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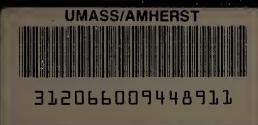
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## LARVAL DISTRIBUTION AND ADULT ACTIVITY OF THE CRANBERRY ROOT GRUB, <u>LICHNANTHE VULPINA</u> (HENTZ) (COLEOPTERA: SCARABAEIDAE)

A Thesis Presented

By

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Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of

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Department of Entomology

## LARVAL DISTRIBUTION AND ADULT ACTIVITY OF THE CRANBERRY ROOT GRUB, <u>LICHNANTHE VULPINA</u> (HENTZ) (COLEOPTERA: SCARABAEIDAE)

A THESIS PRESENTED

BY

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#### **CHAPTER 1**

#### **OVERVIEW OF LITERATURE**

#### **Overview of Literature on Lichnanthe vulpina**

The larvae of the cranberry root grub (CRG), <u>Lichnanthe vulpina</u> (Hentz) are major pests of commercial cranberry (<u>Vaccinium macrocarpon</u> Aiton) beds in Eastern Massachusetts. Larvae feed on the fine roots of the plant, often denuding the main roots so that the vines may be easily lifted and rolled back like a carpet (Franklin, 1950). Franklin (1919) described an infestation as typically being confined to areas within a bed, seldom exceeding 75 - 100 m<sup>2</sup>. The vines do not grow well, look sickly, and do not produce much fruit. Occasionally, the damage is so severe the vines are killed and these weakened or dead areas are usually overtaken by weeds (Franklin, 1950). After an infestation was controlled using methods that are no longer legal, the weakened vines then took two to three years to recover.

In the 1930's, the recommended control measure for CRG was an application of sodium cyanide, which killed a satisfactory percentage within 16 hours (Franklin, 1931). In 1948, there were approximately 15,000 acres of cranberry bogs in Massachusetts that grossed \$16,000,000 (Beattie, 1948). It was also estimated that 42% of this acreage was infested to some degree with CRG. By 1948, there was a "Root Grub Campaign" in place and the Extension Service acquainted growers with control measures. Later, dieldrin (a cyclodiene) was used to control CRG, but this compound was banned in 1972. Now, no control measures are available and populations are re-establishing on many beds. In the summers of 1994 and 1995, a survey of 38 potential grub infestations in 13 towns in

southeastern Massachusetts was conducted (Dunn and Averill, 1996). Grubs were found in 33 of the 38 sites and of these 33 sites, CRG was found at 20 sites in 10 towns. In Carver, all of the grub-infested sites that were surveyed (10) contained CRG.

CRG larvae range in length from 0.5 cm to 3.0 cm depending upon the instar and are flattened dorso-ventrally. The head is deep reddish-brown and the body is covered with fine reddish-brown hair. Laterally, the hair is in tufts and is longer in comparison to the dorsum or venter (Franklin 1950). Adult males are 12.9 mm to 16.0 mm in length and are apparent bee mimics. Their dorsum, except elytra, and their ventral surfaces are clothed with long yellow-orange setae. Females are 13.0 mm to 17.5 mm with the same setation as males, except the color is slightly lighter (Carlson, 1980).

Franklin (1950) described the activity of adult beetles on commercial cranberry beds as follows. The beetles emerge in middle or late June until late July. The adults were never observed feeding on cranberry foliage or fruit. On occasional mornings, the adults emerge on the bed and males can be found flying in great numbers. The females apparently come out primarily to mate and are rarely seen flying. When a female emerges, she is soon found by a male and mating begins. After mating, the beetles generally go back into the soil before noon.

Aside from work done by Franklin, there is little data on the habits of this insect. Some notes were published in 1883 in the Bulletin of the Brooklyn Entomological Society describing the habits of adults not found on cranberries. Blanchard (1883) reported seeing considerable numbers of adults in Lowell, Massachusetts flying at 0500 h over a sandy

area bordering the Merrimac River. He also noted adults clinging to tall grass. Hentz (1883) reported adults resting on flowers and visiting the ground frequently.

Similar to all species of Scarabaeidae, cranberry root grub undergoes three larval instars followed by a prepupal stage. The prepupa occupies an earthen cell prepared by the larva (Tashiro, 1987). In the case of CRG, the prepupa forms in mid-May and pupation occurs in early June (Franklin, 1950). Franklin (1950) also noted that CRG larvae require four, or sometimes five years (depending upon feeding conditions), to complete development. This lengthy generation time is on the high end of the range of time cited by Tashiro (1987) for various turfgrass scarab species which have either two generations a year, one a year, or require two to four years to complete a generation. Of those species requiring multiple years, a three-year life cycle is the most common.

#### **Studies of Scarabaeid Larvae**

Spatial distribution is one of the most characteristic ecological properties of a species and no program of field sampling is viable without an understanding of an organism's underlying distribution (Taylor, 1984). In insects, if the life cycle is known, preliminary work is necessary to gain some knowledge of the distribution of the species (Southwood, 1966). The spatial distribution for some turf-infesting species has been determined (Ng, et. al. 1983a; Burrage and Gyrisco 1954) including control decision rules for sampling of Japanese beetle larvae, <u>Popillia japonica</u> (Newman) (Ng et. al. 1983b). The scarabaeid pests of sugarcane in south Queensland, <u>Antitrogus parvulus</u> Britton, <u>Lepidiota crinita</u> Brenske and <u>L. negatoria</u> Blackburn have been studied by Cherry and Allsopp (1991) and sequential sampling plans were developed for these species plus

<u>Antitrogus mussoni</u> (Blackburn) <u>Lepidiota noxia</u> Britton, and <u>L. picticollis</u> Lea (Allsopp and Bull, 1989). Cherry (1984) did a similar study for two scarabs pests, <u>Cylclocephala</u> <u>parallela</u> Casey and <u>Ligyrus subtropicus</u> Blatchley in Florida sugar cane.

Some of the factors influencing scarab larvae distributions have been established. Scarab larvae are capable of burrowing through the soil at rates up to 11 cm per day (Fellin, 1986) and are influenced by soil moisture and temperature (Fellin, 1986; Villani and Wright 1988). Cherry and Allsopp (1991) determined that soil texture was responsible for the distribution of the two species of sugarcane scarabaeids. Villani and Nyrop (1991) used radiographic techniques to study the influences of gravity, host plant position, external disturbances, and age dependent movement patterns of P. japonica and the European chafer, Rhizotrogus (Amphimallon) majalis (Razoumowsky) larvae. They found that all stages of R. majalis larvae displayed random vertical movement with some arrestment in or near sod. Second instar P. japonica larvae behaved similarly to R. majalis larvae. However, third instar P. japonica larvae displayed an innate downward movement in the soil microcosms, except those tested in late winter or early spring. Third instar larvae tested in late winter showed random movement with some arrestment in sod and those tested in early spring exhibited upward movement and arrestment in sod. In cranberry, L. vulpina grubs are found within 8 to 10 cm of the surface in late spring or early summer. As the summer progresses and the bog becomes drier, the grubs go deeper and may be found 20 to 25 cm from the surface and do not approach the surface again until the bed is wet (Franklin, 1950).

