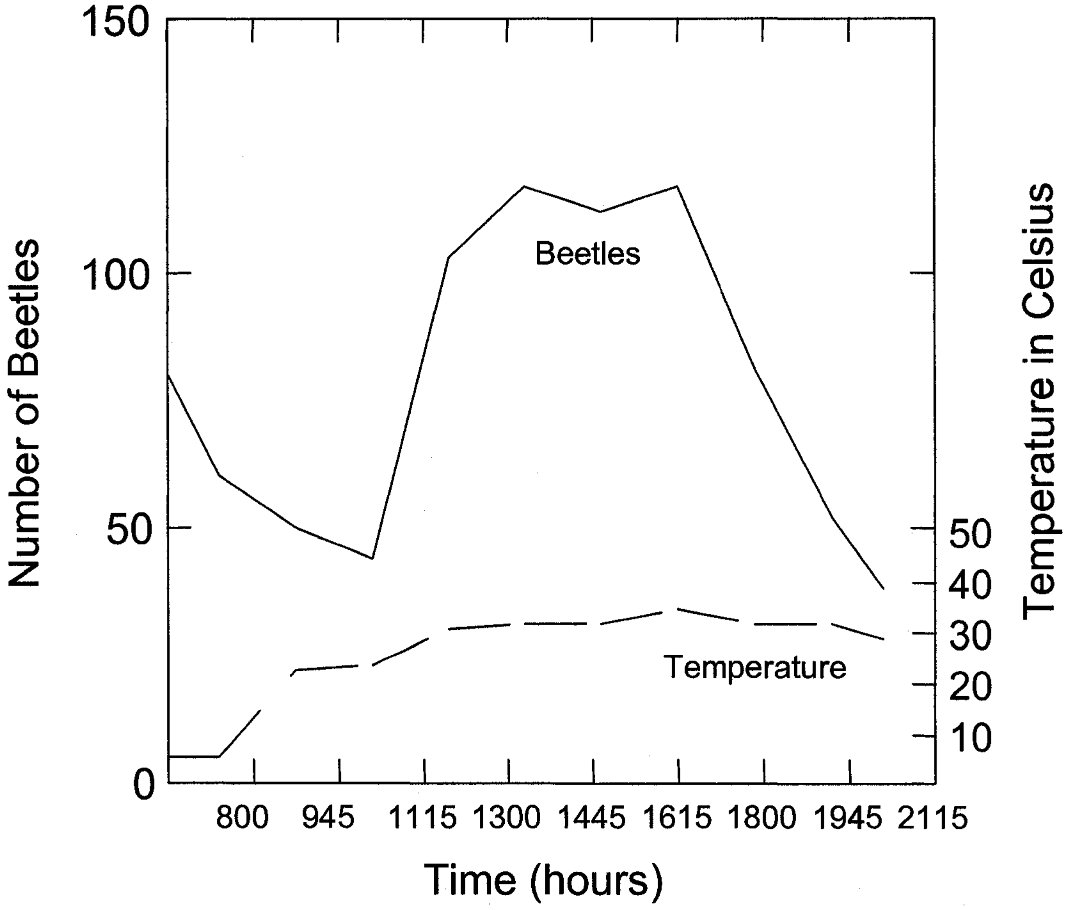

Figure 2.7: Number of beetles (solid line) visible on 30 *Centaurea diffusa* plants ($n_{\text{beetles released}} = 600$) and the temperature (dotted line) in Celsius every 90 minutes from 0600hrs to 2100hrs on 14 August 2003.

Beetle movement in response to storage and frass

To determine if my storage methods affected movement distances throughout the season, data from the laboratory bioassay were tested. There was a significant decrease in dispersal distances over time (days) ($p=0.030$) (Table 2.2; Figure 2.8). However, there was much variation among beetles and a poor fit of the model to those data ($r^2=0.048$). There was a non-significant trend of increasing movement with a higher frass density (Figure 2.9).

Table 2.2: Analysis of deviance table for TOTAL DISTANCE moved (cm) by *Cyphocleonus achates* as a function of the frass DENSITY, DATE and a DENSITY x DATE interaction in the 2004 laboratory arenas.

Term	df	Deviance	F-value	p(F)
Null	98	2045771		
Frass DENSITY	2	40059	0.976	0.380
DATE	1	97870	4.773	**0.031** *
DENSITY x DATE	2	1044	0.025	0.975
Residual	93	1906799		

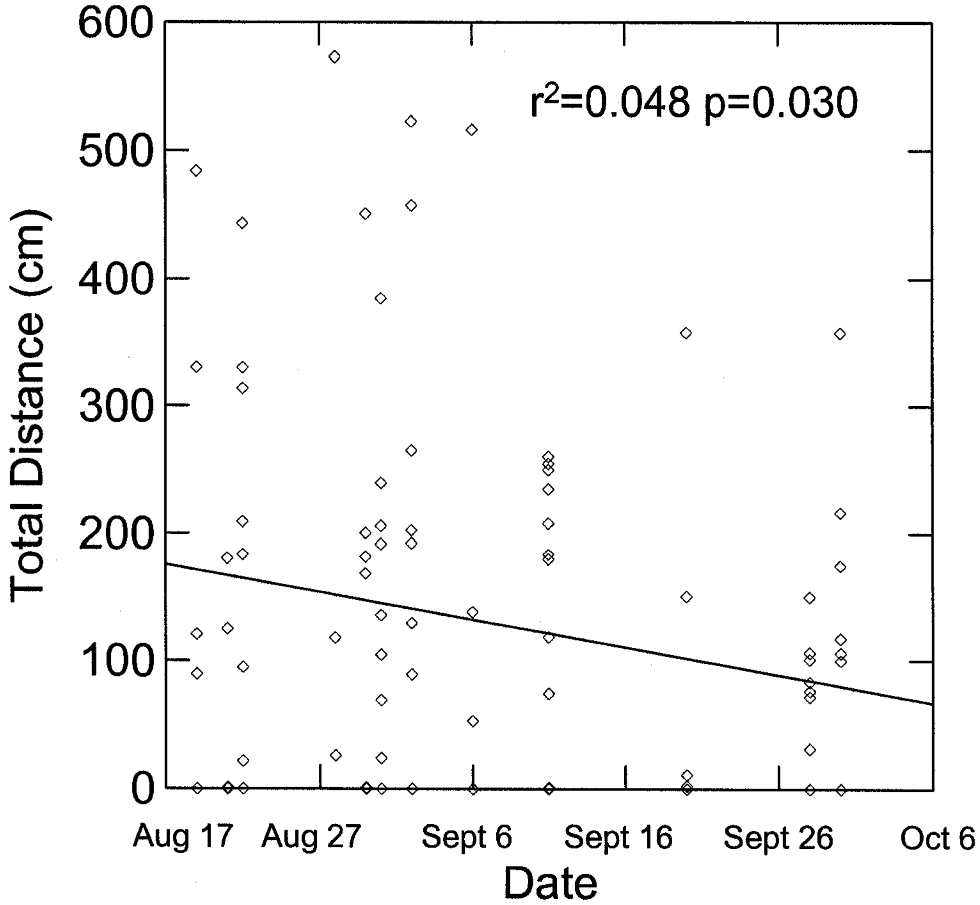

Figure 2.8: Total distances (cm) that each beetle (n=99) moved during thirty minutes in 2004 laboratory arenas.

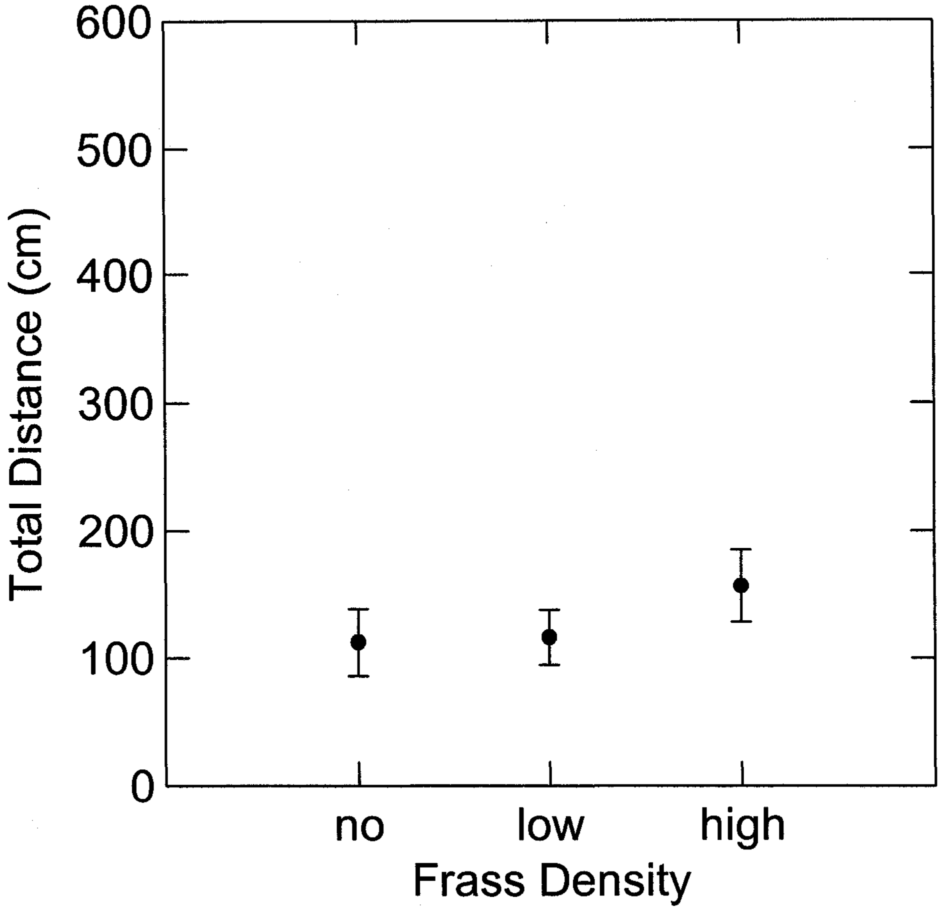

Figure 2.9: Mean total distances moved (cm) in 2004 laboratory arenas in response to beetle frass densities. Error bars are standard errors.

Predation on marked beetles

There was no significant difference in predation of marked and unmarked beetles in the arenas (Table 2.3). The mean number of beetles two days after release on 30 September 2005 in the marked beetle arenas was 7.5 beetles and the mean number of beetles in the unmarked beetle arenas was 7.8 beetles. On 4 October 2005, the mean number of marked beetles was 3.1 and the mean number of unmarked beetles was 4.6. The decrease in number of beetles found between 28 September and 4 October 2005 was likely due to beetle escape rather than to predation because few elytra were found in the arenas.

Table 2.3: Analysis of deviance table for *Cyphocleonus achates* (BEETLE) number as a function of MARKING, ARENA and DATE beetles were monitored.

Term	**df**	**Deviance**	**p(Chi)**
Null	47	112.388	
MARK	1	0.336	0.640
ARENA	11	19.603	0.332
DATE	1	26.378	**< 0.0001*****
Residual	34	66.072	

Testing factors affecting beetle movement using field experiments

High percentages (52-60%) of beetles were recaptured for all of the experiments with the highest percentage recaptured in the Fall 2005 trial (80% beetles recaptured) (Table 2.4).

Table 2.4: Number of *Cyphocleonus achates* released and the percentage of beetles that moved for the 2003-2005 experiments.

Experiment	**Released (N_{total})**	**Recaptured Total (%)**	**Moved Once (% recap)**	**Moved Multiple Times (% recap)**
Diurnal Movement 2003	600	52.00	n.a.	n.a.
Potted Patch				
2004 Summer	502	57.37	26.04	3.82
2005 Summer	754	59.55	54.12	14.03
2005 Fall	756	80.03	42.48	10.08
Natural Patch 2004	598	53.18	27.36	4.09

The mean total distances traveled were lowest in the Natural Patch (2.67m +/- 5.62 sd) (Figure 2.10) and highest in the Potted 2005 Summer trial (31.86 +/- 38.27 sd) (Figure 2.11). The maximum distances recorded from the same trials (39.06m and 271.32m for Natural and Potted patches respectively) exhibit the same trend as the mean total distance (Table 2.5).

