AN ABSTRACT OF THE THESIS OF

Greg S. Fitzpatrick for the degree of Master of Science in Entomology presented on January 9, 1995. Title: The Effects of Intraspecific Plant Competition and Insect Herbivory on Ragwort (Senecio jacobaea) Populations.

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Abstract approved:_____

Peter B. McEvoy

I conducted field studies to determine the effect of insect herbivory and intraspecific plant competition on ragwort Senecio jacobaea. The objectives were to determine the patterns and causes in the distribution of the ragwort flea beetle Longitarsus jacobaeae foraging among varying densities of ragwort, to measure the behavioral and numerical responses of the beetle to changes in ragwort density, and to estimate the impact of insect herbivory and intraspecific competition on ragwort performance. Host density was manipulated by planting 1, 4, 8, or 16 plants per 0.5 x 0.5 m patch. Beetles were counted in each patch to assess the effect of host density on the beetle population. I measured four components of reproductive success represented by growth rate, development rate, reproduction, and annual survivorship to assess the effect of herbivory and intraspecific plant competition on ragwort performance

In the first experiment, beetle populations were manipulated by establishing equal numbers of beetles in patches with unequal number of hosts (1, 4, 8, 16 plants per patch), which were then subsequently allowed to move freely about. Beetles rapidly re-distributed themselves, such that the number of beetles was strongly and positively correlated with the number of hosts. This indicates that ragwort flea beetles are highly sensitive to local distribution of their food plants.

In the second experiment, host density was manipulated by planting ragwort in densities of 1, 4, 8, 16 plants per patch, and beetles were then allowed to colonize the

experimental patches. Beetle behavioral response to a change in host density was dependent on host population size: the numbers of colonizing beetles increased asymptotically with increasing plant density. The number of beetle-days ranged from 261 for 1-plant patches to 1822 for 16-plant patches. In contrast, the numerical response (represented as observed multiplication rate per capita per generation per year) appears to be inexplicably low in the single plant population and levels off in the 4, 8, and 16 plant patches (grand mean for multiplication rate 1 was 5 and for multiplication rate 2 was 10.4 progeny per individual per generation). Combining these results, the beetles apparently respond to spatial variation in the density of hosts primarily by changes in their movement behavior rather than by changes in their per capita reproductive rates. These results highlight the importance of a natural enemy's colonizing behavior for controlling a sudden upsurge in pest abundance.

Both insect herbivory and intraspecific competition had an effect on ragwort performance. For example, over approximately one year, ragwort's rate of biomass accumulation was 48% lower, and seed-head production was18% lower in exposed compared to protected plots, while intraspecific competition reduced ragwort's rate of biomass accumulation and seed-head production, such that a 16-fold increase in host density (in protected patches) led to a 12-fold decrease in biomass per plant and a 11-fold decrease in the number of seed-heads per plant. Herbivore effects were independent of host density: variation in plant density from 1 to 16 plants led to no detectable change in magnitude of the herbivore effect. This suggests there is no density-dependent refuge for host plants operating at these local scales of observation.

Keywords: Host density effects, behavioral response, reproductive response, biological control agent, Longitarsus jacobaeae, Senecio jacobaea.

The Effects of Intraspecific Plant Competition and Insect Herbivory on Ragwort (Senecio jacobaea) Populations

by

Greg S. Fitzpatrick

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed January 9, 1995
Commencement June 1995

Master of Science thesis of Greg S. Fitzpatrick presented on January 9, 1995

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I understand that my thesis will become part of the permanent collection of Oregon State University Libraries. My signature below authorizes release of my thesis to any reader upon request.

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Greg S. Fitzpatrick, Author

I dedicate this thesis to my wife Joy and my son Jesse,

for their constant support and their sense of humor-- many thanks

ACKNOWLEDGMENTS

I wish to thank all those people who helped with this project. I want to especially thank my major advisor, Peter McEvoy, for his guidance and patience throughout this project. Thanks also to other members of my committee, Jeff Miller and Chris Mundt, for their support and helpful suggestions throughout this research; to Manuela Huso for her assistance in the field and advice on the statistical analysis; to Zhi-Qiang Zhang for his many helpful discussions and comments on an earlier draft of the thesis; and to the US Forest Service for use of the Cascade Head Scenic Research Area. This research was made possible by the National Science Foundation grant BSR-9020483 and the OSU Agricultural Experiment Station.

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The Effects of Intraspecific Plant Competition and Insect Herbivory on Ragwort (Senecio jacobaea) Populations

I. INTRODUCTION

The ragwort flea beetle Longitarsus jacobaeae (Waterhouse) (Coleoptera: Chrysomelidae) is considered the key factor in the biological control of tansy ragwort Senecio jacobaea L.(Asteraceae), a weed that displaces valuable forage and is toxic to livestock. This system has emerged as a classic example of successful biological control. Following the introduction of three biocontrol agents (cinnabar moth Tyria jacobaeae, ragwort seed-head fly Botanophila seneciella, and ragwort flea beetle L.jacobaeae) in the Pacific Northwest, a survey of 42 sites over a ten-year period showed that ragwort declined to 1-3 % of its former abundance at lower elevations in western Oregon, and has been replaced by a more desirable vegetation, predominantly composed of perennial grasses (McEvoy et al. 1991). The ragwort system is characterized by disturbances which open up space and recycle limiting resources, which in turn sets the stage for colonization by ragwort (McEvoy et al. 1993, McEvoy and Rudd 1993). Recent experiments have shown that ragwort populations are regulated (1) more strongly by the ragwort flea beetle than by the cinnabar moth or the seed-head fly and (2) by competition from background vegetation, which limits resources that would otherwise be available to ragwort. The various responses and interactions between ragwort, background vegetation, and the beetle do not happen immediately, but have time delays. When disturbances are local in time and space, the time delay in the resource limitation effect by the background vegetation is short compared to the time delays in the natural enemy effect. Resource limitation arrests plant growth and allows time for the herbivores to catch up.

Prior experiments have found that other species of plants and herbivores cause no detectable modification of the interaction between ragwort flea beetle and ragwort

(McEvoy et al. 1993). This suggests that the dynamic behavior of this biological control system can be understood and predicted based on analysis of a subset of organism interactions consisting of ragwort, its resource base, and its most potent natural enemy, the ragwort flea beetle.