The effect of soil moisture has been studied extensively in scarabaeid biology and has been shown to influence oviposition as well as the survival of eggs and first instar larvae. Eggs of the southern masked chafer, Cyclocephala immaculata Olivier, require soil moistures of 12.5 % or more to develop normally. Oviposition in soil moisture gradients showed the depth that eggs are laid can vary in response to moisture levels (Potter, 1983). Eggs and first instar P. japonica were found to be resistant to extreme moisture conditions in various types of soils and delays in egg development were observed in saturated soils (Régnière et. al. 1981). P. japonica did not oviposit in soils with less than 5% moisture, and in choice tests, laid more eggs in wetter soil. The rose chafer, Macrodactylus subspinosus (F.) exhibited the same preferences (Allsopp et. al. 1992). Phyllophaga crinita (Burmeister) adults did not oviposit in very wet or very dry soils. Eggs and larvae survived in a wide range of soil moistures, but survival was poor in very wet or very dry conditions (Gaylor and Frankie, 1979). Oviposition and survivorship of two species of sugarcane grubs were also influenced by soil moisture. Oviposition rates were best at the midrange of soil moistures and approached zero at moisture extremes. Tests showed a greater flood tolerance in third instar C. parallela Casey when compared to L. subtropicus (Blatchley) (Cherry et. al. 1990). Cranberries require irrigation on a regular basis, which makes the beds an ideal habitat for the development and survival of scarab larvae.

#### **Studies of Scarabaeid Adults**

The seasonal and within-day periods of adult activity have been established for some species of scarabaeidae. Tashiro (1987) described the adult activity patterns of many turfgrass species, most of which are nocturnal. Asiatic garden beetles, <u>Maladera</u>

castanea (Arrow), begin emerging in the third week of June at dusk when temperatures are  $18 - 21^{\circ}$ C and adults become very active when temperatures exceed  $21^{\circ}$ C.

Beginning in the second or third week of June, R. majalis emerge from the ground at about 2030 h with peak activity around 2100 hours. May or June beetles (Phyllophaga) emerge around dusk particularly when the day is warm and cloudy and the temperature is above 15.5°C. Oriental beetles, Anomala orientalis (Waterhouse), emerge from late June into August and Facundo (1994) found peak activity occurs around sunset. Flights of the northern masked chafer, Cyclocephala borealis Arrow, begin in early to mid-June with the flights of southern masked chafer occurring at least one week later. The activity period for the southern masked chafer, C. immaculata begins at dusk and ends about 2 hours later, while peak activity for northern masked chafer occurs between midnight and 0400 hours (Potter, 1980). On warm evenings, the black turfgrass ataenius, Ataenius spretulus (Haldeman), is active between 1600 and 1800 h and light precipitation just before or during this period increases activity (Tashiro, 1987). There are two generations per year in some areas. The first emergence occurs in late June to early July; second generation emergence occurs in August (Wegner and Niemczyk, 1981).

For day-active scarabs, the most notorious is <u>P. japonica</u>. In the northeast, emergence begins the last week of June and continues through September. In the early morning, beetles usually sit on plants until the air temperature reaches about 21°C, at which time the beetles begin flight (Fleming, 1972). Green June beetles, <u>Cotinis nitida</u> L., in Virginia begin emerging around mid-June and disappear by the first week in September.

The females appear around daybreak and settle in the grass. Flying males are seen by 0700 h and only an occasional beetle is observed by afternoon (Tashiro, 1987).

The effects of environmental conditions on the activity of adult scarabs have also been studied. Many different factors have been found to correlate with flight patterns, and often in combinations. As examples, studies of two species of <u>Phyllophaga</u> showed that air temperature and light intensity influenced the nocturnal flight activity patterns of adults (Guppy, 1982). Gaylor and Frankie (1979) found that adult flights of <u>P. crinita</u> were closely related to rainfall patterns. For <u>P. japonica</u>, there is little flight activity on cloudy days, windy days, and no flight on rainy days. A passing cloud caused a beetle in flight to seek a resting place immediately (Fleming, 1972). Potter (1981) found that <u>C. immaculata</u> and <u>C. borealis</u> emergence was influenced by rainfall patterns as well as air and soil temperatures.

In addition to the damage caused by feeding larvae, some species are also pests as adults. <u>P. japonica</u> is known to feed on over 280 species of plants in the United States (Vittum, 1986), and is an important pest on many of these. For example, adults can cause extensive damage on the foliage of fruit and shade trees, the epidermis and branches of asparagus, the maturing silk of corn plants and also attack the fruit of early ripening varieties of apples and peaches (Fleming, 1972). Some species of May or June beetle (<u>Phyllophaga</u>) adults are serious pests of shade trees and where these species are abundant, adults can defoliate entire trees. The favored host trees include oak, hickory, walnut, elm and poplar (Tashiro, 1987). <u>C. nitida</u> adults injure many thin-skinned fruits, especially figs, peaches and grapes (Tashiro, 1987). <u>M. castanea</u> adults are a pest of some

ornamental shrubs, flowers and vegetables. When feeding is heavy, entire leaves may be eaten, leaving only the midrib (Hallock, 1933; Tashiro, 1987). Adult <u>A. orientalis</u> occasionally cause damage by feeding on the flowers of some plants including roses, hollyhock, phlox, and dahlias, but feeding is never extensive enough to cause appreciable injury (Hallock, 1933).

The presence of a female sex pheromone has been demonstrated for several species of Scarabaeidae including <u>C. nitida</u> (Domek and Johnson, 1987), <u>A. orientalis</u> (Zhang, 1994), <u>P. japonica</u> (Ladd, 1970), <u>Costelytra zealandica</u> (White) (Henzell and Lowe, 1970), <u>C. immaculata</u> and <u>C. borealis</u> (Potter, 1980) and CRG (A. Zhang, personal communication; O'Donnell, unpublished data). In addition to the long-range female sex pheromone, CRG males also walk excitedly when contacting extracts of the female body on a Kimwipes<sup>®</sup> tissue, which suggests the presence of a contact pheromone as well (P. Robbins, personal communication).