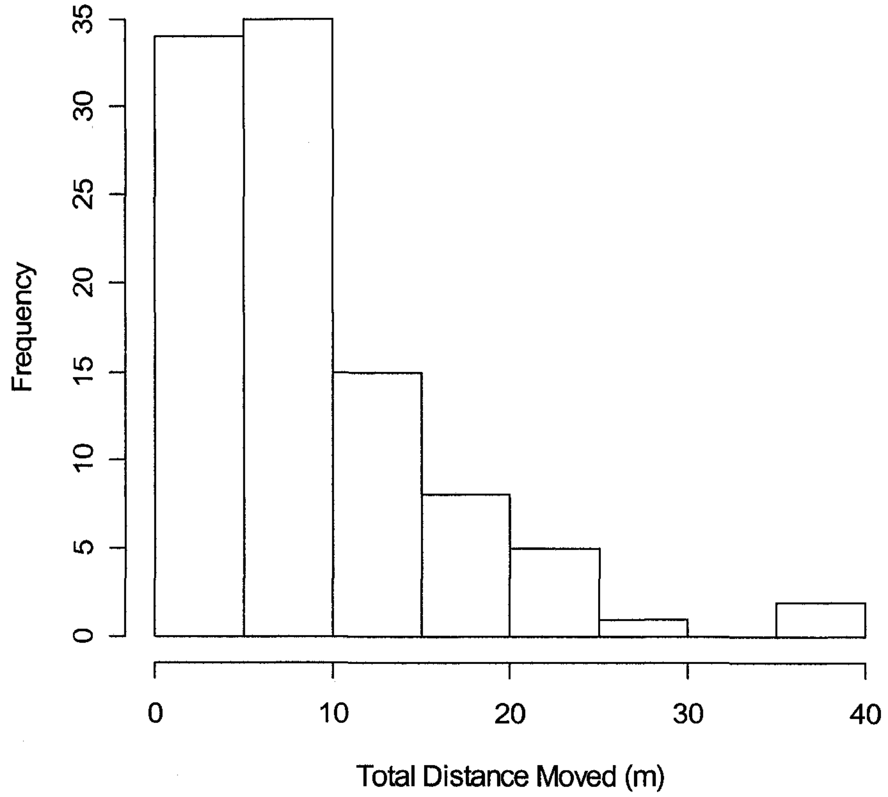

Figure 2.10: Frequency distribution of total distance moved (m) by individual *Cyphocleonus achates* that moved in the Natural Patch experiment ($n_{\text{moving beetles}}$=100). The number of individuals that did not move totaled 218 (not shown).

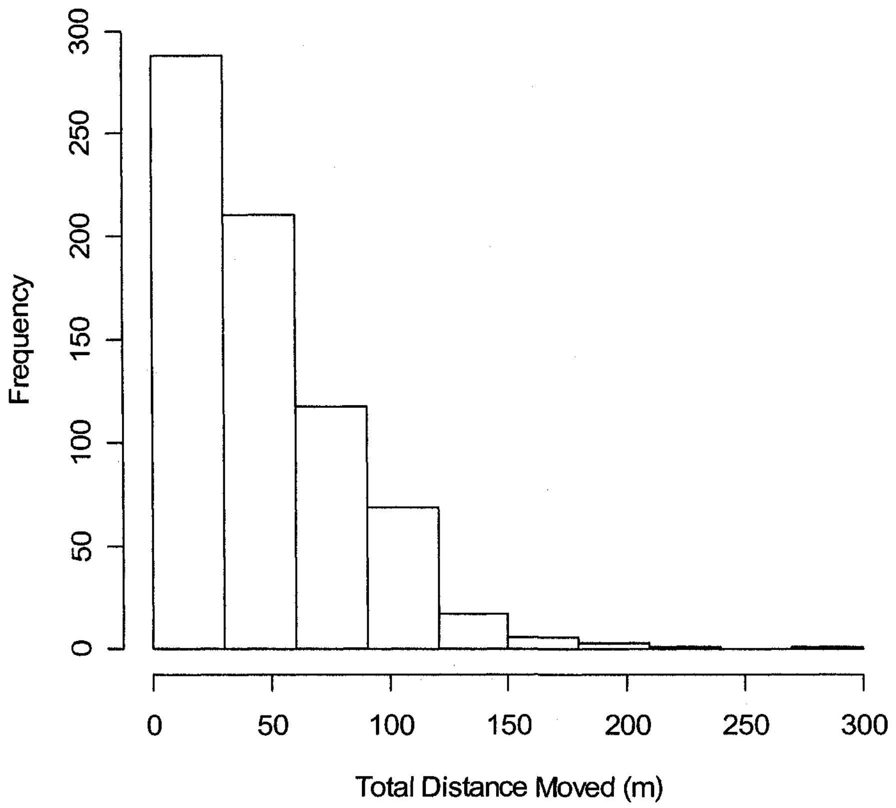

Figure 2.11: Frequency distribution of total distance moved (m) by individual *Cyphocleonus achates* that moved in the combined Potted Patch trials ($n_{\text{moving beetles}}$=710). The number of individuals that did not move totaled 632 (not shown).

Table 2.5: Mean and maximum total distances (m) moved by individual *Cyphocleonus achates* over ten days in each field experiment.

Experiment	**Number of Beetles**	**Mean Total Distance (m)**	**Standard Error**	**Maximum Distance (m)**
Potted Patch – all	1341	23.20	0.930	271.32
2004 Summer	287	11.82	1.389	107.20
2005 Summer	449	31.86	1.806	271.32
2005 Fall	605	22.18	1.352	215.06
Natural Patch 2004	318	2.67	0.315	39.06

Agitation Dispersal

Handling and marking of insects for mark-recapture experiments can cause initial dispersal immediately after release that is faster or slower than what it would occur in normal situations (Turchin, 1998). This change in movement depending on the time after the insects are released is referred to as agitation dispersal. There were no significant differences for *C. achates* between the MOVELENGTHs over 24hrs for each DAY after release for either the Potted Patch trials (Table 2.6) or the Natural Patch (Table 2.7). There was a significant effect of release DATE in the Natural Patch, where the MOVELENGTHs are longer later in the season. This result is related to the date beetles were released in the time of release experiment discussed in Chapter 3.

Table 2.6: Analysis of deviance table for MOVELENGTH by *Cyphocleonus achates* as a function of DAY after release, TRIAL and a DAY x TRIAL interaction in the Potted Patch experiments.

Term	**df**	**Deviance**	**F-value**	**p(F)**
Null	129	57014		
DAY after release	1	928	2.02	0.1582
TRIAL	2	759	0.82	0.4411
DAY x TRIAL	2	546	0.59	0.5544
Residual	124	54782		

Table 2.7: Analysis of deviance table for MOVELENGTH by *Cyphocleonus achates* as a function of DAY after release, release DATE and a DAY x DATE interaction in the Natural Patch experiment.

Term	**df**	**Deviance**	**F-value**	**p(F)**
Null	387	1406.88		
DAY after release	1	3.80	1.09	0.2964
Release DATE	5	72.49	4.17	**0.0011** **
DAY x DATE	4	20.86	1.50	0.2012
Residual	377	1309.74		

Effect of beetle sex and size on movement

A total of 5318 beetles were collected from British Columbia and marked for the experiments 2003 to 2005. Female beetles were significantly heavier than were males and weight differed significantly between years (Table 2.8; Figure 2.12). Beetles were heavier in 2004 and 2005 when compared to 2003 and there was a significant interaction between sex and year due to the large sample size.

Table 2.8: Analysis of deviance table for WEIGHT of *Cyphocleonus achates* as a function of SEX, YEAR and a SEX x YEAR interaction of collected 2003-2005 beetles.

Term	**df**	**Deviance**	**F-value**	**p(F)**
Null	5317	6.195		
SEX	1	0.0941	101.75	< **0.0001** ***
YEAR	2	1.1809	638.34	< **0.0001** ***
SEX x YEAR	2	0.0066	3.58	**0.028** *
Residual	5312	4.913		

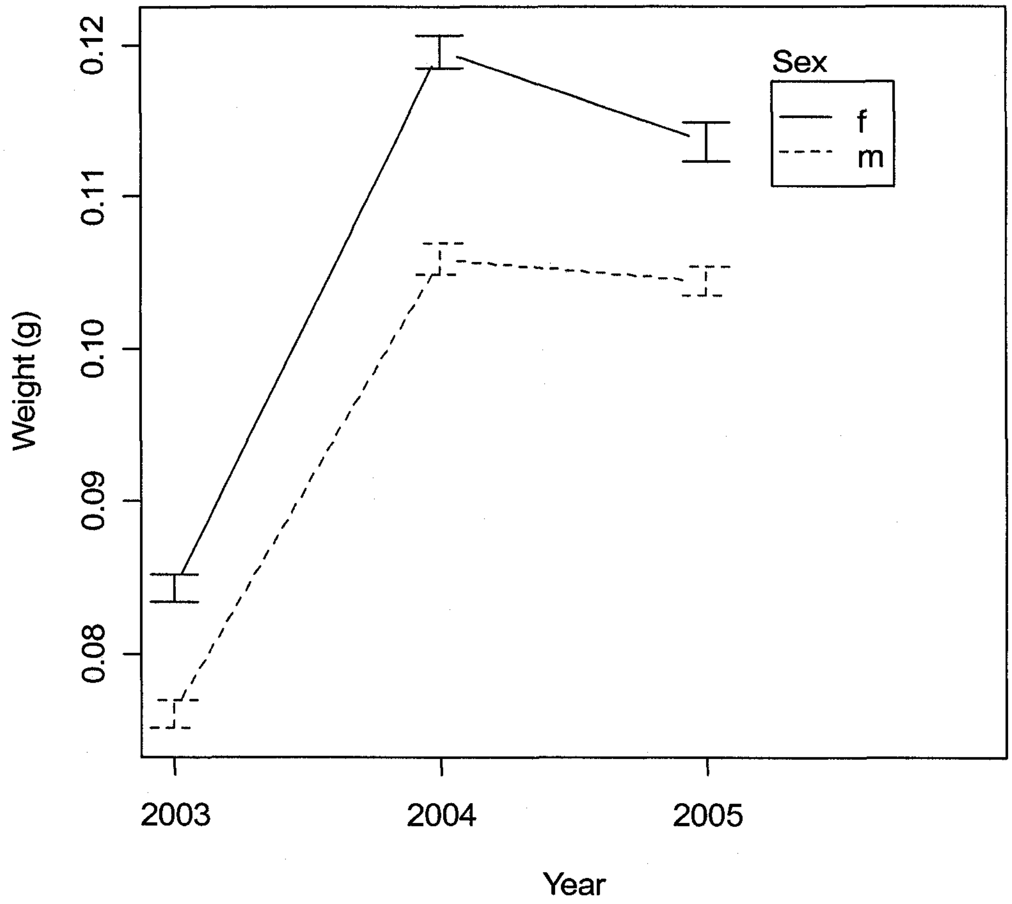

Figure 2.12: Interaction plot of the weight (g) between the sexes and years of collected *Cyphocleonus achates* 2003-2005 ($n_{beetles} = 5318$). Error bars are standard errors.

There was no difference between male and female movement in the 2005 Summer trial, however in the 2004 Summer and 2005 Fall trials, female beetles were more likely to disappear after release whereas males were more likely to stay on the release plant in (Table 2.9).

Table 2.9: Number of male and female *Cyphocleonus achates* that exhibited different movement behaviours for each of the high density Potted Patch trials.

Trial	Sex	Never Found	No Moves	Move Once	Multiple Moves
2004 Summer	Male	58	**85**	29	5
	Female	**99**	66	34	6
2005 Summer	Male	117	46	95	29
	Female	132	46	91	18
2005 Fall	Male	42	**125**	95	25
	Female	**76**	91	95	28

(2004 Summer: x^2=11.6, df=3, p=0.009; 2005 Summer: x^2=3.56, df=3, p=0.312; 2005 Fall: x^2=15.30, df=3, p=0.002)

To determine if beetle traits affect movement, I examined the effect of beetle SEX and WEIGHT (g) on the TOTAL DISTANCEs (m) moved. RELEASE DATE was found to be significant whereas WEIGHT and SEX of the beetles had no significant effect on the distances moved by *C. achates* (Table 2.10).

Table 2.10: Analysis of deviance table of TOTAL DISTANCE moved (m) from a release plant by individual *Cyphocleonus achates* for the complete model including RELEASEDATE, beetle WEIGHT and beetle SEX in the Natural Patch experiment.

Term	df	Deviance	F-value	p(F)
Null	315	10427		
RELEASEDATE	1	1053.2	35.12	**< 0.0001** ***
WEIGHT	1	49.9	1.66	0.1981
SEX	1	8.3	0.28	0.5989
RELEASEDATE:WEIGHT	1	0.9	0.03	0.8599
RELEASEDATE:SEX	1	0.6	0.02	0.8917
WEIGHT:SEX	1	54.6	1.82	0.1782
RELEASEDATE:WEIGHT:SEX	1	19.2	0.64	0.4244
Residual	308	9240.3		

Effect of plant traits on movement

In the Natural Patch experiment, seventeen percent of the non-release knapweed plants had beetles found on them during the ten days after beetles were released. There was a significant effect of the proximity of the nearest release plant to the bolted plant on beetle presence. However, there was no effect of plant size or the number of rosettes surrounding the bolted plant on whether beetles occupied the plants (Table 2.11). The mean distance to the nearest release plant was 7.0m for plants with beetles and the mean distance to the nearest release plant was 12.4m for plants without beetles (Figure 2.13). This difference was small compared to the size of the Natural Patch plot (65m by 50m). There were no non-linear effects of plant SIZE, number of ROSETTEs or distance to the nearest RELEASE plant on beetle occupation of a plant (PRESENCE) (Table 2.12).

Table 2.11: Analysis of deviance table for the full model of *Cyphocleonus achates* PRESENCE in response to the distance to the nearest RELEASE plant, plant SIZE and number of ROSETTEs in the Natural Patch after beetle release.