How foragers respond to a distribution of hosts may have important implications on herbivore-plant interactions (Kareiva 1985, Bach 1988), and the efficiency of biological control (Murdoch et al. 1985, Harris 1991, Reznik 1993, McEvoy et al. 1993). Introduction programs in recent years have begun selecting natural enemies based on desirable control organism attributes (Ehler 1990). Huffaker et al. (1971) suggest that these attributes include: (1) adaptability to the prevailing physical conditions of the environment; (2) superior searching ability; (3) high reproductive potential relative to the prey; (4) host specificity; (5) synchronization with host.

I conducted two field experiments that measured the importance of beetle searching ability, beetle colonization behavior, and ragwort resource limitation in regulating ragwort populations. In experiment I, I investigated the distribution of flea beetles foraging among varying densities of ragwort. The second experiment was broken into two parts. In the first part I investigated the behavioral and reproductive responses of the ragwort flea beetle to changes in host density. In the second part I examined the role of intraspecific plant competition and insect herbivory on ragwort performance. The purpose of the second experiment was to weigh two attributes, colonization and multiplication rates of the ragwort flea beetle, that may contribute to its efficiency as a regulator of ragwort abundance. Ragwort density was manipulated by planting 1, 4, 8, or 16 plants per 0.25 m^2 patch. In the field, ragwort mean density can range from 0-157 plants/m^2 (McEvoy 1985).

HISTORY

Tansy ragwort S. jacobaea is native to Europe and was accidentally introduced to North America in the early 1900's (Harris et al. 1971) and first recorded in Oregon in 1922 near Portland (Isaacson 1971). Ragwort is toxic to livestock and tends to replace forage in pastures. Since ragwort infests low-value land such as dry pastures and clearcuts (Isaacson & Schrumpf 1979), the application of herbicides for control is generally not practical or economical. As an alternative to chemical control, a biological control program was initiated in 1960, which has since successfully controlled ragwort in the Pacific Northwest.

BIOLOGY OF THE ORGANISMS

Ragwort is a biennial but may become a short-lived perennial when exposed to stresses of crowding, cutting, or defoliation (Cameron 1935, Cox and McEvoy 1983, Dempster 1982, Crawley and Gillman 1989). The biology of ragwort is described by Harper & Wood (1957) and Wardle (1987). Litter and growing vegetation suppress ragwort germination (Cameron 1935, Crawley and Gilman 1989), and disturbance is critical for ragwort colonization (Cameron 1935, Crawley and Nachapong 1985, McEvoy et al. 1993). Germination can occur both in the spring and fall (van der Meijden and van der Waals-Kooi 1979, McEvoy et al. 1993). Ragwort spends the first year as a rosette, and in our area, bolts and flowers in the second year between July and September, and then dies. Mortality rate declines with each stage of development: it is estimated that 57% of plants die as seedlings, 29% as single rosettes, 6% as multiple rosettes, and the remaining 8% die after flowering (Forbes 1977). The average number of capitula per

plant (flower heads) has been estimated to be 68-2489 by Cameron (1935) and 110-392 by Harper and Wood (1957) with an average of 70 seeds per capitulum (McEvoy 1984).

The life history of the ragwort flea beetle has been reviewed by Newton (1933), Frick (1970a, 1971), Frick and Johnson (1972, 1973) and Windig (1993). Introduced from Italy, the beetle was released in California near Fort Bragg in 1969 (Frick 1970b, Frick and Johnson 1973, Hawkes and Johnson 1978), and in Oregon near Trask River in 1971 (Isaacson 1978). The beetle is now generally prevalent in Western Oregon where ragwort is abundant. The flea beetle is univoltine (Fig. 1). Beetles lay eggs both in the soil and on the plant. Larvae develop through three instars boring into the roots, petioles, and stems feeding on plant tissues mainly during the winter and spring months. In late spring larvae bore out of the plant and pupate in the soil. The adult emerges during the summer and feeds through the fall primarily on the leaves leaving tiny circular 'shot holes' as evidence of their feeding.

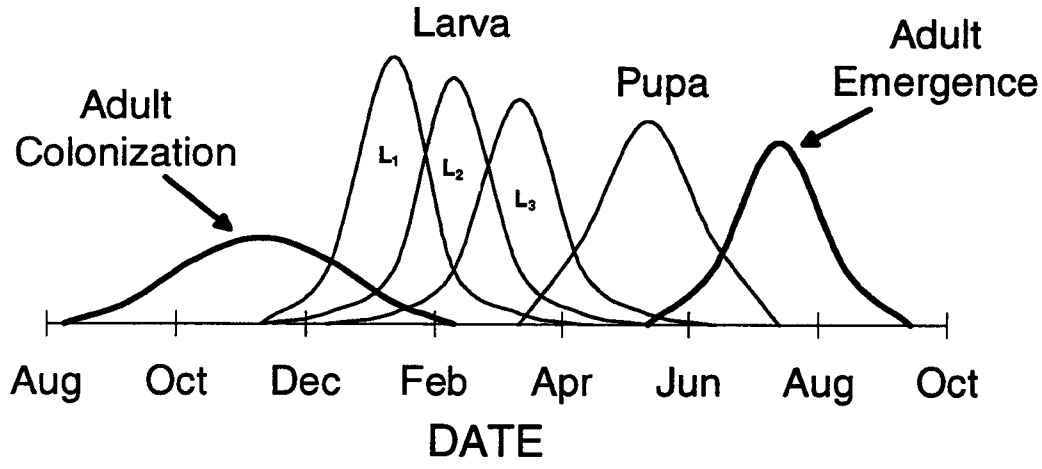


Figure 1. Life cycle of the ragwort flea beetle L. jacobaeae. Adult beetles colonize in the fall, 3 instars and pupa develop during the spring and winter, and new generation adults emerge in the summer.

I begin this study by investigating the equilibrium distribution of ragwort flea beetles L. jacobaeae (Coleoptera: Chrysomelidae) foraging among patches differing in density of ragwort S. jacobaea. Next I examine the behavioral and reproductive responses of the flea beetle to changes in ragwort density. This is followed by an investigation of the intraspecific plant competition and flea beetle effects on ragwort. Finally, I summarize the major findings of this thesis research.

II. EQUILIBRIUM NUMBERS OF ADULT BEETLES LONGITARSUS JACOBAEAE FORAGING AMONG PATCHES OF HOST PLANTS SENECIO JACOBAEA

INTRODUCTION

We commonly expect, but seldom observe a positive correlation (direct density dependence) between the density of foragers and the density of hosts. A review on density dependence in parasitoids (per individual host) showed that half of the 75 cases reviewed were either directly (17) or inversely dependent (21) on host density, with the remaining cases showing possible evidence of density independence (Walde & Murdoch 1988). Other studies on herbivore plant interactions have shown a diversity in herbivore movement patterns in relation to patch size and host density. A review of 19 herbivore species that were measured in relation to patch size per individual plant, found that 9 species were more abundant and 2 species were less abundant with increasing patch size, and 8 species revealed no effect (Kareiva 1983). A review of 13 species that were measured in relation to host density per individual plant, found that 2 species increased and 9 species decreased as host density increased, and 2 showed no change (Kareiva 1983). These studies suggest that herbivores often do not conform to Root's (1973) resource concentration hypothesis that predicts herbivore loads increase with increasing concentration of their food resources.