The mating behavior of some scarabs has been documented. In the turf-infesting species, it is typical that pheromone-producing females are located by flying males and then mating takes place. However, some species exhibit unique behaviors. For example, virgin female <u>P. japonica</u> are very attractive to males, sometimes resulting in an army of males being attracted to a single female. The congregation of beetles resembles an animated ball and consists of a single female and 25 to 200 males (Fleming, 1972). <u>R. majalis</u> emerge from the ground and fly to a tree or other silhouetted object such as a bush, light pole or chimney. The beetles swarm around this object in great numbers, peaking around 1700 h and mating takes place as soon as the beetles come to rest. After

approximately one hour, mating pairs and individuals begin falling from the tree and flights to the ground continue throughout the night (Tashiro, 1987). In Tasmania, the adult pasture beetle, <u>Scitala sericans</u> Erichson, flies to trees around 1950 h. Females land on the limbs and crawl to the edge of a leaf where they remain motionless, attached only by their forelegs. Males landed on the branches, crawled to the female and mated (Hardy, 1976).

#### **Research Objectives**

In order to control a pest insect effectively, an understanding of its ecology and biology is needed if control programs are to be developed and implemented (Pedigo, 1989). In this study of <u>Lichnanthe vulpina</u>, I describe the distribution of larvae within cranberry beds and possible factors influencing the distribution are explored and discussed. The within-day and seasonal activity periods of adults were also determined on commercial cranberry beds as well as on an abandoned bed, which no longer had any cranberry vines. Further, the possible influence of environmental factors on emergence periods was examined. Finally, systematic observations of males and females were carried out to quantify adult behavior. This comprises the first quantitative study of the habits of this insect.

#### **CHAPTER 2**

## LARVAL DISTRIBUTION OF THE CRANBERRY ROOT GRUB, <u>LICHNANTHE VULPINA</u> (HENTZ) (COLEOPTERA: SCARABAEIDAE) Introduction

The cranberry root grub (CRG), <u>Lichnanthe vulpina</u> (Hentz) is a major pest of commercial cranberry (<u>Vaccinium macrocarpon</u> Aiton) beds in Eastern Massachusetts. Heavy infestations of larvae strip the vines of fibrous roots, leaving only the woody roots. Weakened vines can lay like a carpet and complete renovation of the bed may be necessary. If the bed is renovated, it is costly and takes at least three years before a crop can be harvested.

CRG grubs range in length from 0.5 to 3.0 cm depending upon the instar and are flattened dorso-ventrally. The head is deep reddish-brown and the body is covered with fine reddish-brown hair. Laterally, the hair is in tufts and is longer in comparison to the dorsum or venter (Franklin 1950). Adult males are 12.9 to 16.0 mm in length. Their dorsum, except elytra, and their ventral surfaces are clothed with long yellow-orange setae. Females are 13.0 to 17.5 mm with same setation as males, except the color is slightly lighter (Carlson 1980).

Adults are apparent bumble bee mimics emerging during cranberry bloom (Franklin 1950). On commercial cranberry beds, adults are active from the last week in June through mid-July. Adult beetle populations emerge around 0500 h in commercial beds, (O'Donnell, unpublished data) and males, which are active flyers, locate mates through

female- released sex pheromone (A. Zhang, personal communication). After mating, the male flies off and the female usually returns into the soil within the bog.

In 1948, it was estimated that 42% of the acreage in Massachusetts was infested to some degree with CRG (Beattie, 1948). In the following decades, dieldrin (a cyclodiene), was widely used in control programs, but this compound was banned in 1972. With no control measures currently available, populations are re-establishing on many beds.

Prior to this study, no quantitative studies of the biology of this insect have ever been carried out. Here, I report preliminary studies of the spatial distribution of larvae within a bed, which is a key first step in the development of sampling protocols. This insect was previously reported as a cranberry specialist (Franklin 1950), but grower reports of adults flying over turfgrass surrounding some cranberry beds led us to postulate that larvae may be able to feed on plants other than cranberries. I sampled off-bog sites and report these findings as well.

#### **Materials and Methods**

Larval Distribution: Distribution of larvae within a bed was determined in the summers of 1994 and 1995 using sampling plots laid out in a grid on 8 commercial cranberry beds and on an abandoned bed. The vegetation at the abandoned site consisted of Juncus effusus L., Spirea latifolia (Aiton), and a Rubus sp. There was no cranberry vine present. The selection of infested beds was based upon grower willingness to cooperate in this study. Also, due to the destructive nature of sampling and the value of the crop, sampling was limited to one cranberry bed per site. Cranberry bogs are constructed with a network of drainage ditches dug within the bog. The ditches are

approximately 1 m wide and 1 m deep. For this study, a bed was determined as an area of cranberries bordered on all sides by drainage ditches.

Sixty cm by sixty cm plots were laid out over the infested area with a maximum of 5 columns and 6 rows. Columns were separated by either 1.5 m, 3 m, or 5 m, depending upon the size of the bed. Rows were always separated by 10 m to limit the amount of sampling damage. Six rows could not be laid out at all sites due to the size of individual beds, resulting in some beds with fewer samples. Using these guidelines, as many plots as possible were laid out and the first row, (designated Row A) was started 3 m from the edge to ensure the integrity of the bank near the ditch. Each plot was sampled to a depth of 30 cm and the numbers of each instar were recorded.

I tested the goodness of fit of all sites to the Poisson and the negative binomial distributions using a chi-square test described in Southwood (1978). Also, the degree of aggregation was measured using Taylor's Power law (Southwood, 1978).

**Surveying:** Because higher areas of the bed may drain better and, thus, influence grub numbers, surveying was done on 7 of the 8 active cranberry beds using a Sokkia level (Model number 8042-60) surveyor attached to a camera tripod. The remaining bed was renovated before surveying could be accomplished and the abandoned bed was not surveyed because the heights of the different plants varied greatly, making surveying impossible to carry out. The height of each plot was determined and used to create a profile of the beds topography. These heights were compared to grub densities using linear regression.

**Off-bed Sampling:** To assess the possibility that CRG is able to feed on plants other than cranberry vines, two plots with the same dimensions as the plots described above were dug in the turf grass adjacent to the sampled areas of beds 1, 3 and 5. The remaining beds were surrounded by dirt roads with no vegetation. The turf was dug up and the numbers of each instar were recorded.