Term	**df**	**Deviance**	**Resid Dev**	**p(Chi)**
Null	314	291.76		
Nearest RELEASE plant	1	54.75	237.011	< **0.0001** ***
Plant SIZE	1	2.51	234.502	0.113
ROSETTE number	1	0.23	234.275	0.633
DISTANCE:SIZE	1	0.15	234.122	0.696
DISTANCE:ROSETTEs	1	0.002	234.120	0.967
SIZE:ROSETTEs	1	0.47	233.649	0.492
DISTANCE:SIZE:ROSETTEs	1	0.65	233.000	0.420
Residual	307	233.00		

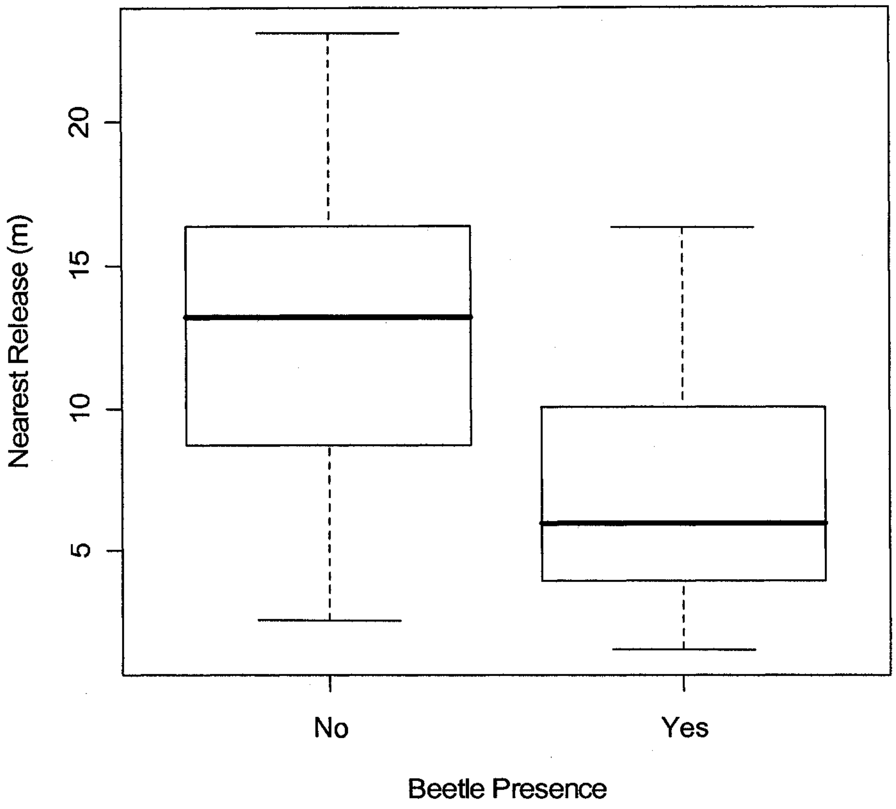

Figure 2.13: Box-plot of the distance between the nearest release plant (m) to individual bolted knapweed (*Centaurea diffusa*) plants with or without *Cyphocleonus achates* in the 2004 Natural Patch experiment.

Table 2.12: Test of non-linear effects on *Cyphocleonus achates* PRESENCE as a function of the distance to the nearest RELEASE plant, plant SIZE and ROSETTE number in the Natural patch experiment after beetle release.

Term	**df**	**Chisq**	**p(Chi)**
Nearest RELEASE plant	1	5.555	0.14
Plant SIZE	1	2.609	0.46
ROSETTE number	1	4.166	0.24

2.4 Discussion

For this study, it was vital that I chose a method of sampling *C. achates* that would not disrupt beetle behaviour and test whether beetle and plant traits affect *C. achates* dispersal. I used bioassays to test individual mark recapture methods (IMR) including lifespan of the beetles after marking, time of day, storage and frass effects on beetle movement and predation of marked beetles. The marking methods used did not affect the lifespan of the beetles or predation on *C. achates* and there was no effect of frass on the total distances moved in the laboratory experiment. Beetle dispersal after storage, however, significantly decreased over time. In addition, I tested factors that may affect beetle movement in the field. I found no effect of agitation dispersal, beetle sex and size and plant traits on beetle movement in the field experiments. In two of the Potted Patch trials, however, male *C. achates* were found to stay on the release plants more often than females who were more likely to disappear after release.

Male and female insects often exhibit different behaviours in the field due to the differences in reproductive strategies. Females tend to disperse further from the release compared to their male counterparts (Kuusaari *et al.*, 1996; Albrectsen and Nachman, 2001; Petit *et al.*, 2001; but see Lawrence, 1988). This difference often results from the need for females to spread oviposition sites over the landscape whereas males tend to compete and stake territories. In this system, *Cyphocleonus achates* females were found to disappear after release more often than males. This finding could be due to *C. achates* being only able to oviposit in the root crown of knapweed rosettes (Story *et al.*, 1996) and because monitoring did not include the roots, females were effectively removed from the sample.

There are few quantitative studies that show correlations between host-plant preference and offspring performance (e.g. Craig *et al.*, 1999; Fox, 1993; Karban and Courtney, 1987; Larsson *et al.*, 1995) and the lack of expected correlation has been linked to limited dispersal in the herbivore (Cronin *et al.*, 2001). In this study, I expected that higher quality host plants (larger plants) or plants with more oviposition sites (higher rosette number) would play a part in plant choice by *C. achates*. However, I found that during the initial dispersal after release, the distance from the nearest release plant was the only significant variable on beetle occupation of bolted plants. This suggests that the limited dispersal ability of *C. achates* rather than beetle preference could be the cause of the inability to detect preferences in this study. Compared to the size of the habitat under study, *C. achates* dispersal capability is relatively small, thus only a fraction of plants could be visited by the beetle and *C. achates* preference of bolted plants is obscured.

The smaller distances moved by *C. achates* in the Natural Patch (0.27m/day) compared to the Potted Patch (2.32m/day) can be attributed to the high densities of different life stages of knapweed in the Natural Patch. The Potted Patch trials contained only 36 bolted plants (2004) or 54 rosettes per trial (2005) while the Natural Patch had 324 bolted plants and numerous rosettes per bolted plant. In the Natural Patch, beetles do not need to move because food and oviposition sites are readily available. The strategy of *C. achates* remaining on good quality plants when plants are abundant has the potential to increase their fecundity. If the insect only moves when food and oviposition sites are unavailable more energy can go to egg production when they are in good quality sites.

Managers can use the information presented here to assess the number of releases needed for a specific knapweed patch. In the Natural Patch, *C. achates* moved an average distance of 0.27m/day. Over a beetle's three-month life span, the maximum intra-patch movement by *C. achates* in continuous knapweed infestations will be approximately 30m. In large continuous infestations of knapweed, multiple releases every 60m would ensure an even distribution of *C. achates* over the entire patch.

Summary

Cyphocleonus achates is a sedentary beetle that moves relatively short distances in patches with high knapweed densities of different stages of knapweed. The beetles are not affected by marking or knapweed traits initially after release and beetle sex and size do not affect movement distances. There is, however, a difference between the number of males and females where females are more likely to disappear after release and males are likely to stay on the release plant.

2.5 Literature Cited

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Chapter 3

The effects of density and time of release on *Cyphocleonus achates* (Coleoptera: Curculionidae) dispersal on the invasive plant, diffuse knapweed (*Centaurea diffusa*) (Asteraceae)

3.1 Introduction

Dispersal after release is important for the establishment and impact of biocontrol agents on weeds and can be manipulated using different release strategies (Fagan *et al.*, 2002; DeClerck-Floate *et al.*, 2005). Two ways of increasing the chances for establishment of a biocontrol agent are by increasing the number of insects released or by manipulating the time of release during the season. The outcome of biocontrol releases in response to the number of insects released has been studied extensively in classical biocontrol. When the number of insects to be released is limited, land managers must trade off between a single large release or multiple releases of the biocontrol agent (Grevstad, 1999; Shea and Possingham, 2000). In some systems, the timing of release is vital in order to synchronize the insects with their new habitat (Van Driesche and Bellows, 1993). However, manipulating the time of releases within the season has been largely ignored in weed biocontrol.

High or low rates of dispersal can have dramatic effects on the success of biocontrol. High dispersal rates prevent starvation resulting from resource depletion and saturation of oviposition sites (Reznik, 1991; Leonard, 1970; Ohgushi and Sawada, 1985). High dispersal rates, however, may result in low local densities of the agents, thereby reducing the probability of successful biocontrol by spreading agents too thinly in the landscape (Fagan *et al.*, 2002). Extirpation of the agents, due to Allee effects, may occur in rapidly dispersing agents because the agents will be too spread out on the landscape to successfully reproduce (Hopper and Roush, 1993). Limited dispersal of biocontrol agents may reduce mortality because high-risk movement between patches would occur infrequently (Comins *et al.*, 1980; Johnson and Gaines, 1990). In addition, agents that exhibit little dispersal can put more energy into egg production leading to higher density of the biocontrol agent and a more rapid impact on the weed. However, reduced dispersal has been linked to reduced local population growth, which can postpone the impact of the biocontrol agents on the weed (Kean and Barlow, 2000; Jonsen *et al.*, 2007). Once resources are exhausted, agents with limited dispersal would be at a higher risk of extirpation.

Increasing the release density of a biocontrol agent can overcome Allee effects and increase establishment of the agent in the patch (Beirne, 1975; Hopper and Roush, 1993; Grevstad, 1999). More agents in a patch will result in higher numbers of weeds affected and a higher population density of agents for the following season. Too high a release density, however, can result in increased dispersal and emigration due to either competition for resources or avoidance of conspecifics (Harrison, 1980; Herzig, 1995). In extreme cases, this will effectively reduce the density of biocontrol agents released in the plot, which will lead to establishment failure.

Mismatched timing of releases into a new habitat can cause establishment failure if hosts are not available at the appropriate life stage (i.e. Youssef and Evans, 1994). If the biocontrol insect is released too early, the insects will be exposed to an increased risk of environmental stochasticity or predation events before they oviposit. Releases too late in the season may not provide sufficient time for oviposition before adults are exposed to frost.

Insects employ different dispersal strategies through the season in response to cues from the environment. The Colorado potato beetle (*Leptinotarsa decemlineata* Say) can disperse up to several hundred metres over a few days early in the season after overwintering, whereas late in the season, only a small proportion can disperse more than 500m (Boiteau *et al.*, 2003). The change in dispersal tactics in this beetle has been linked to a diapause switch caused by photoperiod, cool temperatures or plant senescence later in the season (Boiteau *et al.*, 2003). Using these cues, beetles can switch from long distance flight to walking as the main mode of dispersal (Boiteau *et al.*, 2003), which ensures reproductive success as the environment changes.

Cyphocleonus achates is a biocontrol agent on diffuse knapweed (*Centaurea diffusa*) whose dispersal is not well understood. *Cyphocleonus achates* feed on the bolting and rosette lifestages of knapweed and overwinter in the roots of rosettes (Stinson *et al.*, 2004). A typical biocontrol release consists of 100 beetles per patch (Val Miller, personal communication) and patch sizes of knapweed can range from individual plants to vast monocultures. This beetle is established on knapweeds in British Columbia, Alberta, Wyoming, Washington, Utah, South Dakota, Oregon, Montana and Colorado (Lang *et al.*, 2000). Its univoltine life history and flightlessness have resulted in a slow rate of spread (Story *et al.*, 2006). To increase spread, researchers have developed mass rearing techniques and manual distribution of the beetle (Story *et al.*, 1996). Despite the improved management techniques, there has been little attention paid to the way in which *C. achates* is released with respect to its dispersal.

To increase the initial spread of *C. achates*, I examined release strategies, which may cause increased movement away from the release plants. I conducted mark-release-resight experiments in the field to assess movement in response to 1) beetle release densities and 2) timing of releases during the season on *C. achates* dispersal away from the release site. Understanding the effect of our release strategies on movement of *C. achates* will allow us to modify and improve releases in the field.

3.2 Methods

Study Areas

Mark-release-resight studies were conducted in two locations: on a pre-existing patch of knapweed (2003 & Natural Patch) and on artificial plots (Potted Patches: Summer 2004, Summer 2005 and Fall 2005 trials).

Experiments in 2004 (Natural Patch and Potted Patch: Summer 2004) took place on the Blood Tribe Indian Reserve approximately 20km west of Lethbridge, AB. The Natural Patch experiment was located in a gravel bed 65m by 50m (N:49.7400460 W:112.9888450) approximately 100m from the Old Man River. Diffuse knapweed, open bare spaces, mullein, shrubs and trees characterized this plot. Two biocontrol agents were already present at this site: the seedhead flies *Urophora affinis* and *U. quadrifasciata.*

The 2004 Summer Potted Patch experiment was located on rangeland approximately 2.5km east of the Natural Patch (N:49.7332392 W:112.9753424) in a 50m by 90m plot. The primary vegetation at this site was grasses, wild rose, and sedges. The biocontrol agents present were the seedhead gallflies *U. affinis*, *U. quadrifasciata* and second-generation *C. achates* from a nearby 2003 release.