How foragers respond to a distribution of hosts can have important implications on herbivore-plant interactions (Kareiva 1985, Bach 1988a, 1988b), and the efficiency of biological control (Murdoch et al. 1985, Harris 1991, Reznik 1993, McEvoy et al. 1993). For example, a natural enemy's sensitivity to local distribution of host plants may determine its searching efficiency and therefore its ability to control a pest. McEvoy et al. (1993) showed that when areas are disturbed and ragwort is allowed to invade, a positive

correlation develops between the ragwort flea beetle Longitarsus jacobaeae density (larvae per patch) and host plant density (biomass per patch). Flea beetle distributions among patches differing in host density may be due to a number of factors, such as number and quality of plants, physical conditions, interaction with other organisms, or differential mortality. For a given population, over some period of time, there exists a unique average density (equilibrium) which is appropriate for that population under certain environmental conditions (Murdoch 1970). The perturbation-response method is one approach to isolate possible factors which set the equilibrium in populations. In this study I investigated the distribution of flea beetles foraging among varying densities of ragwort. I perturbed the system by placing equal numbers of beetles in patches with unequal number of hosts and then followed beetle numbers until they reached equilibrium.

METHODS

Study site

Beetle movement was studied in a mowed grass field at Oregon State University Entomology Research Farm, Corvallis, Oregon. Background vegetation consisted mainly of perennial grasses. The experimental site has cool wet winters and warm dry summers. The following averages are based on normals for the period 1961-1990 at the Hyslop Experimental Station, Corvallis, Oregon. The mean monthly temperatures range from 7.5° C in January to 27.3°C in August. Mean annual rainfall is 108 cm, with 72% of the rainfall occurring during the five months of November through March (Oregon Climate Service 1994).

Experimental design and layout

The experiment was a randomized complete block design consisting of 2 blocks with 4 levels of ragwort density (1, 4, 8, 16, plants per 0.25 m^2 patch). Each host density was replicated 4 times within each block. Thus the total number of patches was: 2 blocks x 4 levels of host density x 4 replicates for each host density = 32 patches. Plot size and spacing were as follows. The experimental patches were arranged in 2 blocks (Fig. 2). Blocks measured 9 m x 9 m = 81 m^2 and were separated from each other by 30 m. The patches measured 0.5 x 0.5 m = 25 m^2 and were separated from each other within blocks by 3 m.

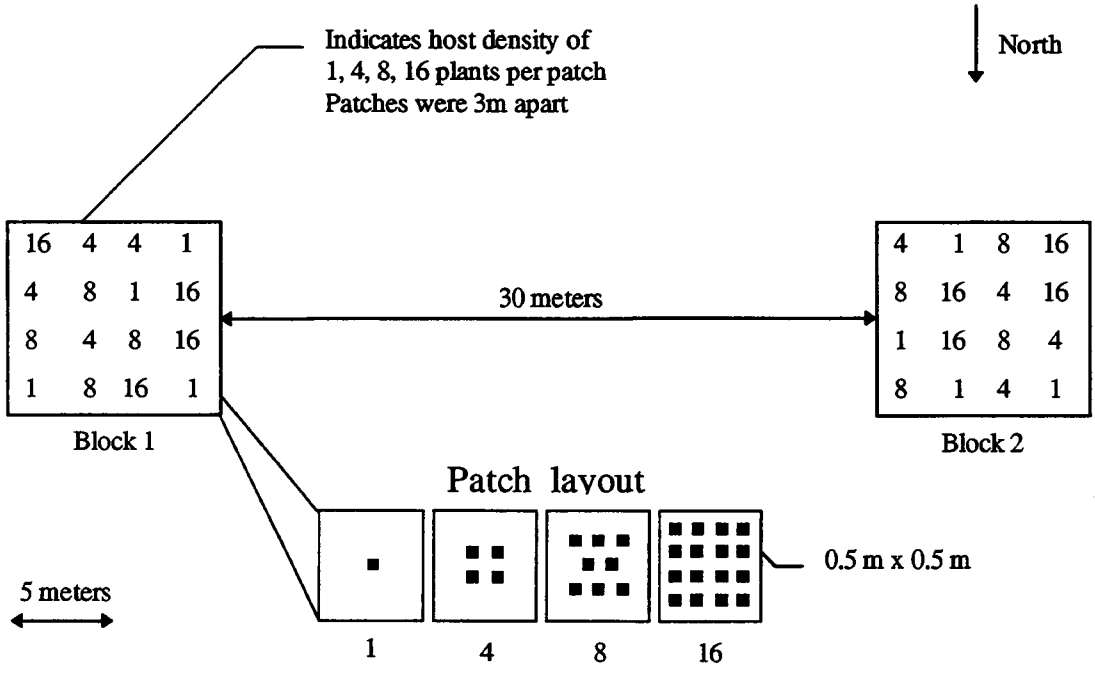


Figure 2. Experimental layout for the 1991 experiment conducted at the Entomology Research Farm, Corvallis, Oregon. Layout shows location of 2 blocks (blocks are 30 m apart) and 16 patches within each block (patches are 3 m apart).

It is not certain whether beetles were flying and/or walking during the redistribution of beetles among the experimental patches. However, results from a preliminary migration study indicate that beetles begin flying sometime in August and continue through October. I did not observe any flying during my 80 hours of field and lab observations from November through December of 1991. These two pieces of evidence suggest that the time of the experiment came after the time of migration and therefore beetles probably moved among the experimental patches on foot rather than on the wing.

Manipulating plant density

Plant density was manipulated by planting ragwort in densities of 1, 4, 8, or 16 plants per 0.5 x 0.5 m plots in the field. Ragwort seeds were collected from Cascade Head Scenic Research Area (CHSRA) and germinated in a greenhouse at Oregon State University, Corvallis, Oregon, during September 1991. Seedlings were later transplanted individually to pulp pots (14 cm square x 12.7 cm deep) containing a soil medium consisting of soil, peat moss, sand, and pumice (1:1:1:2 parts respectively). Plants were fertilized with 20% N, 20% P, 20% K (Grace-Sierra Horticultural Products Company, Milpitas, California), and watered and weeded as necessary. Upon reaching rosette stage (plants that are 10-20 cm tall and not bolted), plants were transplanted to the field and randomly assigned to blocks and treatments on 6 December 1992.