#### **Results**

**Larval Distribution:** All three instars were present at all sites and the larvae were totaled for each sample plot. Chi-square goodness of fit tests showed the data do not fit Poisson or negative binomial distributions at p = 0.05. Taylor's power law showed the grubs are aggregated (b = 1.33).

Larvae were highly aggregated within the sampled area at six of the eight commercial beds and at the abandoned site (Figure 1). The infestation at Site 1 has been established for at least ten years (Mark Sherman, Foreman, Edgewood Cranberry Company, personal communication). The larvae were most abundant in the plots taken from the middle of the bed and the majority of larvae were found within the first 30 meters (starting at row A). Site 2 is a newly established population of grubs occurring within the past five years (Ben Gilmore, Owner, personal communication). This site is located approximately 300 m from Site 1. Larvae were found in low numbers in two edge plots, with a single larva in a second row plot. The populations at Sites 3 and 4 were discovered in 1993. These sites are separated by approximately 600 m. Unlike the other sites where larvae tended to be localized within areas of a bed, here larvae were found throughout both beds, except for the last row in Site 4. The infestation at Site 5 is at least fifty years

Figure 1. Distribution of Lichnanthe vulpina larvae on eight commercial cranberry beds and an abandoned bed. Numbers reflect the

total larvae taken in 60 cm by 60 cm plots laid out in columns (numbers) and rows (letters).

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old (Bob Whiting, Owner, personal communication). The majority of larvae were found in the first two rows with only 1 and 2 grubs found in the third and last row, respectively.

Sites 6, 7, and 8 have been infested since the 1920's, 1940's, and 1950's, respectively (Bob Conway, Foreman, A. D. Makepeace Company, personal communication). The distributions of larvae at Sites 6 and 8 were similar, with most of the larvae found in the first row. The aggregation of larvae in edge plots of the first row at Site 8 was very pronounced. This bed had an irrigation ditch 30 meters from the edge that greatly limited the amount of useable area. However, the distinct nature of this population is still apparent given the constraints of the sample area. The distribution at Site 7 was similar to Site 1, with most of the larvae found in the middle of the bed, although grubs at Site 7 were present in all rows. The abandoned site was taken out of production in the 1920's (Bob Conway, Foreman, A. D. Makepeace Company, personal communication) and no cranberry vine remains. This site is located approximately 100 m away from Site 6 and had a small population of grubs. Most of the grubs were found in the last two rows, with no grubs found in the first row.

**Surveying:** There was no significant linear relationship between grub numbers and plot height in 6 of the 7 beds surveyed (Table 1). At Site 5, the correlation is low but the slope is highly significant. However, a ninety-five percent confidence interval plotted around the regression line shows that this relationship is poor (not shown).

**Off-bed Sampling:** Larvae were found in at least one of the two plots sampled in adjacent turf at all three sites. The numbers of grubs per plot were 0, 5; 0, 1; and 2, 15 at Sites 1, 3, and 5 respectively.

Table 1. Regression values for plot height versus number of Lichnanthe vulpinaIarvae in 60 cm by 60 cm plots sampled throughout commercial cranberry beds.

Slope	r <sup>2</sup>	p-value
1.1300	0.0019	0.8179
-0.0474	0.0001	0.9540
5.5244	0.0026	0.8115
-3.9451	0.0633	0.2250
-18.5152	0.4055	0.0002
6.2283	0.0421	0.3253
20.6976	0.0297	0.6340
	1.1300 -0.0474 5.5244 -3.9451 -18.5152 6.2283	1.1300 0.0019   -0.0474 0.0001   5.5244 0.0026   -3.9451 0.0633   -18.5152 0.4055   6.2283 0.0421

#### Discussion

Other studies have also shown that abundance of Scarabaeidae larvae varies widely among samples within a habitat. Allsopp and Bull (1989) sampled six species of melolonthine larvae in sugarcane and showed that larvae were slightly aggregated. Tashiro (1987) noted that larvae of the European chafer, <u>Rhizotrogus majalis</u> (Razoumowsky), tend to be found in clumps resulting in high and low counts when sampled.

The causal factors underlying our observations of larval aggregation in CRG are not apparent. Variation in larval numbers within a bed may result from choice of oviposition sites. This is the case for other Scarabaeidae where studies have shown that environmental parameters influence oviposition. Fleming (1972) noted that female Japanese beetles, <u>Popillia japonica</u> Newman, select low, poorly drained soils for oviposition during drought years. Also, Allsopp *et al.* (1992) showed in choice tests that Japanese beetles laid more eggs in wetter soils and no eggs in soils with < 5 % moisture content. <u>L. vulpina</u> are excellent fliers and if females prefer an optimum soil moisture for oviposition, they could assess large areas of habitat.

Alternatively, soil conditions may be responsible for differential grub survivorship or may cause grubs to aggregate in favorable areas, resulting in samples with high and low numbers. Soil moistures  $\geq$ 12.5% were necessary for normal development and survival of <u>Cyclocephala immaculata</u> Olivier eggs (Potter 1983). Soil moisture has also been shown to influence the movement patterns of grubs (Villani & Wright 1988). Regarding speed

of larval movement, Fellin (1986) determined that <u>Pleocoma</u> larvae are able to travel up to 11.0 cm per day.

Drainage or soil texture differences within a bog could contribute to variation in within-bog distribution of larvae. The makeup of commercial cranberry bog soils consists mostly of sand with an underlying peat base. There may be differences in drainage due to variation in the underlying peat layer resulting in areas that drain better. Most of the plantings in this study are at least thirty years old, with some over fifty years old. As the years pass, portions of cranberry beds tend to settle and most beds are out of grade. Also, differences in soil compaction or sand content may result in areas of varying moisture. However, irrigation and drainage vary among bogs so it is difficult to say if these factors have any effect on the observed patterns of <u>L. vulpina</u> larval distributions.

The previous report that <u>L. vulpina</u> larvae are cranberry specialists (Franklin 1950) was not confirmed by our studies. The vegetation at the abandoned site where larvae were found had no cranberry vine present. Also, sampling of the turf grasses surrounding commercial cranberry bogs yielded larvae in all stages, sometimes in comparable numbers to bog populations. Adults had been found previously in riparian areas where the sites consisted of grasses (Blanchard 1883) and flowers (Hentz 1883).

Since so little is known of the biology of this insect, our findings may have important implications for cranberry production. For example, because larvae are highly aggregated in some areas, sampling to determine degree and extent of an infestation must be thorough. Further, the apparent ability of larvae to develop in adjacent turfgrasses and other vegetation (abandoned site) means immediate reinvasion of renovated cranberry

beds may occur from off-bog sites. Since populations are increasing in frequency and size on many commercial beds, more research on <u>L. vulpina</u> ecology and behavior is needed if successful management programs are to be developed and implemented.