The 2005 Summer and Fall Potted Patch trials were located on land near the Agriculture and Agri-food Canada Research Centre in Lethbridge, Alberta (N:49.69727

W:112.76255). Knapweed in this plot was planted in two trials on this land during August and September respectively, in field plots 50m by 150m with no knapweed biocontrol agents present.

Experimental Design

Collection and marking beetles

Individual beetles were hand picked or sweep-netted from established populations in British Columbia and transported in containers of 50 individuals each to the Agriculture and Agri-Food Canada Research Centre in Lethbridge, Alberta. Beetles were marked with a two-colour combination of Testors™ enamel model paint in either a four-dot or six-dot pattern. Paints were applied on the elytra with a head of a pin, taking care to avoid marking joints, pronotum, head parts and sealing the elytra. Individually marked beetles were separated by sex, weighed and released in the field. Prior to release, all individual *C. achates* used in these experiments were held in a controlled environment chamber at 5°C night and 10°C day and a photoperiod of 14light:10dark.

Plot Designs

Potted Patch Experiment - Summer 2004, 2005 and Fall 2005 Trials

Centaurea diffusa plants were either collected in the field and potted as bolting plants (2004 trial) or grown from seed in the greenhouse and used at the rosette stage (2005 trials). Bolted field plants could not be used in 2005 because the field collected plants were unavailable due to flooding. Pots were buried in the ground in replicates of four rows (2004 trial) or six rows (2005 trials) (Figure 3.1). Each row consisted of three groups of pots 10m from each other. Each group contained three plants including a central release plant with two target plants 5m on either side. For each group of plants, one of three release densities of beetles (low=6 individuals, medium=24 individuals and high=96 individuals) was assigned to the centre release plant. Release plants were assigned densities in a modified Latin Square design. Release dates were 24 August 2004 (Summer 2004 trial), 19 August 2005 (Summer 2005 trial) and 9 September 2005 (Fall 2005 trial). All plants in each plot were monitored daily with the exception of days with inclement weather (one day for Summer 2005 and two days for Fall 2005).

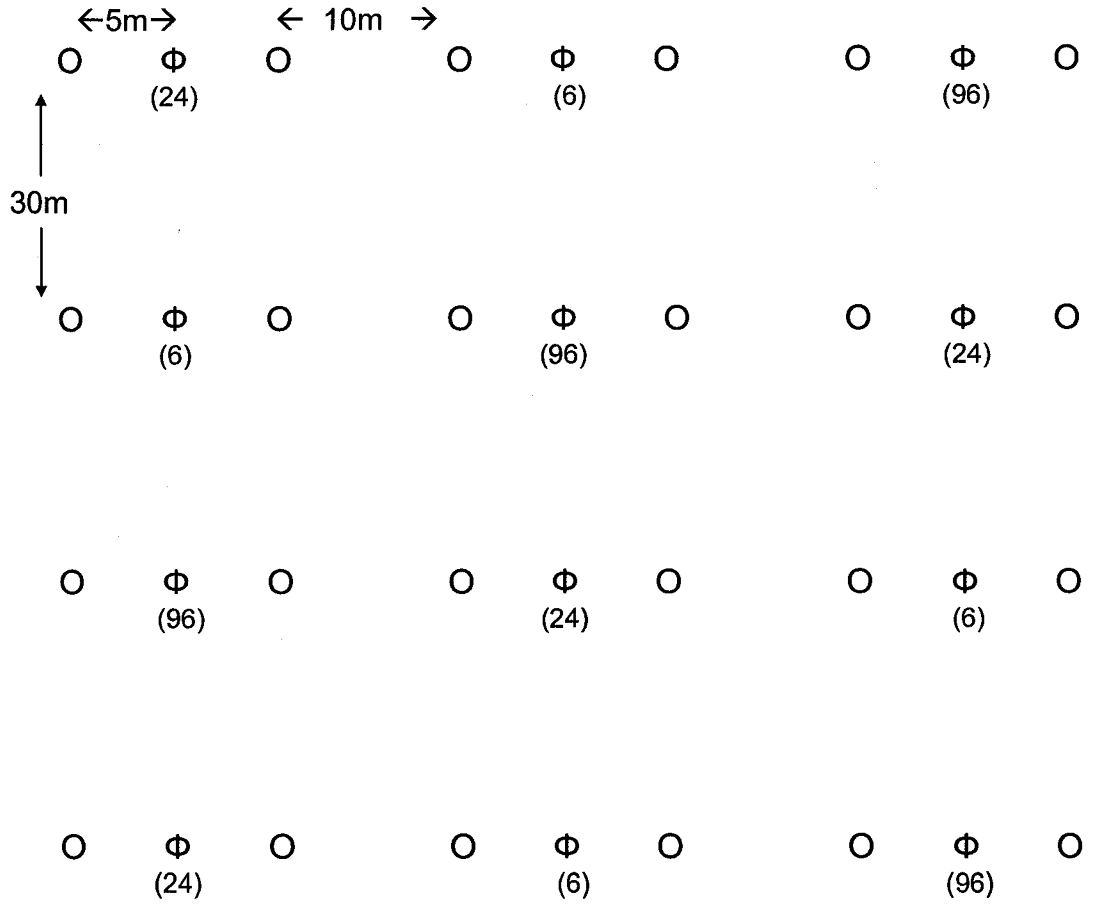

Figure 3.1: Plot design of the Potted Patch trial in 2004 with n=4 rows and three groups of pots per row (n=6 rows in both 2005 trials). The number of beetles released for each group is in parentheses below the central release plant. The symbols O = target plants and Φ = release plants.

Natural Patch Experiment 2004

To record beetle movements between plants in the Natural Patch, each plant was assigned a number and tagged with a metal label at the base of the plant. GPS coordinates for all 324 bolting *Centaurea diffusa* plants as well as height, width of the crown, and number of rosettes within 1m^2 area around each bolted plant was recorded before beetle release.

The number of rosettes and bolted plants did not change during the monitoring period. The plot was divided into six quadrats and one plant was chosen randomly per quadrat for each week (Figure 3.2). One hundred *C. achates* were released on a bolting plant in a randomly chosen quadrat each week from 27 July – 31 August 2004 ($N_{\text{total beetles}} = 600$). One of the pre-determined quadrats could not be used because beetles had already been found in that quadrat. As a result, beetles for the 24 August 2004 release were released on a group of isolated plants without *C. achates*, closest to the unusable quadrat.

Daily beetle monitoring took place between 1300hrs and 1500hrs from 28 July 2004 – 21 September 2004 by scanning the leaves and stalk of each of the bolting plants and the surrounding rosettes for 10 seconds per plant to standardize sampling effort. The markings of each observed *C. achates* were recorded. Elytra from predated beetles were collected and their markings were recorded as an estimate of mortality from predation.

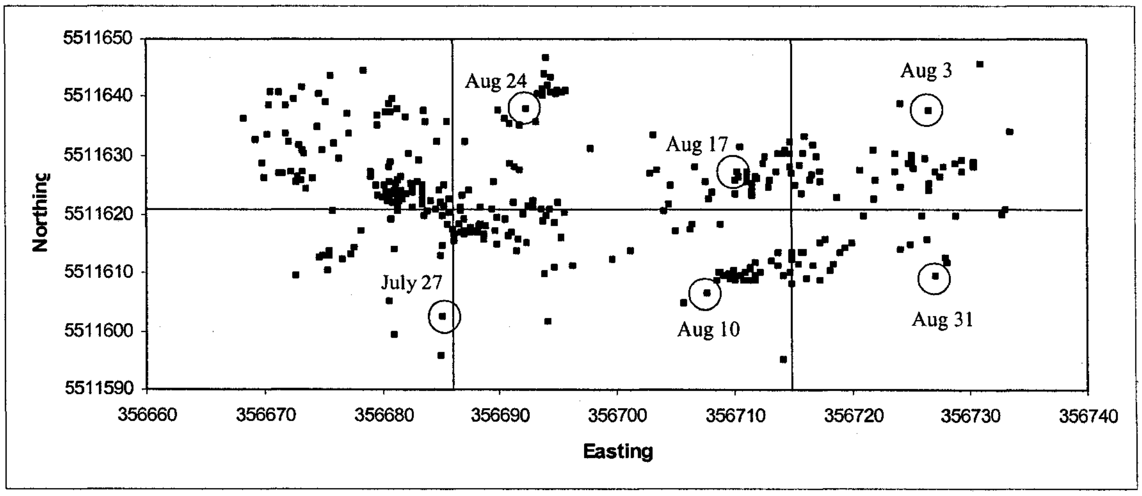

Figure 3.2: GPS coordinates of bolting diffuse knapweed plants (n=324 *Centaurea diffusa*) in the Natural Patch experiment. The plot was divided into six quadrats (solid lines) in which a randomly chosen release plant (circle) was picked for the release of 100 *Cyphocleonus achates* each week (n=6 releases).

Statistical Analyses

I estimated the disappearance RATE[1] off the release plant and calculated the TOTAL DISTANCE beetles traveled between plants in both the Natural and Potted Patches to determine the effects of beetle release density and the time of release on *C. achates* movement. These parameters were chosen because they give two different perspectives on dispersal. The total distances moved by each beetle describe movement per individual *C. achates*. The rate of dispersal off release plants describes movement by the group of beetles released on that plant. The concern is that movement distances of beetles that are found multiple times on the same plant (and therefore have a large number of zeros) would be equal to beetles that disperse away from the patch, beetles that are depredated or beetles that are hiding because they also have large numbers of zeros in the dataset. By using two estimates of dispersal to test for treatment effects, I gain confidence in the analyses when trends are similar. For all analyses, I used R-statistical package (R_{gui} 2.3.1, 2006).

To calculate the disappearance RATE off each release plant in both Natural and Potted Patches, I recorded the number of beetles on each release plant on each of ten days after beetle release (n=4/day for 2004; n=6/day for 2005). The number of beetles was log (x+1) transformed and was regressed against sample date. The slope of the regression line was used as an estimate of the RATE of disappearance of the beetles from each plant over time (see Appendix). The estimated slopes were then used as the dependent variable in an Analysis of Variance (ANOVA) to determine the effects of DENSITY (6, 24 or 96

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[1] Factors included in statistical models are presented in SMALL CAPS font

beetles) and TRIAL (Summer 2004, Summer 2005 or Fall 2005) on the RATE of disappearance.

Potted Patch Trials

The distance (d) between plants were calculated using x, y grid coordinates in the equation d = sqrt $((x_2-x_1)^2 + (y_2-y_1)^2)$. To calculate the TOTAL DISTANCE that individual beetles traveled during the course of the experiment, linear distances between sightings for each individual beetle were summed. I used ANOVA to assess the effect of TRIAL and DENSITY on the TOTAL DISTANCE (m) beetles moved. Differences among trials or densities were assessed using Tukey's Honest Significant Difference (HSD) test. In models with overdispersed data, quasi-likelihood error distributions were used in place of Gaussian error distributions.

Natural Patch

Movement distances were calculated using the GPS coordinates of the bolted knapweed plants with the distance matrices function in R-statistical package (R_{gui} 2.3.1, 2006). RATE of disappearance and TOTAL DISTANCE moved were calculated for each release treatment as in the Potted Patch trials.

Data were collected daily for two months in the Natural Patch, and thus beetle movement histories were truncated to 10 days after release to enable comparisons among releases. The effect of DENSITY, TRIAL, beetle SEX and WEIGHT were assessed in separate ANOVAs for both the RATE of disappearance and for TOTAL DISTANCE (m) moved.

Quasi-likelihood error distributions were used in place of Gaussian error distributions for overdispersed data.

To determine which life stage of knapweed plant *C. achates* preferred during different times of the season, the number of beetles on either bolted or rosette plants were counted during the week after beetle release in the Natural Patch experiment. The proportion of beetles found on the rosettes or bolted plants of the total number found in the plot was plotted for each release date ($n_{releases}$=6). In addition to the experimental time after releases, all beetles in the plot were counted and scored as being on bolted plants, rosette plants or other for the last two weeks of the experiment (8 and 15 September).

3.3 Results

A total of 2,612 beetles were marked and released for the Potted and Natural Patch experiments. The overall mean recapture rate for all field experiments was 64.6%. Total and multiple recaptures were highest in the Potted 2005 Fall trial (80.0%) with the majority of these beetles being recaptured multiple times (55.6%) (Table 3.1).

Table 3.1: Number of *Cyphocleonus achates* released and recapture percentages in the Potted Patch trials and the Natural Patch experiment.