Manipulating herbivore density

Beetles were collected from CHSRA using a 'D-vac'® (a large, gas-powered vacuum net; D-Vac Company, P.O. Box 2095, Riverside, California, 92506) on 23 November 1991. Beetles were stored in covered plastic trays containing ragwort leaves

and put in a refrigerator at a temperature that ranged from 3 to 13 °C. Female beetles were separated into batches of 20 using a dissecting microscope at 10-15 x. Female beetles were used so that mating behavior could be distinguished from searching behavior. Four to five florescent pigment colors (Day-Glo Color Corporation, subsidiary of Nalco Chemical Company, 4515 St. Clair Ave, Cleveland, Ohio, 44103) were applied to each batch of beetles resulting in 32 batches of 20 beetles, each having a unique combination of colors. The pigment was applied using a fine camel hair brush to the beetle elytra and body in general, one color at a time.

On 7 December 1991 I placed 20 marked female beetles uniformly onto each of the 32 ragwort patches, for a total 640 beetles released. To minimize dispersal due to handling and other experimental procedures, beetles were kept in the refrigerator and released in the cool hours of the morning. Since I removed all ragwort plants from the area it is likely that the released beetles were the only beetles present at the time. Beginning the next day I counted (sampling non-destructively) total, resident, and non-resident beetles in each patch for the next 15 days between the hours of 5 PM and 10 PM. Resident beetles were defined as beetles originating from location of release, and non-resident beetles were defined as beetles originating from other locations. I used a free standing AC blacklight or UV spectrum florescent lamp to illuminate the florescent pigments on the beetles, which made the beetles more visible and easier to sample. On 21 December I sampled beetles both during the day and night to compare the efficiency of day and night time sampling.

I was able to measure how total number of beetles per patch varied over time for each level of host density, but I could not distinguish resident beetles from non-resident beetles, or immigration from emigration, as originally planned.

Data analysis

I estimated the time required to reach equilibrium by visual inspection of the graphs in figure 3 that show number of beetles over time for each level of host density. To analyze the response of the beetle to a change in host density I regressed $\log_e$ mean equilibrium number of beetles as a function of $\log_e$ host density. Log-transformation helped linearize the relationship and stabilize the variance. All statistical analyses were performed using SYSTAT for windows, version 5.02 (SYSTAT, Inc. 1992). The significance level used was 5%.

RESULTS

All beetles found and sampled in experimental patches were marked with florescent dye, indicating that no wild beetles colonized from outside the experiment. All patches started with the same number of beetles, and the number of beetles subsequently decreased in all patches (Fig. 3). The rate at which beetles were lost decreased with increasing number of hosts per patch, suggesting that beetles were more likely to remain in patches containing more hosts. It was estimated by visual inspection that beetle number reached equilibrium after about 7 days (Fig. 3). The number of beetles varied across blocks and levels of host density (Table 1). Although initial numbers of beetles were independent of the number of hosts per patch, the regression of equilibrium number of beetles ($\log_e$ mean total beetles per patch for days 7-15) as a function of host density had a positive slope (Table 1, Fig. 4). The number of beetles per patch (back-transformed mean + 95% CI) ranged from 1.9 (0.443, 0.881) in 1-plant patches to 9.4 (8.142, 10.868) in 16-plant patches. There was an interaction between block and host density (Table 1) indicating that the equilibrium number of beetles increasing with density varied by block.

There was no detectable difference in the numbers of beetles sampled during the day or night (ANOVA, $F_{1,63} = 2.588$, $P = .113$; Fig. 5)

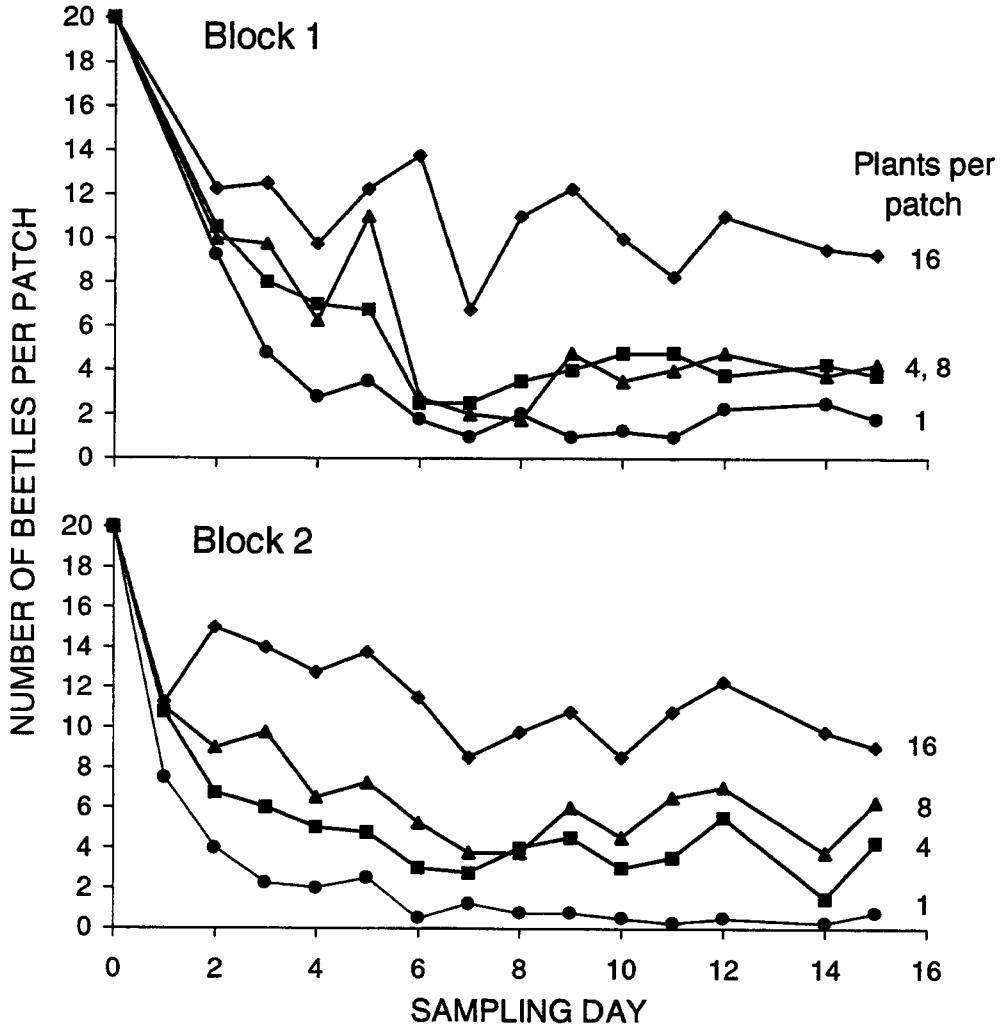


Figure 3. Flea beetle L. jacobaeae redistribution as a function of time for the 1991 experiment conducted at the Entomology Research Farm, Corvallis, Oregon. Numbers of adult flea beetles redistributing within the experimental array (mean number of beetles per patch) as a function of time for each block and level of patch size.