#### **CHAPTER 3**

# ADULT ACTIVITY OF THE CRANBERRY ROOT GRUB, <u>LICHNANTHE</u> <u>VULPINA</u> (HENTZ) (COLEOPTERA: SCARABAEIDAE)

#### Introduction

The cranberry root grub (CRG), <u>Lichnanthe vulpina</u> (Hentz), is a major pest of commercial cranberry (<u>Vaccinium macrocarpon</u> Aiton) beds in all cranberry growing areas of Eastern Massachusetts. Heavy infestations seriously injure the fibrous roots, resulting in weakened or dead vines that can be pulled back like a carpet. Large areas of the cranberry bed may be infested with this insect and complete renovation of the bed may be necessary. If a bed is renovated, it takes at least three years before a crop can be harvested.

In 1948, it was estimated that 42% of the acreage in Massachusetts was infested to some degree with this insect (Beattie, 1948). In the 1950's and 1960's, dieldrin (a cyclodiene) was used effectively in control programs. However, this compound was banned in 1972. Insecticides currently registered for cranberry are not effective controls for this insect, and, as a result, populations are re-establishing on many beds.

Prior to this study, no quantitative study of the biology of this insect had ever been carried out. In this paper, I describe the results of systematic observations that were conducted to determine diurnal and seasonal activity patterns of adults, as well as to observe and quantify adult behaviors.

#### **Materials and Methods**

Observations were carried out on commercial cranberry beds and an abandoned bed in southeastern Massachusetts. The abandoned site was taken out of commercial production in the 1920's and was approximately 100 m away from one of the commercial beds. Currently, there is no cranberry vine present. The vegetation there consisted of <u>Juncus effusus</u> L., <u>Spirea latifolia</u> (Aiton), and a <u>Rubus</u> sp.

**Daily activity:** In the summer of 1993, preliminary studies on the flight activity of adult beetles were conducted using plexiglass sticky traps. Two 1.2 m by 2.4 m plexiglass sheets were fitted together by cutting from the mid-point of the 2.4 m side to the center of the sheets. The two sheets were aligned perpendicular to each other and the cuts slid together. This formed a cross that could stand on its own. Three infested beds were sampled with one sticky trap each. The traps were assembled on the bed and then covered with Tangle-Trap. Each trap was checked four times daily, 0800 h, 1230 h, 1430 h and 1700 h, for the presence of adult beetles and all trapped beetles were removed each time. I was interested in determining the general activity period within a day; therefore, the numbers of beetles caught for each time period were totaled from all three traps.

To more accurately pinpoint patterns of adult flight activity within a day, in the summer of 1994, beetles on 3 commercial beds and at the abandoned site were captured at 15 min intervals. An insect sweep net was used to capture beetles on the commercial beds and flying beetles were netted on the abandoned site. At the abandoned bed and one commercial bed (site 3), beetles were also perched on the flowers of <u>S. latifolia</u>, and these beetles were captured by hand. Observers stood on an infested bed and captured as many

beetles as possible in the time interval. The observation area was limited to the area that an observer could see while standing on a predetermined site on the bed. Following capture of a beetle, the observer returned to the predetermined site before another beetle could be captured. Observations began at 0500 h and ended when no beetles were seen during three consecutive intervals after 0900 h. On two occasions, the temperature was too warm for observers to remain on the bed and on those days, observations were stopped before the three intervals were obtained (6 July and 20 July).

On 28 June and 12 July, 1995, observers remained on the cranberry bed to determine activity within a 24-hour period. The cranberry bed and surrounding non-cultivated areas were checked every 30 minutes for the presence and levels of activity of adults.

Seasonal activity: In the summers of 1994 and 1995, 5 commercial sites and the abandoned site were visited a few times per week to determine seasonal activity of adults. Observations started in mid-June and ended in mid-August and observers remained at the site for 15 minutes during times of peak activity to check for adults. The first and last day that adults were seen on cranberry beds and on the infested abandoned site were established, as well as the period of peak numbers observed during each year. Peak activity was determined by the numbers of beetles caught per day in the daily activity study.

**Environmental parameters and adult activity:** In the summer of 1994, the diurnal adult activity on five commercial cranberry beds and at the abandoned site were assessed. Three 20-min observations were taken each hour. In the first 15 minutes, adults

were captured with a net and sexed. During the remaining 5 minutes, environmental parameters were recorded. Air temperature and relative humidity (Fisher Thermo-Hygro, Fisher Scientific, Pittsburgh, PA), soil temperature at depths of 5 cm and 10 cm (Fisherbrand Monitoring Thermometer, Fisher Scientific, Pittsburgh, PA), wind speed (m/s) (TurboMeter by Davis Instruments, Hayward, CA) and light intensity (lux) (Digital Light Meter, ExTech Instruments, Boston, MA) were measured. For each day, single and multiple regression were used to determine relationships between number of beetles caught in each 15 minute period and the measured environmental conditions in the subsequent 5 minute period. Because factors that influenced adult emergence within a day were sought in this part of the experiment, the environmental data collected on days when no beetles were observed were excluded.

Adult Behavior: In the summer of 1995, observations of adult activity were carried out on two commercial cranberry beds and on the abandoned site. Observations were conducted using a micro-cassette recorder and a digital watch. An individual beetle was chosen at random and the type and duration of behaviors were recorded for 15 minutes or until the beetle was lost. I was aware that informative censoring influenced these data, that is, beetles engaged in some behaviors are more likely to be lost during the observation interval (flying for example), and thus, will have shorter observation times (J. Buonaccorsi, personal communication). This biases the proportions because different length observation times are treated the same. However, for ease of interpretation and lack of a better method, the average proportion spent in each behavior per individual was determined as follows:

 $\mathbf{T}_{i}$  = total time that beetle was observed

 $\mathbf{B}_{i}$  = time in the behavior

 $\mathbf{P}_{i}$  = the proportion of time beetle i spent in the behavior

 $\overline{\mathbf{P}}$  = the average proportion across all individuals observed Therefore,

$$\mathbf{P}_{i} = \mathbf{B}_{i} / \mathbf{T}_{i}$$

 $\overline{\mathbf{P}} = \sum_{i} \mathbf{P}_{i} / \mathbf{n}$ 

Using  $\mathbf{P}$ , the ten behaviors with the highest proportions for each sex were determined for adults on commercial cranberry beds and on the abandoned site.