Experiment	**Released (N_{total})**	**Recaptured Total (%)**	**Recaptured Once (%)**	**Recaptured Multiple Times (%)**
Potted Patch				
2004 Summer	502	57.37	26.49	30.47
2005 Summer	754	59.55	24.01	35.54
2005 Fall	756	80.03	24.47	55.56
Natural Patch 2004	598	57.86	28.26	29.60

Potted Patch Trials

There was a significant effect among the DENSITY(ies) and the TRIALs on the RATE of disappearance off the release plant (Table 3.2; Figure 3.3). There was a significant but weak interaction between the medium and high density releases for the 2005 Summer and Fall trials. The medium density release was similar to the high density release in Fall 2005 and had a slower disappearance rate in the Summer 2005. There was a greater rate of disappearance at higher density for all trials and the Summer 2004 trial had a faster disappearance rate than the Summer 2005 and the Fall 2005 trials (Figure 3.4). The difference in disappearance rate between years can be attributed several different factors. Most importantly, different plant life stages were planted in 2004 (bolted plants) versus 2005 (rosettes). Beetles may move faster off bolted plants than off rosettes because rosettes provide oviposition sites as well as food whereas bolted plants only provide food. Another difference between the 2004 and 2005 trials is the location the plants were planted. In 2004, the site chosen was rangeland, where a natural patch of diffuse knapweed was found approximately 100m away from the experimental pots and seedhead flies and second generation *C. achates* were present. The 2005 trials took place on a weedy field where no diffuse knapweed plants were nearby and therefore no biocontrol agents present.

Table 3.2: Analysis of deviance table for RATE of movement from a release plant by individual *Cyphocleonus achates* as a function of DENSITY, TRIAL and a DENSITY x TRIAL interaction in the Potted Patch trials.

Term	**df**	**Deviance**	**F-value**	**p(F)**
Null	47	0.2218		
DENSITY	2	0.1056	50.53	**< 0.0001** ***
TRIAL	2	0.0622	29.76	**< 0.0001** ***
DENSITY x TRIAL	4	0.0132	3.15	**0.0245** *
Residual	39	0.0408		

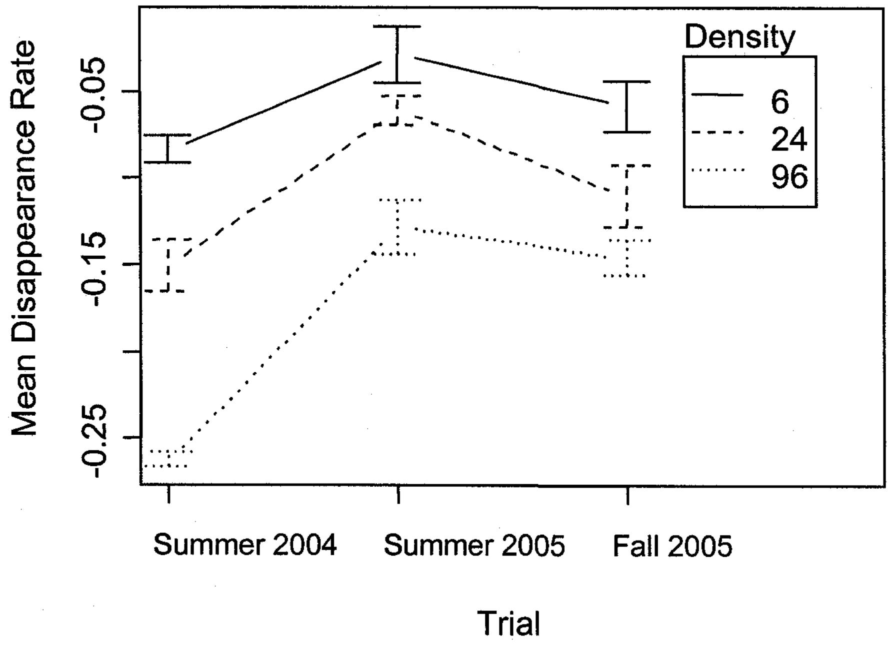

Figure 3.3: Interaction plot of the mean rate of *Cyphocleonus achates* moving off release plants in the Potted Patch trials. Sample sizes for each release density was n=4 in the Summer 2004 trial and n=6 per trial in the Summer and Fall 2005 trials. The error bars are standard errors.

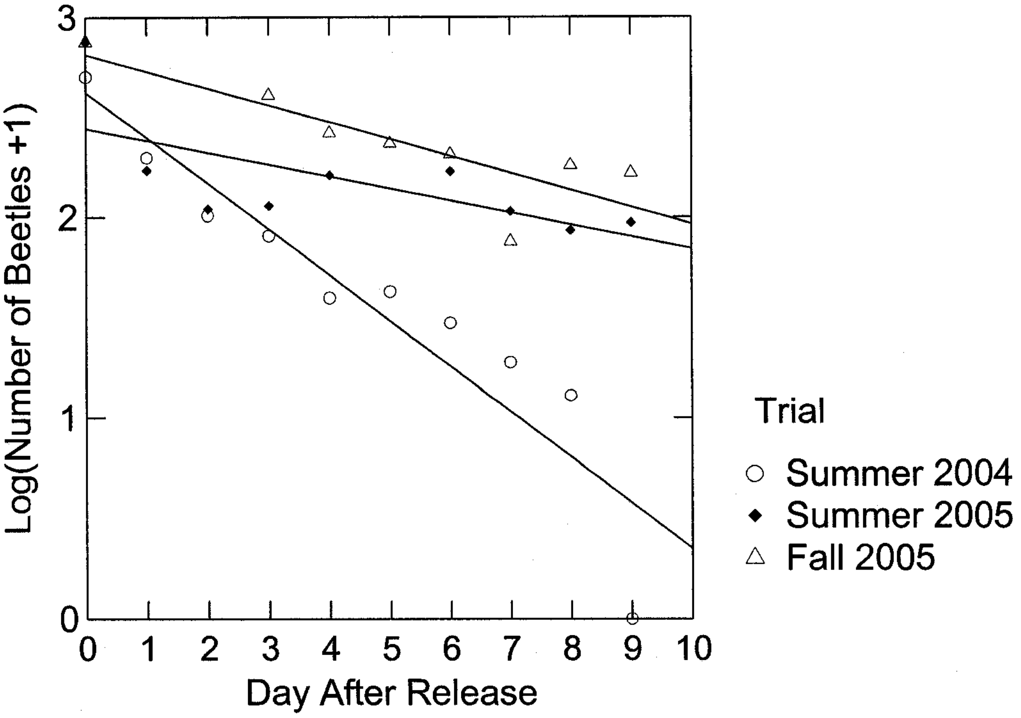

Figure 3.4: Disappearance rates (slope) of *Cyphocleonus achates* for each trial in the Potted Patch experiments. Each data point is the log transformed mean number of beetles +1 found for each replicate in each trial (2004: n=4, 2005: n=6).

The TOTAL DISTANCE beetles moved in the plot differed significantly among TRIALs and DENSITYs, but there was no significant DENSITY by TRIAL interaction (Table 3.3). Beetles moved farther at high conspecific densities than at low-density releases (Figure 3.5). Beetles also moved farther in the 2005 trials compared to the 2004 trial but there were no significant differences between the Summer and Fall TRIALs (Figure 3.6). The increase in dispersal distances between years of the total distances moved could be due to the scale dispersal was measured for each of the trials. The larger number of replicates in 2005 (n=6) versus 2004 (n=4) resulted in a larger plot size for 2005 (2004: 90m by 50m 2005: 150m by 50m).

Table 3.3: Analysis of deviance for TOTAL DISTANCE moved (m) from a release plant by individual *Cyphocleonus achates* as a function of DENSITY, TRIAL and a DENSITY x TRIAL interaction in the Potted Patch trials.

Term	**df**	**Deviance**	**F-value**	**p(F)**
Null	47	7282.1		
DENSITY	2	1335.6	7.33	**0.0020** **
TRIAL	2	1780.9	9.78	**0.0004** ***
DENSITY x TRIAL	4	614.8	1.69	0.172
Residual	39	3550.8		

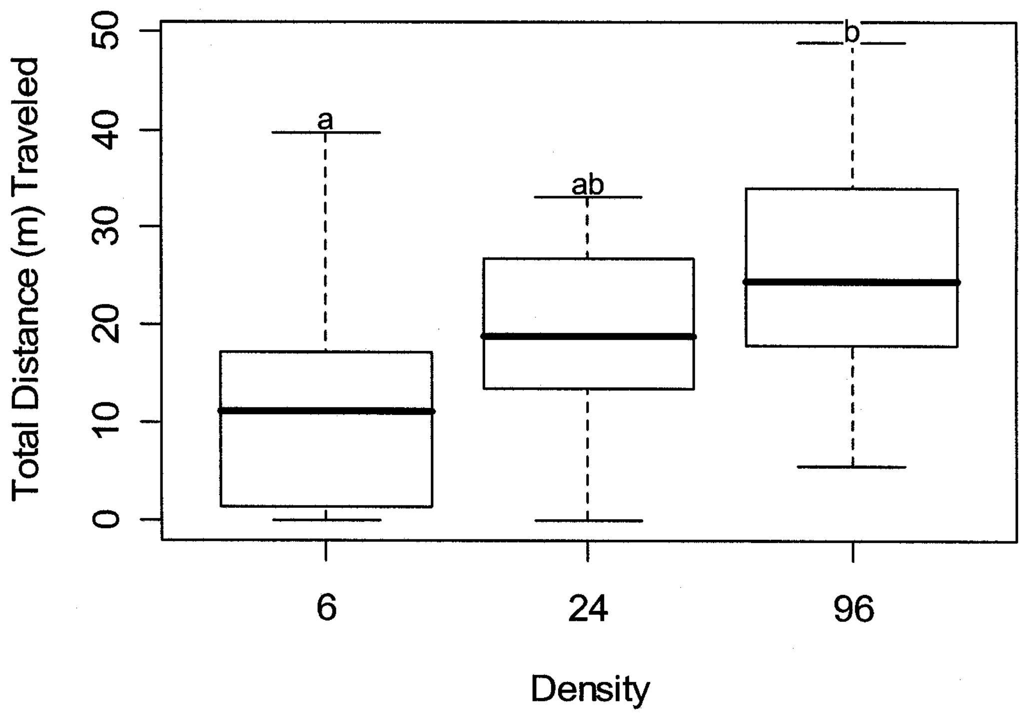

Figure 3.5: Box-plot of total distances (m) traveled by individual *Cyphocleonus achates* from a release plant for each release density in the Potted Patch trials. Boxes that have the same letter do not differ significantly from each other (Tukey's HSD).

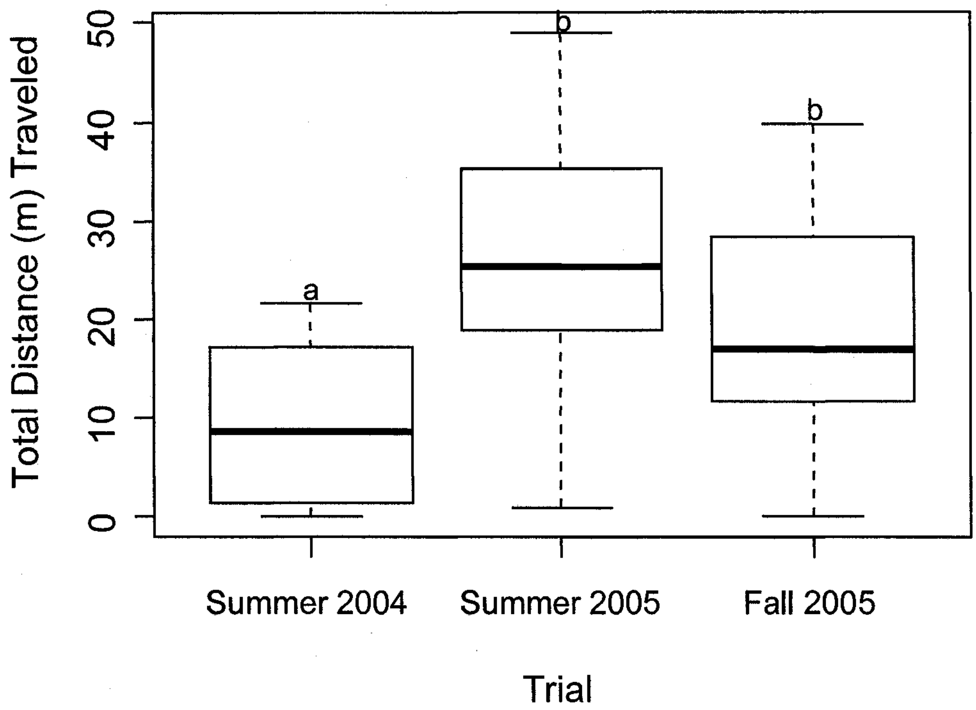

Figure 3.6: Box-pot of the total distance (m) traveled by individual *Cyphocleonus achates* from a release plant for each trial in the Potted Patch experiment. Different letters above the boxes signify significantly different means (Tukey's HSD).

There was a higher proportion of predation in the medium (24) density beetle releases when compared to both the low (6) and high (96) density beetle releases in the 2004 Summer Potted Patch trial (Figure 3.7). This is most likely due to the way in which generalist predators search for beetles in the field. At very low densities, predators do not come across beetles often and at high densities predators may be satiated before a significant proportion of beetles are eaten.