Table 1 ANCOVA on number of flea beetles L. jacobaeae (log_e mean number of beetles per patch) in relation to host density (loge) for the 1991 experiment conducted at the Entomology Research Farm, Corvallis, Oregon.

Source of variation	df	MS	F	P
Host density (H)	1	10.861	109.321	<0.001
Block (B)	1	.481	4.845	.036
H x B	1	.510	5.131	.031
Error	28	.099		

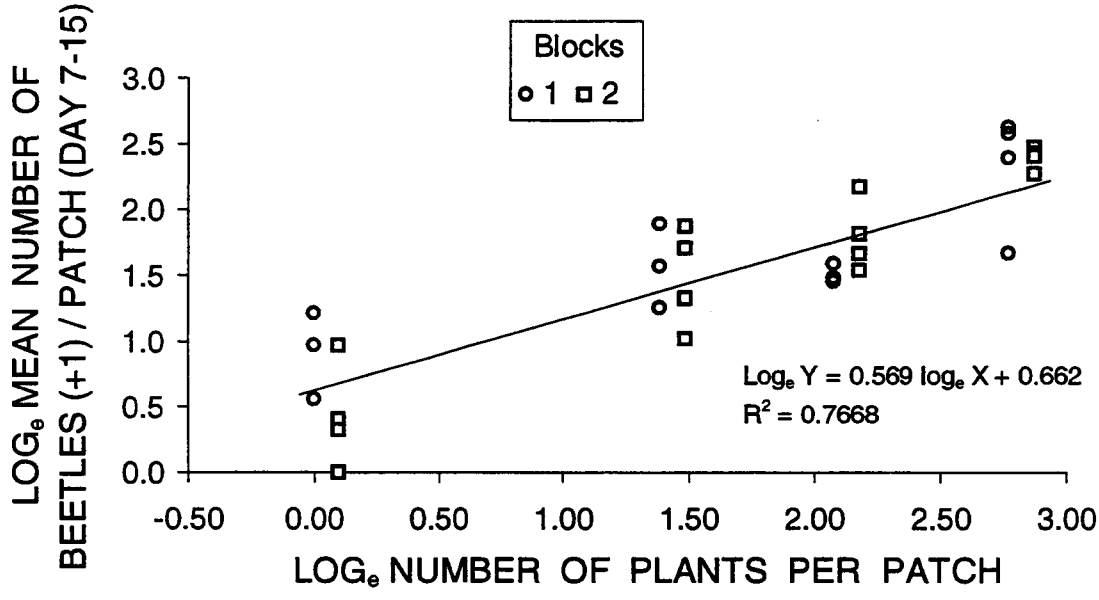


Figure 4. Flea beetle L. jacobaeae redistribution as a function of host density for the 1991 experiment conducted at the Entomology Research Farm, Corvallis, Oregon. Numbers of adult flea beetles redistributing within the experimental array (log_e mean number of beetles per patch for day 7-15) as a function of patch size (log_e).

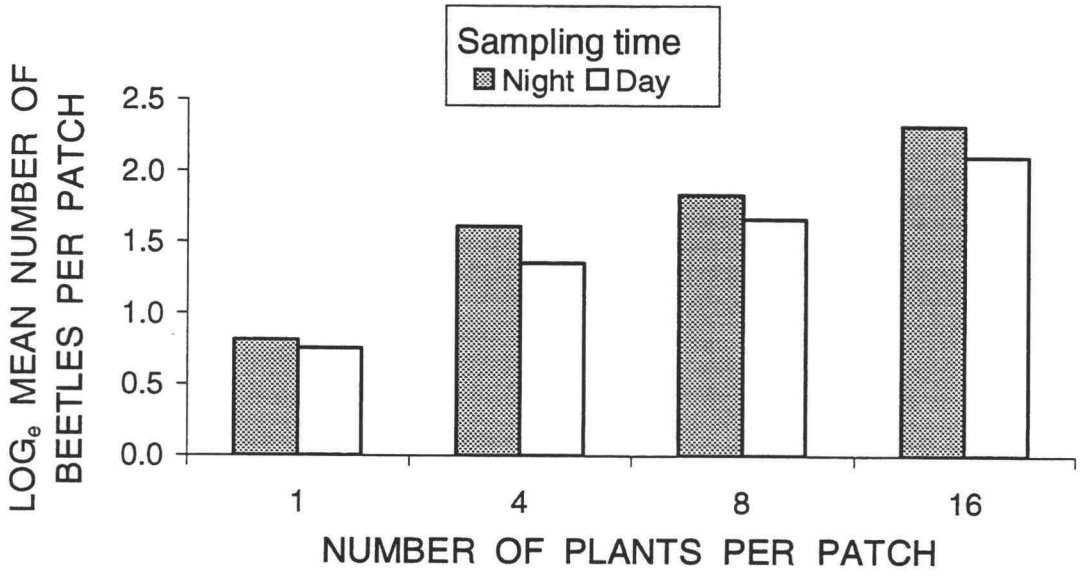


Figure 5. Comparison of day and night sampling of the ragwort flea beetle L. jacobaeae for the 1991 experiment conducted at the Entomology Research Farm, Corvallis, Oregon. Numbers of adult flea beetles ($\log_e$ mean number of beetles per patch) as a function of patch size for both night and day sampling periods.