#### Results

**Daily activity:** Most beetles were caught on the sticky traps between 0830 h and 1230 h and very few beetles were caught during the other time periods (Figure 2). On both days where observers spent 24 continuous hours in the field, beetles were primarily found on the cranberry bed, with a few flying over the turfgrass surrounding the bed. On both days, adults began emerging from the soil around 0700 h and then returned to the soil by 1500h (28 June) or 1130 h (12 July).

The numbers caught in 15 minute intervals on 4 of the 5 days show that the activity period on commercial beds began between 0530 h and 0730 h with peak activity occurring between 0600h and 1000 h. There was little activity from 1000 h to 1200 h except for at site 3 on 6 July where activity extended until 1355 h and observers had to leave because of the heat (Figure 3). The activity period for adults found on flowers began between 0730 h and 0830 h. Peak activity occurred around 1000 h on two of the

Figure 2. Total number of Lichnanthe vulpina (Hentz) adults caught during each time interval on three commercial cranberry beds using sticky plexiglass traps. 0800 h numbers represent beetles captured from 1700 h on the previous day.

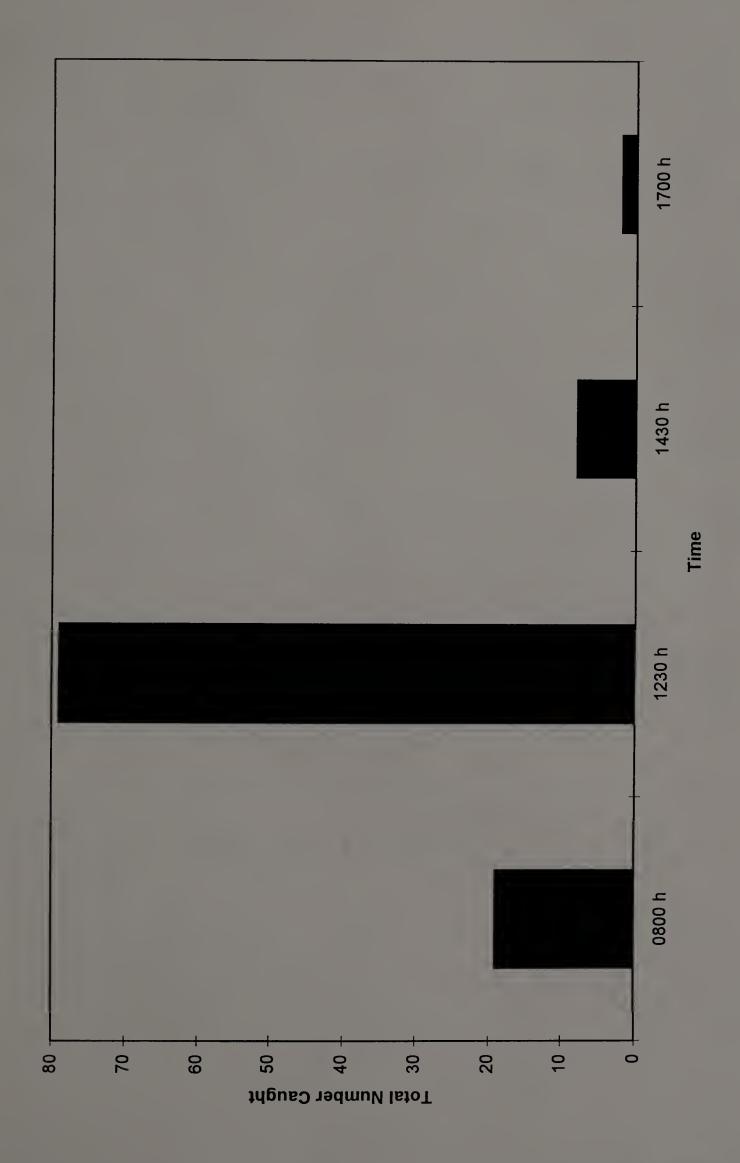
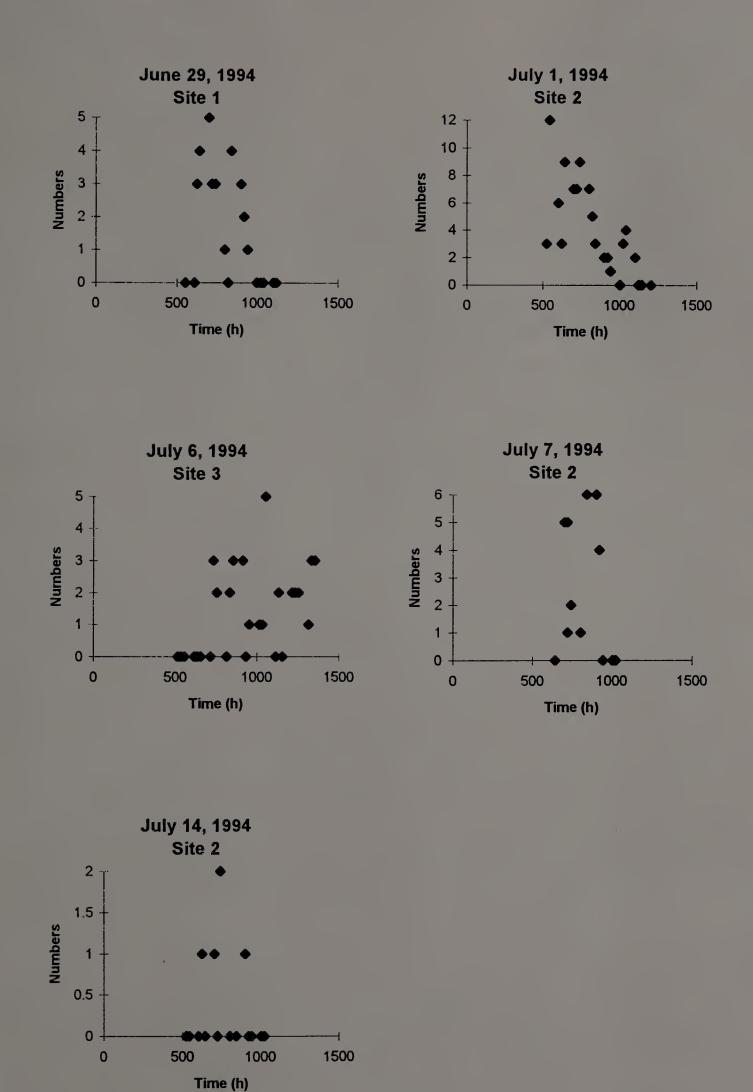


Figure 3. Number of <u>Lichnanthe vulpina</u> adults caught over time at 15 minute intervals on three commercial cranberry beds using a net.



three observation days. At site 3, which is a commercial bed, activity on flowers ended at 1145 h. At the abandoned site on 20 July, activity extended until 1535 h and observers had to leave because of the heat. On 29 July, activity was very low and activity ended at 0845 h (Figure 4).