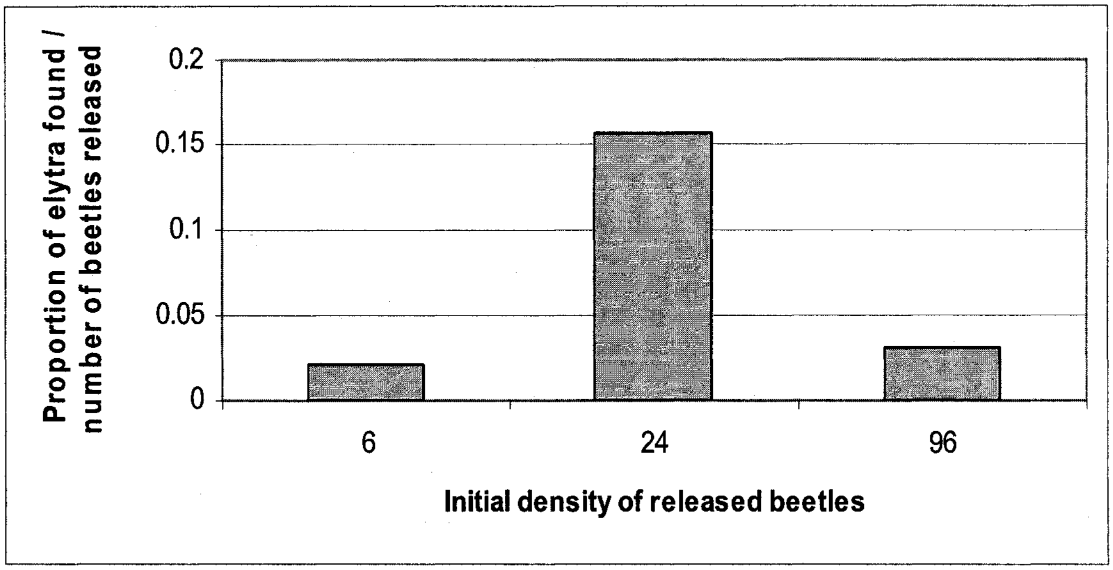

Figure 3.7: Proportion of elytra found as a function of the number of *Cyphocleonus achates* released for each density of plants in the 2004 Summer Potted trial.

Natural Patch

For the Natural Patch, there was an increasing RATE of disappearance by *C. achates* from each plant over the first three releases in the season (slope: July 28 = -0.25, Aug 4 = -0.35, Aug 11 = -0.54, Aug 18 = -0.47, Aug 25 = -0.49 and Sept 1 = -0.48). The last three weeks of releases beetles have similar disappearance RATES (Figure 3.8). This pattern suggests a change in behaviour over the season.

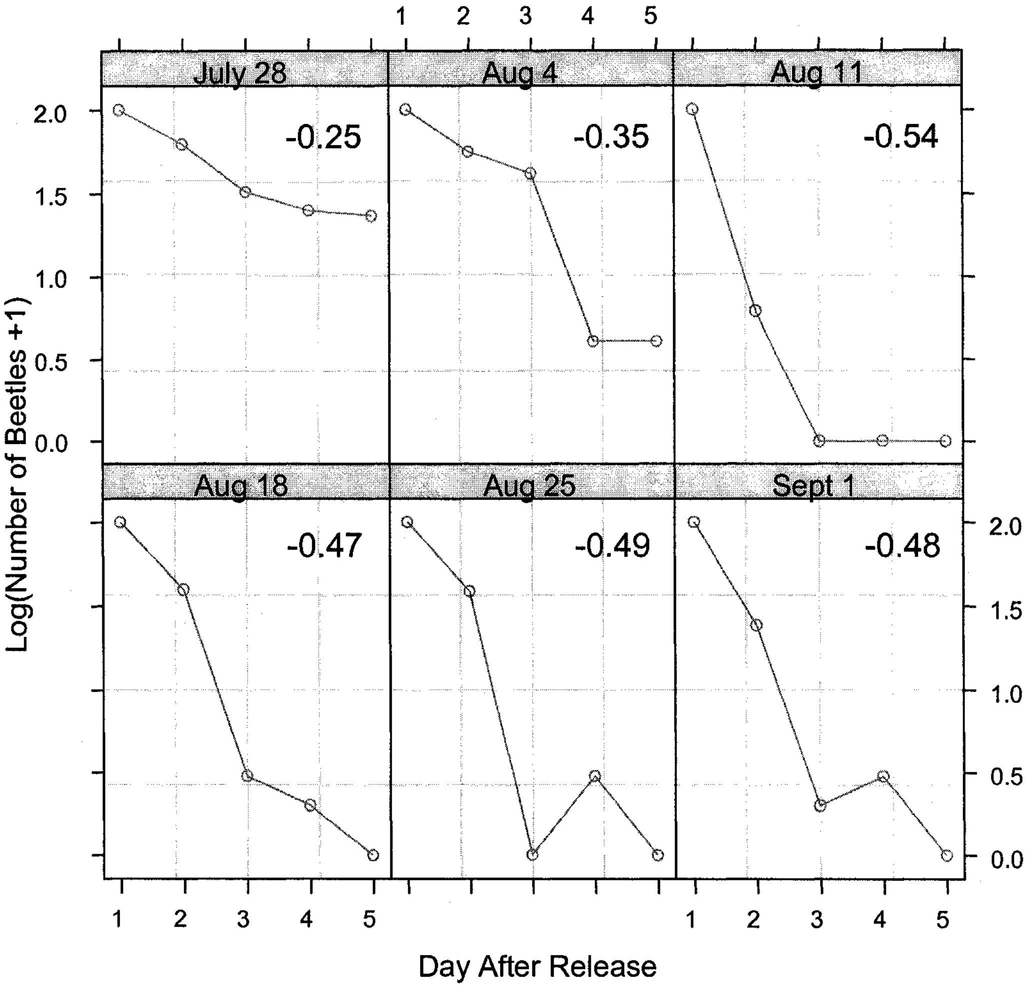

Figure 3.8: Rate of disappearance of *Cyphocleonus achates* from each release plant in the Natural Patch experiment (n=100 beetles/release). Rates are indicated by the number in each panel and are the slopes of the lines.

There was a significant effect of RELEASEDATE on the TOTAL DISTANCE beetles moved for ten days after release (F-value=35.12, p(F)<0.0001). SEX and WEIGHT of the beetles had no significant effect on TOTAL DISTANCE moved (Table 3.4). Longer distances were traveled on the last release day (31 Aug) compared to the other five release dates (Figure 3.9). Later season movement corresponds with the switch of beetle preference from the bolted plants to rosettes on this date (Figure 3.10). Monitoring of all the beetles occurred for two weeks after the releases ended (8 Sept and 15 Sept). During the last two weeks most beetles remaining in the plot spent time on the rosettes and not on bolted plants.

Table 3.4: Analysis of deviance table for TOTAL DISTANCE moved (m) from a release plant by individual *Cyphocleonus achates* as a function of the full model RELEASEDATE, WEIGHT, and SEX in the Natural Patch experiment.

Term	**df**	**Deviance**	**F-value**	**p(F)**
Null	315	10427		
RELEASEDATE	1	1053.2	35.12	< **0.0001** ***
WEIGHT	1	49.9	1.66	0.1981
SEX	1	8.3	0.28	0.5989
RELEASEDATE:WEIGHT	1	0.9	0.03	0.8599
RELEASEDATE:SEX	1	0.6	0.02	0.8917
WEIGHT:SEX	1	54.6	1.82	0.1782
RELEASEDATE:WEIGHT:SEX	1	19.2	0.64	0.4244
Residual	308	9240.3		

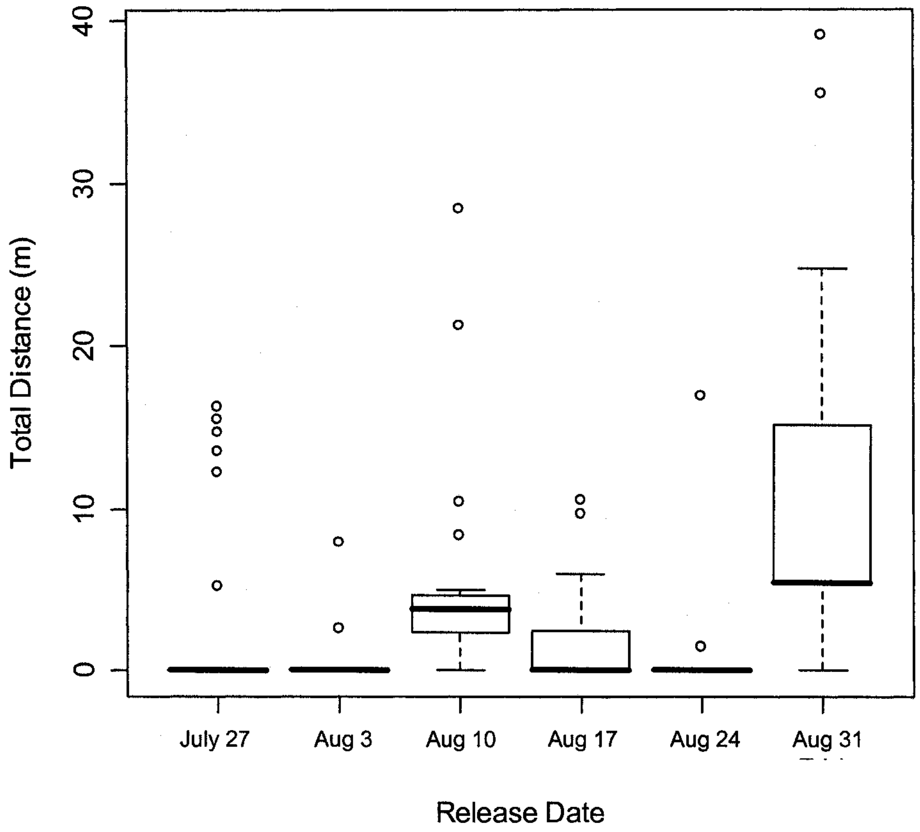

Figure 3.9: Box-plot of total distance (m) traveled by individual *Cyphocleonus achates* for each release date in the Natural Patch Experiment. One hundred beetles were released on each date. Total distances were calculated by summing all of the movelengths observed over ten days of monitoring for each beetle (N_{total}=600).

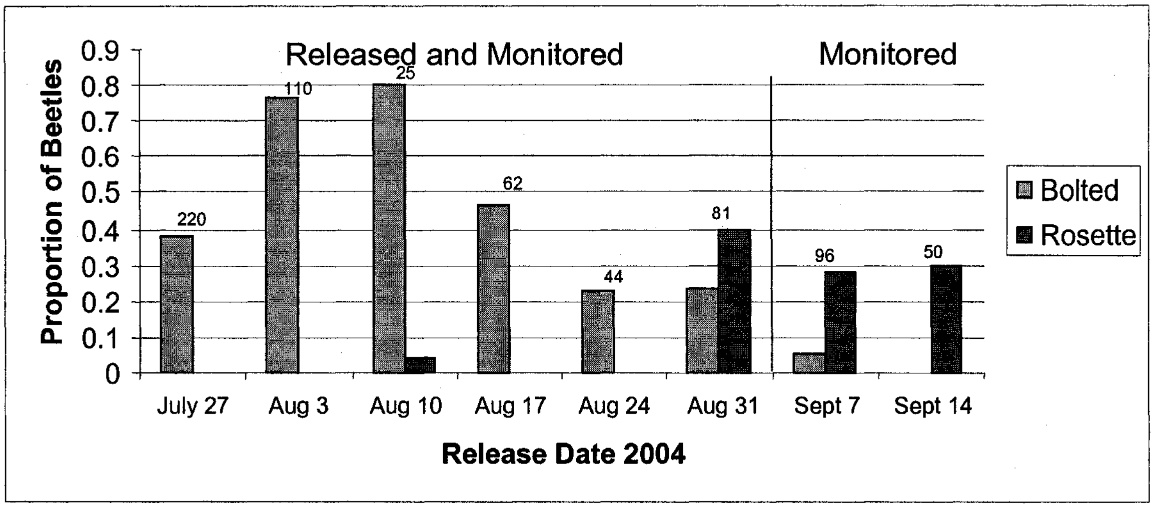

Figure 3.10: Proportion of *Cyphocleonus achates* found on bolted and rosette *Centaurea diffusa* for each release date in the Natural Patch experiment (n=100 beetles/release). The total numbers of beetles found during monitoring are above each release date. After the six release dates (27 July – 31 Aug) all of the beetles in the plot were monitored for two weeks (7 Sept and 14 Sept) and are represented by the last two bars.

3.4 Discussion

Dispersal in response to release density

Cyphocleonus achates is sensitive to high conspecific density, which triggers a dispersal response. This response is demonstrated by an increased rate of disappearance off the release plant and increased distances moved. Density-dependent factors are a common mechanism responsible for insect behaviour shifts. For mobile species, increased densities of conspecifics provide information on the suitability of the environment. For beetles that are slow moving and cryptic, increasing population densities could result in increased predation or parasitism, decreased food availability and decreased availability of oviposition sites (Harrison, 1980). These factors can lead to reduced fecundity or mortality if insects cannot respond immediately to the change in population size (Price, 1997).