DISCUSSION

The results of this experiment show that ragwort flea beetles were highly sensitive to local distribution of their food plants. When beetle numbers were perturbed by establishing equal numbers of female beetles in patches with unequal number of hosts, the beetles promptly re-distributed themselves to restore a persistent configuration in which the number of beetles is strongly and positively related to the number of hosts. That the concentration of foragers increases with the concentration of resources is perhaps not surprising. What is surprising is how rapidly beetles move to establish this relationship: the number of beetles went from equal numbers of beetles among patches to increasing

beetles with increasing host density in about 7 days. The positive relationship between number of beetles and the number of hosts in this study is consistent with a prior experiment that showed beetle density (larvae per patch) was positively correlated with host plant density (biomass per patch) (McEvoy et al. 1993).

By manipulating host density, this experiment effectively isolated the effect of host density from the effects of potentially confounding variables like environmental conditions or interactions with other organisms (mates, competitors, or natural enemies). By restricting the population of searching adults to females, I avoided the confounding that can occur when foragers respond to both hosts and mates. By equalizing the initial number of beetles per patch, I avoided possible confounding between the effects of host density and beetle density. Natural history observations during the study found no evidence of the influence of natural enemies on flea beetle populations.

The mechanisms by which these beetles respond to reestablish a relationship with host density requires further investigation. The observed beetle equilibrium probably reflects a balance between immigration and emigration rates rather than other factors. It is assumed (1) that no births occurred since the time interval of the experiment was so short, and (2) that no wild beetles immigrated from outside the experiment since no unmarked beetles were sampled. Disappearance was probably explained by emigration, although some beetle mortality may have occurred.

Although movement is the likely explanation for the observed spatial variation in beetle density among patches varying in host density, it is not clear whether density dependence lies in the immigration or emigration rates. Zhang and McEvoy (in press) found that ragwort flea beetles, in lab wind tunnel experiments, orient towards upwind host plants over a distance of 60-300 cm, however the strength of the beetle response to hosts did not increase with an increase in hosts from one to six plants. This suggests that an increase in beetle density with increasing host density is not due solely to density

dependent immigration. Kareiva (1985) found that when numbers of the crucifer-feeding flea beetles were perturbed from equilibrium levels, they rapidly returned to equilibrium in which the number of beetles increased with host density. The equilibrium in beetle numbers per patch reflected a balance between immigration and emigration rates: beetle immigration rate was independent of host density, while beetle emigration rate decreased with increasing host density. In this study I found that the rate at which beetles were lost decreased with increasing hosts per patch, suggesting that beetles are more likely to remain in patches containing more hosts. Insects encountering suitable hosts may adjust local rates of movements to an "area restricted search" and settle more frequently where resources are more concentrated (Kareiva, 1983; Sheehan & Shelton, 1989). Area restricted search, which may be due to increases in turning frequency and reduced move lengths, has been reported for numerous phytophagous insects (Morris & Kareiva, 1991; Turchin, 1991). Enhanced emigration in smaller patches could also result from perimeter : area ratios increasing with decreasing host density and thus the border across which emigration occurs becomes disproportionately large (Kareiva, 1985). In other words small patches may be "easier to lose" than larger patches. The actual mechanisms contributing to more beetles in areas of higher host density could not be evaluated in this study. Future experiments on insect dispersal could test the alternative hypothesis by estimating beetle immigration and emigration rates for different host densities.

I will conclude with some remarks concerning methodology. Results suggest that night and day sampling were equally reliable, assuming that the single day/night period that I compared is representative of other sampling periods. For this species under the prevailing climatic conditions, the marking methods using powdered dyes was adequate for distinguishing marked from unmarked beetles, but not for distinguishing treatments in a multi-factorial design using a unique mixture of different colors for each treatment combination. There were two primary problems associated with marking flea beetles with

powdered dyes: (1) the powdered dyes washed off the beetles in the rain (2) the unique mixture of different colors ran together. As a consequence beetles could not be distinguished from one another after a few days in the rain. Future mark-recapture experiments of this type should use more permanent marks and reduce the number of treatment combinations so that only one or two colors have to be applied.

III. BEHAVIORAL AND REPRODUCTIVE RESPONSES BY THE RAGWORT FLEA BEETLE, LONGITARSUS JACOBAEAE TO CHANGES IN DENSITY OF RAGWORT SENECIO JACOBAEA

INTRODUCTION

The purpose of this study was to identify attributes of the ragwort flea beetle that may contribute to its efficiency as a regulator of ragwort abundance. New weed outbreaks occur from time to time, and place to place. A successful control organism must have high colonization and/or multiplication rates to discover and destroy incipient pest outbreaks before they develop into a wider infestation. In this study I measure and compare the behavioral (colonization) and reproductive (multiplication rate) responses of Longitarsus jacobaeae to a change in density of its host Senecio jacobaea in a field experiment conducted in western Oregon. Host density was manipulated by planting 1, 4, 8, or 16 rosette-stage plants (plants that are 10-20 cm tall and not bolted) per 0.5 x 0.5 m patch in fall 1992, and the responses to these manipulations were measured over one beetle generation.

Plant populations often exhibit a patchy distribution. How herbivores respond to a patchy environment can have important implications for herbivore-plant interactions (Kareiva 1985, Bach 1988a, 1988b), and the efficiency of biological control (Murdoch et al. 1985, Harris 1991, Reznik 1993, McEvoy et al. 1993). Studies have shown a diversity in herbivore movement patterns in relation to patch size and host density. A review of 19 herbivore species that were measured in relation to patch size per individual plant, found that 9 species were more abundant and 2 species were less abundant with increasing patch size, and 8 species revealed no effect (Kareiva 1983). A review of 13 species that were measured in relation to host density per individual plant, found that 2 species increased and 9 species decreased as host density increased, and 2 showed no change (Kareiva

1983). These studies suggest that herbivores often do not conform to Root's (1973) resource concentration hypothesis that predicts herbivore loads increase with increasing concentration of their food resources. However, in many of these studies the results depended on the temporal, spatial, and organizational scale of observation, and changes in host quality (e.g. size of hosts) were often confounded with changes in host quantity (e.g. number of hosts) (Kareiva 1983). More recent studies have tried to unravel these confounded effects. For instance, Horton and Capinera (1987) found host density effects on the Colorado potato beetle disappeared after correcting for variation in plant size, i.e., by changing the sampling unit from the plant to leaf area. Bach (1988a) reported no change in abundance of the striped cucumber beetle Acalymma vittatum in relation to patch size after correcting for plant size. The time scale of most studies has been too short to measure and compare behavioral and reproductive responses of herbivores to changes in host density. An exception was a study by Bach (1988a), who examined the effects of host patch size on the density of the spotted cucumber beetle over two generations and found that beetle densities per plant were greater in small patches for the first generation but became greater in large patches for second generation. It was suggested that this pattern may have been due to oviposition rates and/or larval survivorship being much lower in small patches.