Seasonal Activity: On commercial beds, adults first appeared in the third week of June (June 20 in 1994: June 23 in 1995), reached peak numbers the last week of June through the first week of July and ended in the second week of July (July 14 in 1994: July 13 in 1995).

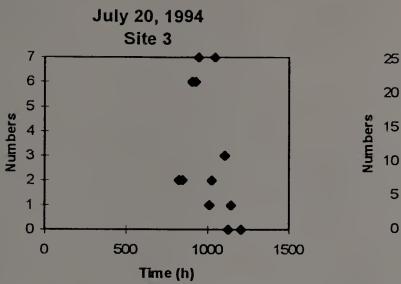
At the abandoned site, adults first appeared in the second week of July (July 14 in 1994: July 19 in 1995), and reached maximum abundance in the third week of July. The last beetle was seen during the last week of July (July 29 in 1994: July 31 in 1995).

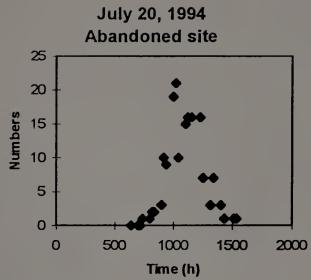
Environmental parameters and adult activity: On commercial cranberry beds, a significant relationship was found only when all parameters were put into the model (p = 0.02) (Table 2). A significant relationship was found for relative humidity alone (p = 0.04); however, the confidence interval of the slope contains zero. Therefore, the relationship is weak and the regression has no predictive value.

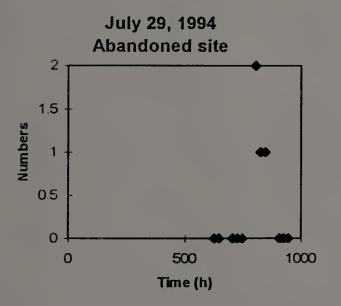
For adults caught at the abandoned site, the slope of the regression line was significant in all cases ( $p \le 0.05$ ) except wind speed (p = 0.13) (Table 3). In cases where the relationship was significant, the slope was positive for all parameters except relative humidity.

Adult Activity: The ten most common behaviors of adults are presented in Tables 4 and 5. On commercial cranberry beds, males spent the majority of the time

Figure 4. Number of <u>Lichnanthe vulpina</u> caught over time at 15 minute intervals on the flowers of <u>Spirea latifolia</u> (Aiton) on a commercial cranberry bed and an abandoned bed.







	p value	0.12	0.04	0.09	0.25	0.30	0.21	0.02
Confidence Interval	Upper 95%	0.02	0.07	0.72	0.92	0.07	0.18	
Confiden	Lower 95%	-0.13	0.00	-0.05	-0.25	-0.23	-0.83	
	Slope	-0.06	0.04	0.34	0.33	-0.08	-0.32	
	12	0.03	0.05	0.06	0.02	0.01	0.02	0.26
	Parameter	Air temp.	R. H.	5 cm Soil	10 cm Soil	Light	Wind	All

Table 2. Regression values for number of Lichnanthe vulpina adults observed on cranberry beds in relation to environmental parameters. Table 3. Regression values for number of Lichnanthe vulpina adults observed on Spirea latifolia in relation to environmental parameters.

	p value	0.00	0.00	00.0	0.01	0.01	0.13	0.00
Confidence Interval	Upper 95%	0.45	-0.07	0.82	1.01	1.25	0.34	
Confide	Lower 95%	0.21	-0.16	0.20	0.15	0.16	-2.51	
	Slope	0.33	-0.12	0.51	0.58	0.71	-1.08	
	r2	0.41	0.38	0.19	0.14	0.13	0.05	0.61
	Parameter	Air temp.	R. H.	5 cm Soil	10 cm Soil	Light	Wind	IIA

Table 4. Proportion of time spent by Lichnanthe vulping in the ten most prevalent behaviors observed for each sex on cranberry beds. 

Females (n = 34)	Proportion	0.4064	0.1389	0.1369	0.1184	0.1010	0.0358	0.0210	0.0180	0.0067	0.0055
Females	Behavior <sup>a</sup>	Mate	Mate-walk	Corkscrew	Sit	Walk	Fly	Mate-dig	Buzz.	P-A	ELY-PA
(= 271)	Proportion	0.6908	0.0881	0.0635	0.0584	0.0215	0.0151	0.0138	0.0136	0.0055	0.0042
Males $(n = 271)$	Behavior <sup>a</sup>	Fly	Sit	Mate	Walk	Buzz	M-H	P-ANT	ELY-PA	P-A	P-ABD

<sup>a</sup> Buzz, wings beat against foliage; H-M, head motions left to right; P-ANT, preen antennae; Corkscrew, twist head first into the soil; Mate-dig, female digging in soil while mating ELY-PA, elytra half open and pumping abdomen; P-A, pump abdomen; P-ABD, preen abdomen; Mate-walk, female walking while mating;

Table 5. Proportion of time spent by Lichnanthe vulpina in the ten most prevalent behaviors observed for each sex at an abandoned cranberry bcd. 

Females (n = 63)	Proportion	0.6278	0.1194	0.0824	0.0553	0.0494	0.0276	0.0182	0.0067	0.0045	0.0043
Females	Behavior *	Feed	Mate-feed	Sit	Mate	Walk	Mate-walk	Fly	SP-5	P-ANT	Corkscrew
1 = 73)	Proportion	0.3745	0.2908	0.1970	0.0804	0.0289	0.0089	0.0081	0.0081	0.0029	0.0003
Males $(n = 73)$	Behavior <sup>a</sup>	Fly	Feed	Mate	Sit	Walk	P-ANT	Corkscrew	P-ABD	Ely-pabd-hm	M-H

P-ANT, preen antennae; Corkscrew, twist head first into the soil P-ABD, preen abdomen; Ely-pabd-hm, elytra half open, pumping abdomen, head motions left to right; H-M, head motions left to right; Mate-feed, female feed on flowers while mating; Mate-walk, female walking while mating; SP-5, sitting pumping abdomen once in approximately 5 second intervals. flying (69%), with less time spent sitting, mating, and walking (5 - 8%). Females were typically observed mating (41%), walking while mating, corkscrewing and sitting (12 - 14%). Corkscrewing occurred after mating and involved twisting their bodies head-first into the soil. The majority of males at the abandoned site were observed flying (37%), feeding, mating, and sitting on the flowers of <u>S. latifolia</u> (8 - 29%). Females were typically seen feeding (62%), mating while feeding and sitting on the flowers (8 - 12%).