Escaping detection by parasitoids and predators is important for biocontrol releases if agents are released in high densities. Specialist and generalist parasitoids have been reared from *C. achates* in Europe (Müller *et al.*, 1989) but to date, there are no reports of parasitoids in North American populations. The increased movement in response to high conspecific density could be an evolutionary remnant from European populations, but may also be an advantage for avoiding generalist predation. Generalist predators exploit high-density ephemeral resources like the artificial high densities of individuals created during biocontrol releases. Story *et al.* (1996) found rodent predation in high density rearing corrals for mass rearing of *C. achates*. I observed similar predation in my release plots. The medium density releases had the highest proportion of depredation when compared to the low and high densities. This trend would exist if predators were unable to detect beetles at low densities and if predators were saturated at sites with high-density releases. High rates of adult predation have the potential to reduce the effectiveness of the biocontrol program and even cause extirpation from the patch.

In the high density releases of *C. achates* there were an increased rate of disappearance from the release plant, which may have been caused by extensive feeding close to the release. The effect of starvation on dispersal distances has been studied extensively in insects. For example, Ferro *et al.* (1991) found that a higher percentage of unfed Colorado potato beetle (*Leptinotarsa decemlineata* Say) females on flight mills flew earlier than did fed females. Barker *et al.* (1989) further found that starvation promotes flight muscle development in *Listronotus bonariensis* Kuschel (Coleoptera: Curculionidae). Herzig (1995) also reported that a reduction in goldenrod quality, due to defoliation, caused increased dispersal by the goldenrod leaf beetle, *Trirhabda virgata* LeConte. In extreme cases, crowding results in production of winged morphs of aphids (Lees, 1967) and planthoppers (Denno, 1985) increasing dispersal potential of these individuals.

At high densities, oviposition sites are likely limiting for *C. achates*. Only one or two larvae can survive to adulthood in any single rosette root (Stinson *et al.*, 1994), thus being able to judge conspecific densities would be beneficial for *C. achates*. Beetles that experience limiting oviposition sites must be able to trade off between staying in the patch and risking larval mortality from overexploiting resources or reduced fecundity because of dispersal. I observed higher rates of movement off release plants and longer distances moved at high densities, suggesting that beetles are ovipositing further from the release plant than they do at low densities. Beetles such as female *T. virgata* also exhibit oviposition choice, laying fewer eggs when fed conspecific grazed plants than when fed whole plants (Herzig, 1995).

Releasing *C. achates* at high densities will encourage dispersal from the release plant, which has several advantages. High-density movement may reduce predation or environmental stochasticity by spreading agents throughout the patch. Spreading agents out evenly means that more plants can be visited and impact on the weed will be more extensive. High-density releases also increase the number of beetles in the patch. These releases directly increase the beetle population within the patch, which can contribute to a more rapid population increase over years. If population densities of the agent can be increased rapidly, impact will be observable earlier and biocontrol success is more likely.

Dispersal in response to time of release

Releases later in the season resulted in longer movement distances and a faster rate of movement off release plants compared to releases early in the season. This pattern is accompanied by a switch in knapweed life stage preference by the beetles, from bolted plants in the summer to the rosettes in the fall. Changing preference between plant types is likely related to oviposition because *C. achates* must oviposit on rosettes. This late season oviposition behaviour could be correlated with egg maturation. Although I did not dissect beetles for eggs in my study, Stinson *et al.* (1994) reported that laboratory beetles begin laying eggs 14-20 days after emergence and VanHezewijk (2006, personal communication) found no eggs in dissected field-collected beetles until the third week of August.

Storage in cool temperatures was intended to keep beetles at similar day-degree ages across all release dates and therefore aging related processes such as egg maturation did not trigger the switch in beetle preference from bolted to rosette plants. Researchers have suggested that beetles stay on bolted plants after release because they are maturing eggs, calling mates or basking to harden the cuticle (Stinson *et al.*, 1994; Story *et al.*, 2006). Instead, I suggest that beetles could be using environmental cues such as photoperiod, senescing bolted plants or changes in physiology of rosettes as cues to increase dispersal and move to rosette plants to oviposit. This delay could be caused by the need to synchronize larval development with rosette growth. *Cyphocleonus achates* larvae are not dependent on a diapause to complete development (Goodman *et al.*, 2006). Thus, adult females could delay laying eggs, and ensure that larvae remain as small first or second instars over winter and complete development in spring. Early development of the larvae to the large, feeding third and fourth instars could lead to larval starvation during the winter months because the plant itself would not provide sufficient food for the larvae.

Summary

Cyphocleonus achates was found to increase dispersal when released at high densities or later in the season. This beetle also moves to rosettes later in the season. The delay in oviposition has implications for biocontrol releases because there is a greater risk of environmental stochasticity and predation wiping out a release population when the individuals do not move from the release plant for an extended period. Managers using *C. achates* to control knapweed infestations would benefit from releasing beetles at high densities, late in August to early September when beetles are ready to lay eggs. Releasing later in the season and at high densities will promote movement away from the release having the added effect of reducing predation and environmental stochasticity.

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Chapter 4

General Conclusions

Dispersal is an often overlooked, but important aspect of a biocontrol agent's ability to control weeds in a new environment. I used individual mark-recapture (IMR) techniques in field and laboratory studies to determine the factors affecting the movement of *Cyphocleonus achates* and identified what release strategies would increase the dispersal of the beetle in the field. In Chapter 2, I test the methodology of IMR and describe movement of the beetles in response to beetle and plant traits. In Chapter 3, I determine the effect of release density and date of release on *C. achates* movement.

There were no significant effects of the IMR techniques on the lifespan or predation of the beetles in the field. However, in the laboratory bioassay there were decreasing distances moved by *C. achates* over time (Figure 2.8) which can be attributed to the storage methods. This finding was potentially problematic because the time of release experiment in Chapter 3 assumed that storing and cooling beetles did not change their dispersal behaviour. In Chapter 3, however, the beetles were found to have increasing movement distances over time (Figure 3.10). Therefore, the decreasing movement of the beetles after storage observed in Chapter 2 had a minimal effect on the beetles compared to the increasing distances moved in Chapter 3.

Dispersal resulting from high-densities may occur because individuals sense the number of conspecifics directly (i.e. through mechanical stimulation) or indirectly (i.e. through frass densities) rather than from reduced resources such as food or oviposition sites. I found no significant increase of movement distances when frass densities were increased (Chapter 2), however, a trend of longer movement distances when exposed to higher densities of frass was observed. Unfortunately, this was not a significant trend. There were, however, a large number of zero movements for each frass density treatment and a significant pattern may emerge if tested again with a larger number of beetles. In Chapter 3, I found a similar trend of increasing dispersal rates at when beetle densities increased. This trend was significant. The results from Chapter 2 and 3 suggest that beetles could be sensing the increased density of conspecifics through frass but further experiments are needed.

This research provides empirical evidence that *C. achates* is a relatively sedentary beetle which agrees with previous studies that assessed dispersal capability indirectly. Researchers have theorized that flightlessness is the key to the sedentary nature of *C. achates* (Stinson *et al.*, 1994; Story *et al.*, 1996). However, other flightless insects have been found to move longer distances than *C. achates* and beetles that are capable of flight have been found to travel similar distances as *C. achates,* in good habitat. For example, the flightless bush cricket, *Pholidoptera griseoaptera* De Geer , disperses an average of 7.5m/day (Diekötter *et al.*, 2005), which is much longer than *C. achates* dispersal distances of 0.27m/day in the Natural Patch. The flight capable Colorado potato beetle has a mean inter-patch movement distance of 0.31m/day (Schmera *et al.*, 2006), which is similar to the Natural Patch estimate of 0.27m/day. Therefore, the differences between beetle movement distances may have less to do with flight capability and more to do with finding high quality hosts. There is further evidence for this in my experiments. I found lower total distances moved in the Natural Patch (0.27m/day) when compared to the Potted Patches (2.32m/day). The Potted and Natural patches were similar in plot size but differed dramatically in plant density. The Natural Patch contained 324 bolted plants and numerous rosettes compared to the Potted Patch, which only contained 36 (in 2004) 54 (in 2005) rosette plants. Having lower densities of plants may force *C. achates* to increase movement distances to find appropriate oviposition sites or food due to the increased competition for plants in the Potted Patch.

Implications for Population Biology

The individual's ability to disperse has potentially a great impact on metapopulation dynamics and persistence of the population in its new environment (Hastings and Harrison, 1994; Gyllenberg and Hanski, 1997). Large or small amounts of dispersal can result in different outcomes for both establishment and spread of the biocontrol agent and impact on the target plant. Biological control agents that disperse widely can distribute themselves in a patchy manner where the agent inhabits only a small percentage of the host population at any one time (Lewis and Pacala, 2000). Host plants in this situation reproduce unchecked and although the impact of the agent may be noticeable in occupied plants or patches, there is no reduction in population growth of the pest species at a larger scale. In contrast, agents that have limited means of dispersal may be excluded from larger population structures such as metapopulations, which exhibit the rescue effect

(Brown and Kodric-Brown, 1977). The rescue effect can facilitate population persistence because beetles can immigrate into vacant patches after beetles are extirpated. Without this immigration, beetle populations may be extirpated more easily than in a system where the agent has the ability to disperse between patches (Hanski, 1999). Weed managers can create this effect by choosing to manually spread the agent to new patches. This can ensure spread and persistence of the agent in a landscape, but at a substantial economic cost.

A concern in all IMR studies is the choice of scale. Dispersal distances may be affected when plot sizes change due to the distance between the furthest monitored plants. Schneider (2003) found that increasing the spatial scale of the study results in a correlated increase in mean movement distances recorded for butterflies. In my experiments, the difference in total distance moved between the Potted Patch trial in 2004 versus the 2005 trials can be attributed to the larger plot size in 2005. When patch sizes increased from 90m by 50m in 2004 to 150m by 50m in 2005, beetle dispersal distances also increased. Further experiments at multiple scales are required to confirm if this is biologically significant for *C. achates* or an artifact of the scale choice.

Implications for Biocontrol

The trade off between dispersal and fecundity is well documented in many insect species (Gu *et al.*, 2006). If *C. achates* is sensitive to this trade off, beetles would stay in an area of high knapweed densities until high beetle populations within a patch triggers dispersal. This may increase biocontrol success rates because the limited dispersal of *C. achates* has the effect of keeping beetles in the patch and more individuals would be available to feed and oviposit on plants. However, due to the high densities of knapweed in North American stands, beetle populations may be slow to increase to the very high densities needed before land managers observe impact. To supplement this, land managers may find they have better success by releasing beetles yearly on the same patch, artificially increasing *C. achates* populations.

Delayed oviposition behaviour in *C. achates* can reduce the potential fecundity of beetles in a knapweed patch. This behaviour exposes beetles to greater predation and mortality before oviposition can occur. While the reason behind this delay is unclear, I suggest that it may be caused by a need to synchronize larval development with the appropriate condition of rosettes. The cues beetles use to determine an appropriate time to oviposit need further investigation. This can include observations of beetle oviposition under differing photoperiods, seasonal temperatures or rosette ages.

Biocontrol managers can use the information presented in this thesis to modify releases to increase dispersal away from the point of release, in order to increase chances of establishment and reduce predation of the beetles. To do this, choosing either many small releases or one large release, will increase the proportion of beetles available to oviposit in the field. Because I found that beetles only move between 0.3 – 2.3m/day within a patch, managers may find that releasing several groups of 100 beetles on larger continuous patches will result in a rapid, even distribution of *C. achates* throughout the patch.

Future Research

Knowledge about the dispersal of both the invasive plant and the biocontrol agents involved is useful in developing management practices and can be used to predict spread or impact (Stinner *et al.*, 1983; Cronin *et al.*, 1999). Calculating a rate of spread of *C. achates* to neighbouring patches would be useful for predicting where releases should be spaced in the landscape. Biocontrol managers are currently redistributing beetles to unoccupied patches in British Columbia and Montana. From an estimate of immigration, the distribution of these releases can be planned so that overlapping releases or missed patches in the landscape is reduced. In addition, calculating the spread of knapweed on a landscape scale would also be useful to determine if the beetle can keep pace with knapweed spread. If these slowly dispersing beetles do not have a similar rate of spread as the weed, steps must be taken to increase dispersal in the patch, or other control agents with the ability to disperse between patches, should be considered to work in concurrence with *C. achates*.

The results presented adequately describe movement of *C. achates* within 10 days after a release. However, my results do not describe how adult beetles disperse after establishment on a patch. Beetle movement may be quite different when beetle densities per plant are lower than they are immediately after release and this will be important with respect to intra-patch movement and dispersal between patches. To understand dispersal between patches and within the patch once beetles are released, beetle movement at low densities should be investigated.

In North America, we may find that *C. achates* are more sedentary than their Eurasian counterparts. When beetles disperse in North America, knapweed patches are continuous and densities of plants are high, thus beetles have numerous plants to feed and oviposit on. Wikeem *et al.* (1999) found that patch-wise larval densities in British Columbia are well below the carrying capacity of knapweed roots. I found that higher densities of *C. achates* within a patch will result in higher dispersal distances and rates of dispersal. To predict the outcome of very high density beetle patches, beetle dispersal in response to higher conspecific densities should be assessed.