It has been shown that the ragwort flea beetle readily colonizes new host patches, and strong positive correlations develop between insect density and host density per patch (McEvoy et al. 1993). Prior results suggest that (1) the number of beetles increases with increasing host density; (2) both behavioral (adult colonization) and reproductive responses (emergence) contribute to congregation of beetles in areas of high host density, but it remains to distinguish their relative contribution. The objective of this study was to measure colonization, emergence, and reproductive rates of the flea beetle in response to varying densities of ragwort (1, 4, 8, and 16 plants per patch).

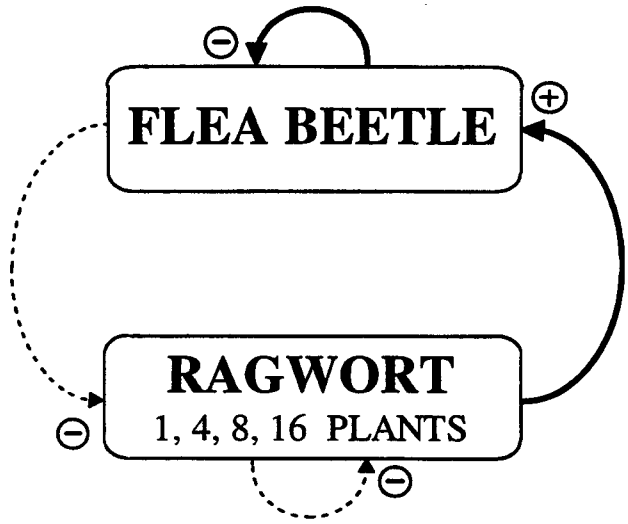


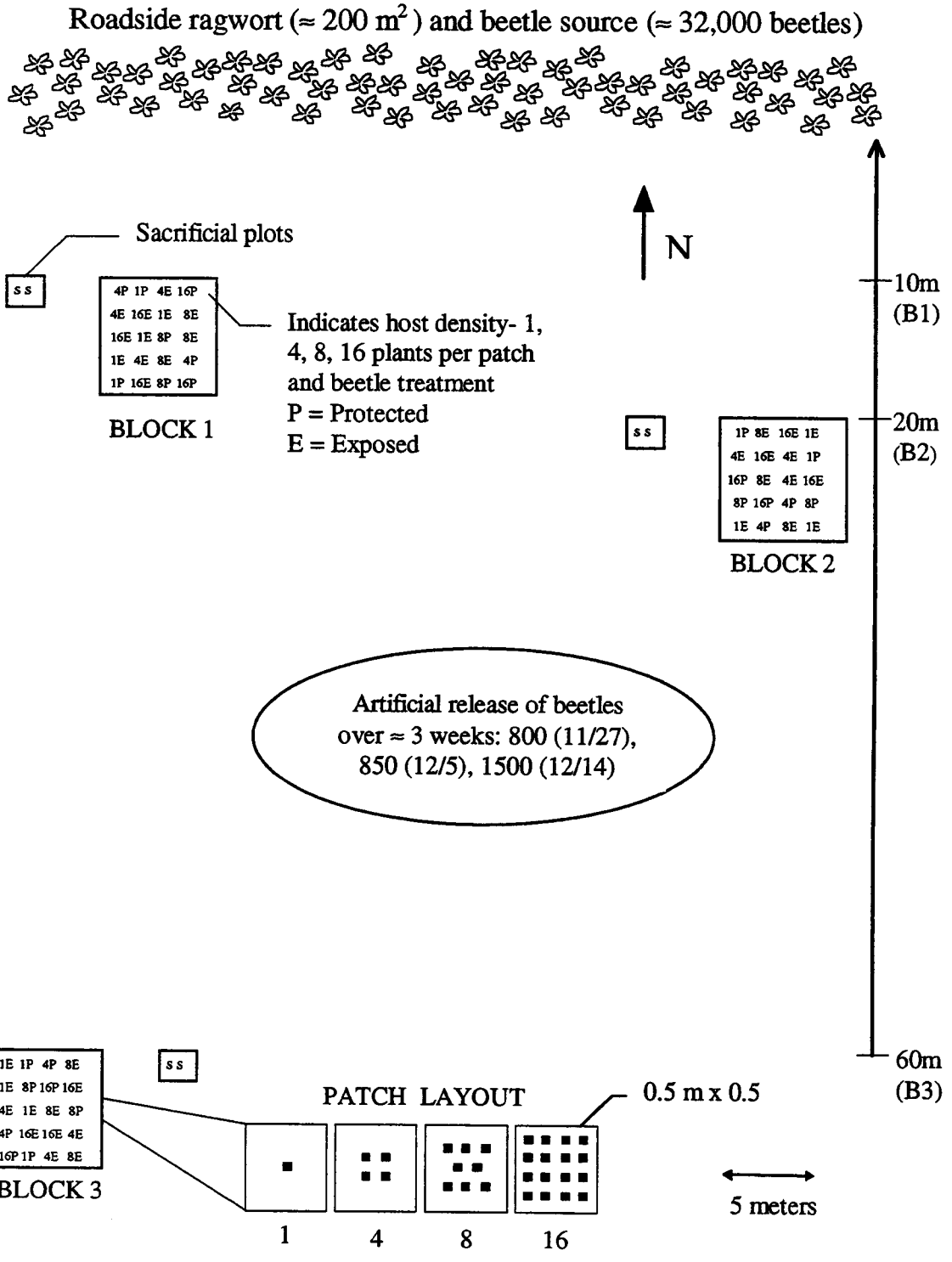
Figure 6. Signed diagraph representing the feedbacks in the ragwort-flea beetle L. jacobaeae interaction for part 1 of the second experiment. This study is represented by a solid line. The expectations are that an increase in plant density causes an increase in insect density, which in turn leads to a decrease in plant density. Increase in each population also has a negative feedback on itself.