At the abandoned site, large numbers of mating pairs were observed on <u>S. latifolia</u>. Males often landed on or near feeding females and began mating. While mating, the female continued feeding, carrying the male on her back from flower to flower. After mating, the male flew off and the female usually remained on the flowers feeding. On the commercial beds, females were difficult to find and when they were found, they were usually mating. After mating on the bog, males usually flew off and females returned to the soil. At the abandoned site, females were very abundant and far out-numbered the males on flowers. Females were rarely seen flying.

## Discussion

Seasonal activity of adults varied between the commercial beds and the abandoned site. Adults on commercial cranberry beds appeared approximately two weeks earlier than adults at the abandoned site. The thick vegetation at the abandoned site created a very dense cover over the soil. Commercial cranberry is far less dense and the vines grow to a height of approximately 15 cm. Sunlight likely penetrates the cranberry canopy better, resulting in warmer soil temperatures earlier in the season and this possibly contributes to the earlier adult activity on commercial beds. Temperature dependent development of

insects, including scarabs, has been studied extensively. The relationship between temperature and development has been used to predict adult emergence in important scarab pests including the Japanese beetle, <u>Popillia japonica</u> Newman (Régnière et. al., 1981) and northern and southern masked chafers, <u>Cyclocephala borealis</u> Olivier and <u>C.</u> <u>immaculata</u> Arrow (Potter, 1981).

On commercial cranberry beds, the results show that  $\underline{L}$  vulpina remain in the soil and came out briefly in the early morning and solely to mate. At the abandoned site, adults tended to appear later in the morning not only to mate, but also to feed. The mechanism responsible for early-morning activity on commercial beds and mid-morning activity on the abandoned bed is unknown at this point. However, the mid-morning activity at the abandoned site may coincide with pollen availability on <u>S</u>. latifolia. In early morning, the flowers are covered with dew and it may be difficult for the beetles to harvest the pollen as compared to when it is warmer and the flowers have dried off.

It is reported that adults of <u>L. vulpina</u> never feed (Franklin, 1950). However, adults found at the abandoned site on <u>S. latifolia</u> clearly moved their mouthparts over the flowers and their midguts were full of pollen (O'Donnell, unpublished data). Because studies have shown that feeding plays an important role in female longevity and egg production in other scarabs (Ladd, 1987, Domek and Johnson, 1991), parallel studies should be carried out on this insect to see if females at the abandoned site differ from those at commercial sites in longevity or number of eggs laid.

On commercial cranberry beds, adults are present during cranberry bloom but have never been observed feeding on cranberry flowers. At one commercial cranberry site,  $\underline{S}$ .

latifolia was present on the border of the woods surrounding the bed. However, it did not bloom until one week after the last beetle was seen on the bog and no beetles were found feeding on it. At another commercial bed (site 3), <u>S. latifolia</u> was growing as a weed on the cranberry bed and was also abundant in the drainage ditches surrounding the bed. Here, adults were observed feeding on the flowers of <u>S. latifolia</u> in low numbers. This site was surrounded by a large area of turfgrass that is infested with this insect; therefore, it is unclear if the beetles found on <u>S. latifolia</u> originated from the cranberry bed or the surrounding turfgrass.

On the commercial beds, I observed beetles between 0530 and 0730 h, sometimes in large numbers. However, given this pattern, the number of beetles caught on the sticky traps in mornings until 0830 h is fewer than expected. Differences in irrigation patterns may account for the discrepancy between the two data sets. Visual observations were conducted at sites that were irrigated on a periodic basis, and always very early in the morning. In contrast, the sticky traps were located in beds that were irrigated almost daily, usually until 0700 h. Because I have observed that beetles begin to appear ca. one hour after the water is turned off, the frequent irrigation probably substantially delayed flight activity on the beds where the traps were located.

Distinct daily activity periods for many turfgrass scarabs have been determined. Drab-colored scarabs, such as Asiatic garden beetles, <u>Maladera castanea</u> (Arrow), May or June beetles, <u>Phyllophaga</u> species, and European chafers, <u>Rhizotrogus majalis</u> (Razoumowsky), have nocturnal activity periods (Tashiro, 1987). In contrast, similar to <u>L. vulpina</u>, the brightly colored scarabs such as the green June beetle, <u>Cotinis nitida</u> L.,

and Japanese beetle, <u>P. japonica</u>, have diurnal activity patterns. <u>L. vulpina</u> appear to be bee mimics, so it is not surprising that they are day-active.

At commercial sites and the abandoned site, males were observed flying in large numbers, probably searching for females producing a sex pheromone (A. Zhang & P. S. Robbins, unpublished data). By 0700 h, male <u>L. vulpina</u> flew rapidly 15-45 cm above the cranberry canopy with a buzzing sound resembling that of bumblebees. This activity is similar to behaviors of the male green June beetle, <u>C. nitida</u>, described in Tashiro (1987). The flight of male <u>L. vulpina</u> was an undulating, meandering flight that became more focused near a calling female, where it consisted of a casting or zigzag pattern. This pattern is similar to that described by Fleming (1972) for <u>P. japonica</u>. Wind tunnel studies of Scarabaeidae also describe a casting pattern of flight for male scarabs (Facundo et. al., 1994, Domek et. al., 1990).

On commercial bogs, the female is commonly within the cranberry vine and is not visible from above; therefore, the males sometimes flew past the female and circled downwind and repeated the casting pattern. When females were positioned on a cranberry upright or a weed above the cranberry vine, males were observed landing on females and then copulating, which suggests the use of visual cues as well. If females were within the vine, males landed within a few centimeters of them and then crawled to them to mate. Preliminary studies suggest the presence of a female contact pheromone as well, that may aid in recognition of females within the vines(A. Zhang & P. S. Robbins, unpublished data).

The underlying mechanism responsible for the differences in the daily and seasonal activity patterns exhibited by the cranberry and non-cranberry populations could be attributed to genetic divergence, a phenomenon that has been documented in other species where host races have evolved (Ridley, 1993). However, the abandoned bed and a commercial bed are located within several meters of each other and <u>L. vulpina</u> adults are extremely mobile, factors that would allow a high level of gene flow between populations. Further, the observed differences in activity pattern can be largely accounted for by variation in the environmental conditions in each habitat. Therefore, I feel that genetic divergence is unlikely in the case of these <u>L. vulpina</u> populations.

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