In Chapter 3, I determined that density and time of release caused increased dispersal. The next step is to determine whether these release strategies affect the actual impact of the agent on the knapweed patches. In particular, given that *C. achates* move more in response to higher density and later season releases, tests should be done to determine whether increased dispersal translates into increased larval densities or knapweed mortality.

Summary

The importance of studying dispersal in biocontrol agents can not be overlooked. Dispersal data not only describes insect movement but also can be used to elucidate behaviour, which may provide insights into the biology of the insect. In this thesis, I found unique behaviours exhibited by *C. achates* in response to its environment and give suggestions that may improve release strategies. The next step is to predict spread and distribution of the beetles in a landscape and to determine if *C. achates* dispersal is comparable to its host, *C. diffusa,* for long-term control.

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Appendix

The rate of disappearance off the release plant was parameterized for each release density in the Potted Patch trials to use in Chapter 3. The rate of disappearance of beetles was highest for plants with the highest release density (96 beetles) relative to the medium and low release densities (slope = -0.26, -0.13, -0.15; for Summer 2004, Summer 2005 and Fall 2005 respectively). The disappearance rates for the medium release density (24 beetles) were -0.15, -0.06, -0.1 and for the low release densities (6 beetles) were -0.08, -0.03, -0.05 for Summer 2004, Summer 2005 and Fall 2005 respectively. The slopes for the target plants (0 beetle releases) had a positive slope, which demonstrates that *C. achates* is moving off of the release plants over time (Figures A, B and C).

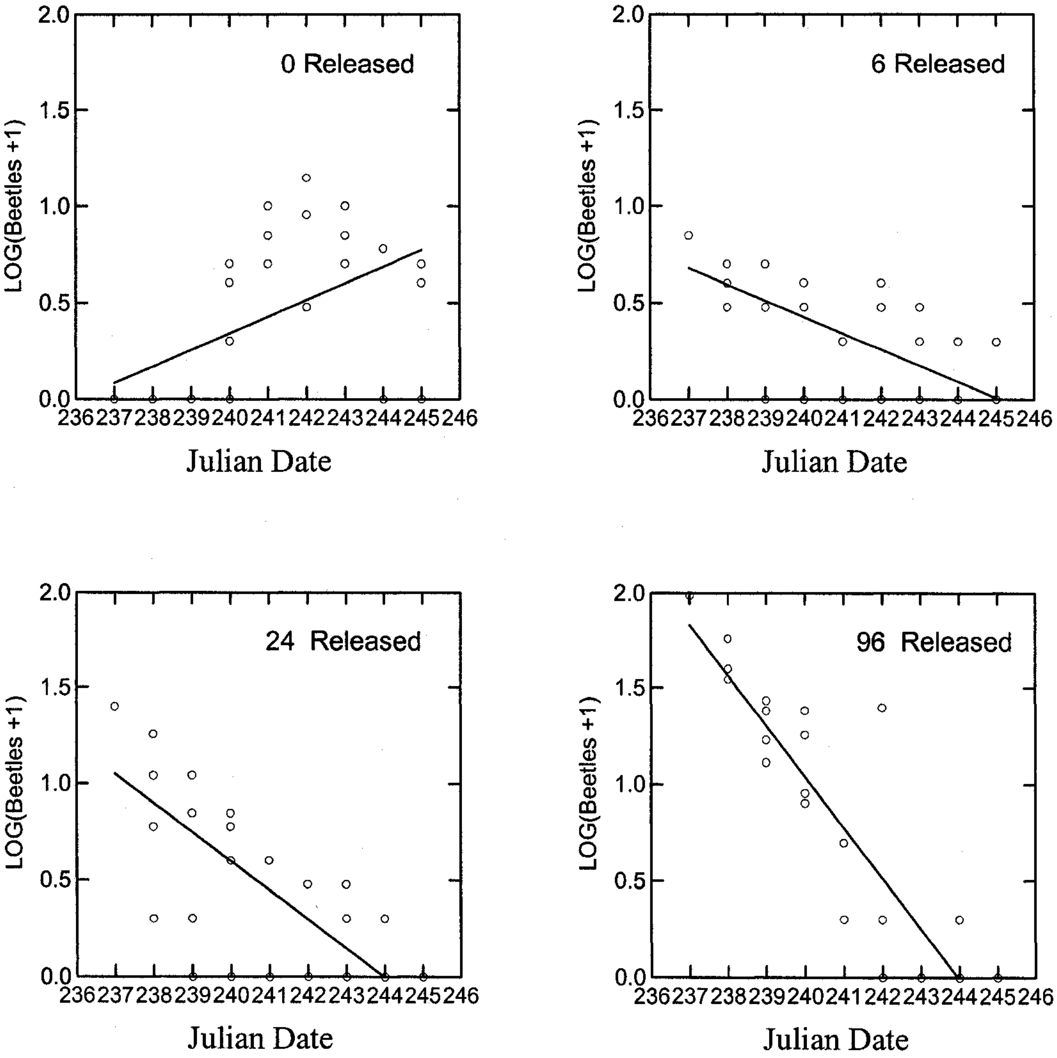

Figure A: Number of *Cyphocleonus achates* on *Centaurea diffusa* plants in the Summer 2004 Potted Patch trial from 24 August – 1 September 2004 ($n_{\text{release plants}}$=4/day: $n_{\text{target plants}}$=8/day (0 beetles released)).

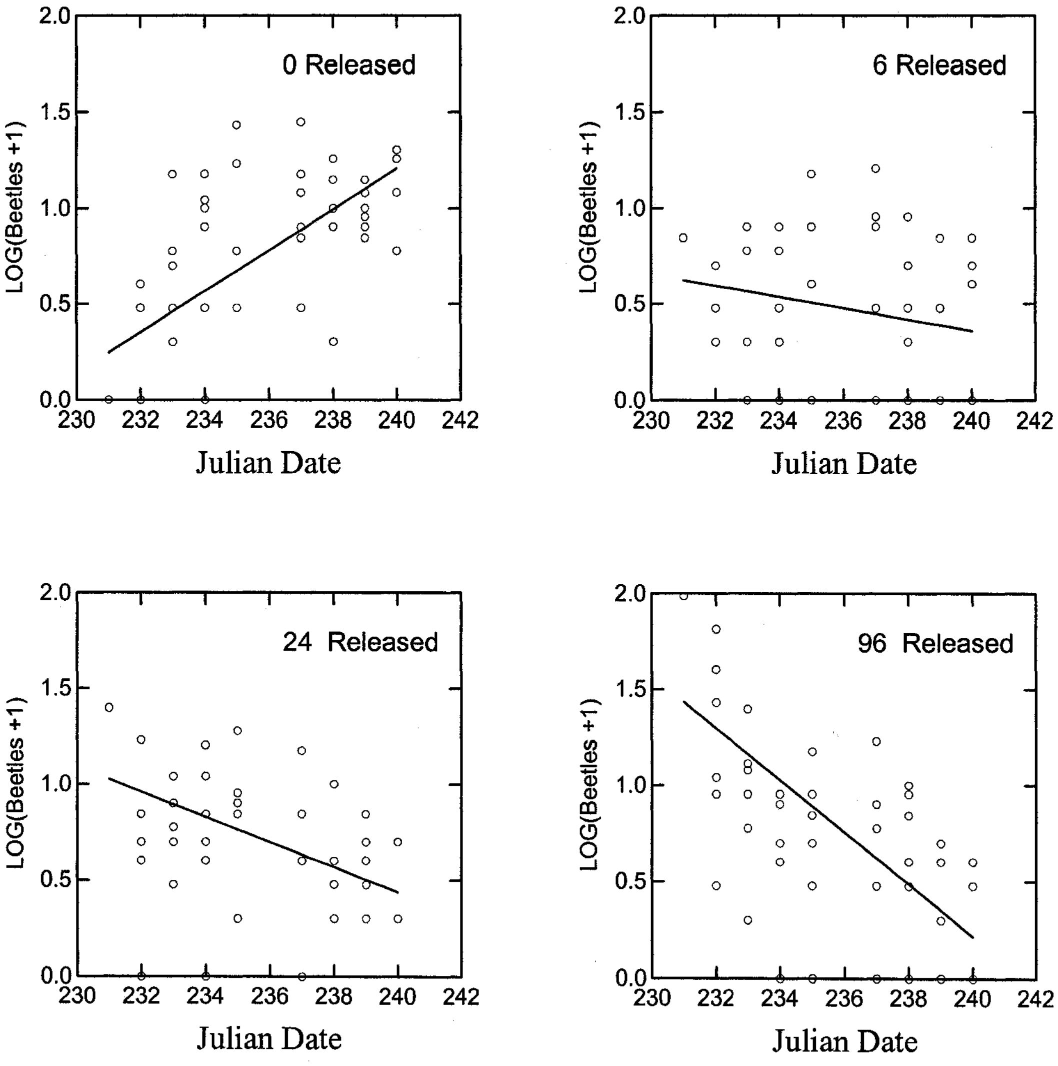

Figure B: Number of *Cyphocleonus achates* on *Centaurea diffusa* plants in the Summer 2005 Potted Patch trial from 19 - 28 August 2005 ($n_{\text{release plants}}$=6/day: $n_{\text{target plants}}$=12/day (0 beetles released)).

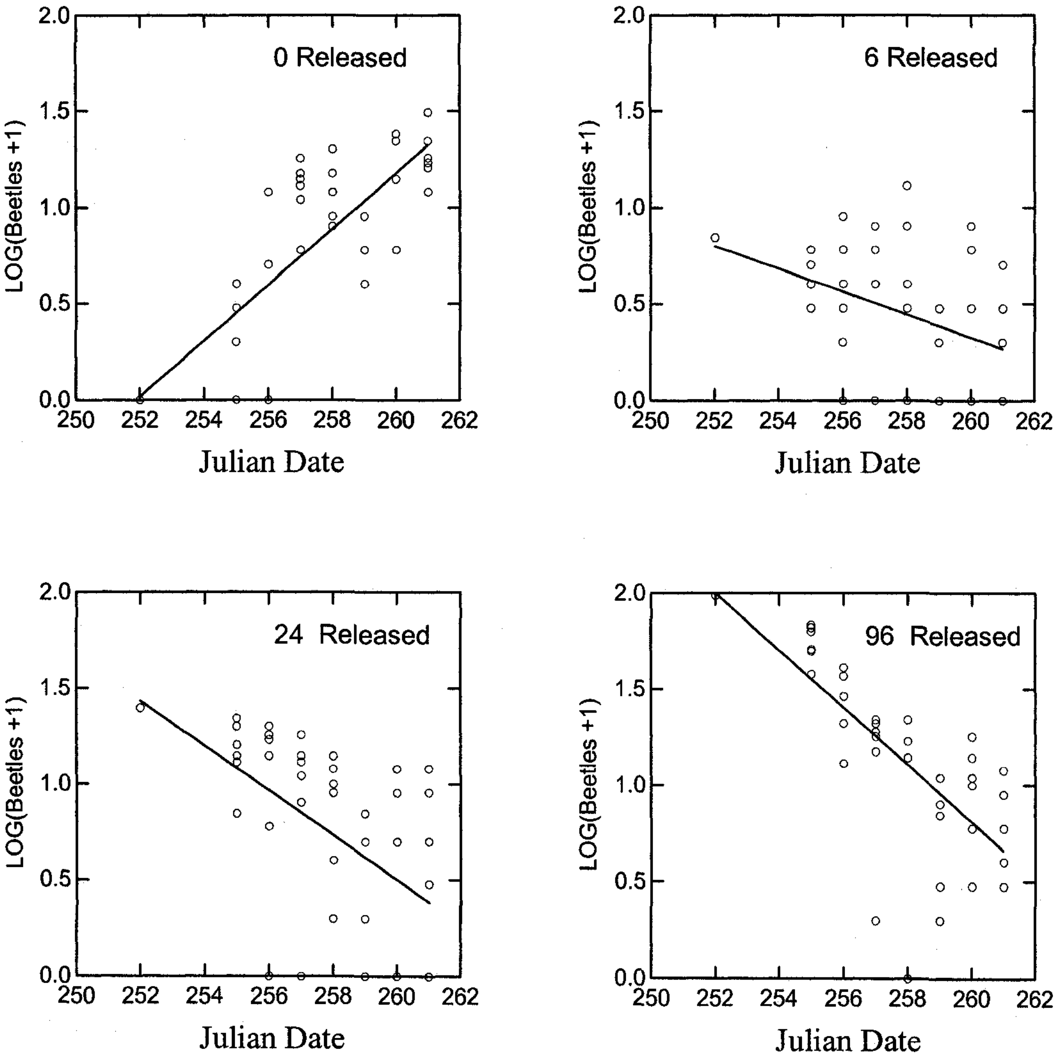

Figure C: Number of *Cyphocleonus achates* on *Centaurea diffusa* plants in the Fall 2005 Potted Patch trial from 9 – 18 September 2005 ($n_{\text{release plants}}$=6/day: $n_{\text{target plants}}$=12/day (0 beetles released)).