METHODS

Study site

This is the first-part of a two-part field experiment manipulating insect and host densities to estimate the effect of (1) host density on the insect populations and (2) insect and host density on the host populations (Fig. 6). The first part of the experiment investigates the effect of increasing host density (1, 4, 8, 16 plants per patch) on insect populations. The experiment took place during 1992 and 1993 in a 0.9 ha meadow (Three Rocks Road South, 45°2' 2" N, 123° 58' 42" E) in Lincoln county on the central coast of Oregon within the Cascade Head Scenic Research Area (CHSRA). The meadow, last grazed by cattle in 1977, is currently grazed by elk Cervus elaphus and deer Odocoileus

Figure 7. Experimental layout for the 1992-1993 experiment conducted at CHSRA. This figure shows the location of the 3 blocks, (20 plots within each block), the allocation of 8 (flea beetle density x ragwort density) treatment combinations among plots within blocks, and 3 sacrificial plots outside of blocks reserved for destructive sampling. Locations of potential sources of colonizing beetles at the roadside and central release site (3 batches of beetles were released) and patch layout are also indicated. Distance of blocks from roadside beetle source is shown on the right


Roadside ragwort (≈ 200 m²) and beetle source (≈ 32,000 beetles)
Sacrificial plots
S S
4P 1P 4E 16P
4E 16E 1E 8E
16E 1E 8P 8E
1E 4E 8E 4P
1P 16E 8P 16P
BLOCK 1
Indicates host density- 1, 4, 8, 16 plants per patch and beetle treatment
P = Protected
E = Exposed
N
10m (B1)
20m (B2)
60m (B3)
S S
1P 8E 16E 1E
4E 16E 4E 1P
16P 8E 4E 16E
8P 16P 4P 8P
1E 4P 8E 1E
BLOCK 2
Artificial release of beetles over ≈ 3 weeks: 800 (11/27), 850 (12/5), 1500 (12/14)
1E 1P 4P 8E
1E 8P 16P 16E
4E 1E 8E 8P
4P 16E 16E 4E
16P 1P 4E 8E
BLOCK 3
S S
PATCH LAYOUT
0.5 m x 0.5
1
4
8
16
5 meters

spp. with some disturbance by fossorial rodents, mainly moles Neurotrichus or Scapanus spp. The vegetation consists mainly of perennial introduced grasses including Holcus lanatus L., Festuca arundinacea Shreb, Dactylis glomerata L., and Anthoxanthum odoratum L., with ragwort at very low density of 1-2 flowering plants per ha.

The experimental site has a maritime climate consisting of cool wet winters and warm dry summers. The following averages are based on normals for the period 1961-1990 (Oregon Climate Service 1994). The mean monthly temperatures range from 4.9°C in January to 20.3°C in August. The mean annual rainfall is 247 cm, with 69% of the rainfall occurring during the 5 months of November through March. Maximum, minimum, and mean temperatures for the period of this study were obtained from the Otis weather station (near Lincoln City, OR, approximately 8 km from the site).

Experimental design and layout

The experiment was a randomized complete block design consisting of 3 blocks with 4 levels of ragwort density (1, 4, 8, 16, plants per 0.25 m^2 patch) and 2 levels of exposure to beetles (exposed, protected). The exposed treatment was replicated three times and the protected treatment replicated twice within each block across all ragwort densities. Thus, the total number of patches was: 3 blocks x 4 levels of ragwort density (1, 4, 8, 16 plants per patch) x 2 levels of flea beetle density (plant patches either exposed or protected) x variable replication for each treatment combination (3 exposed, 2 protected) = 60 patches. Results reported in this paper are from the exposed level of the beetle treatment.

Plot size and spacing were as follows (Fig. 7). The experimental patches were arranged in 3 blocks. Blocks measured 6.5 x 5 m = 32.5 m^2 and were separated from each other by ~ 50 m. The patches measured 0.5 x 0.5 m = 0.25 m^2 and were separated from each other within blocks by 1 m. Host density was varied by planting different

number of equally-spaced plants within a fixed area. Since I maintained constant spacing between plants within a patch, increasing the number of plants in a patch increased the area of the plant stand. Henceforth, the number of plants per $0.25m^2$ patch will be referred to as host density.

The spacing of plots was appropriate for both practical and scientific reasons: (1) it allowed use of prior experimental plots which were protected from disturbance by fossorial rodents using buried hardware screen (McEvoy et al. 1993); and (2) the spacing of plots was close enough to allow a thorough mixing of the beetle population from which colonists were recruited. The area between patches was mowed twice, and patches were weeded 4-5 times throughout the experiment to exclude interspecific plant competition. Two other patches, referred to as sacrificial patches, were planted without rodent exclusion screen in the vicinity of each of the three experimental blocks (Fig. 7). These patches were harvested destructively prior to harvesting experimental patches to monitor the stage of larval development.

Manipulating plant and insect density

Manipulating plant density. I fixed the carrying capacity for the plant by clearing and tilling a standard area within the background vegetation. Plant density was manipulated by planting ragwort in densities of 1, 4, 8, or 16 plants per 0.5 x 0.5 m plots in the field. Ragwort seeds collected from CHSRA were germinated in a greenhouse at Oregon State University, Corvallis, Oregon, during July and August 1992. Seedlings were later transplanted individually to pulp pots (14 cm square x 12.7 cm deep) containing a soil medium consisting of soil, peat moss, sand, and pumice (1:1:1:2 parts respectively). Plants were fertilized with 20% N, 20% P, 20% K (Grace-Sierra Horticultural Products Company, Milpitas, California), and watered and weeded as necessary. Upon reaching

rosette stage (plants that are 10-20 cm tall and not bolted), plants were transplanted to the field and randomly assigned to blocks and treatments on 7 and 8 November 1992.

Manipulating herbivore levels. The second part of this experiment, investigating the effect of the beetle on ragwort, required the use of cages to create two levels of beetle density (plants were either exposed and protected). In this study I sampled beetles only from exposed plots. Plots were either protected from herbivores using closed cages or exposed to herbivores using open, sham cages. The use of sham cages helped ensure that any unwanted side effects of cages would be experienced equally by both exposed and protected patches. Cages and sham cages were similar except the screen walls of sham cages were rolled up from the ground about 15-25 cm (the rolled screen stayed in place by friction) to allow full beetle access. The cages measured 61 cm x 61 cm x 61 cm (outside dimension) and were constructed of 22 mm (OD) PVC pipe sections with couplers and covered with fine mesh screen "Leno weave" bags (open spaces in the mesh were 0.6 x 1.0 mm). To accommodate plant growth, cages were extended in height using PVC pipe sections and couplers. Frames were 61 cm, 122 cm, or 183 cm high depending on the height of the ragwort plants.

Beetles were allowed to colonize, from the surrounding area, the experimental patches assigned to the exposed level of the beetle treatment. A survey of the area to the north of the study site showed various densities of ragwort and beetles depending on location. For example, the 200 m^2 area along the roadside just north of the study site (Fig. 7) averaged 203g/m^2 (SE = 32.4) of ragwort and an average of 163 beetles/m^2 (SE = 35.1).

To increase the potential number of beetles colonizing the plots I collected beetles from an area to the North and West of the site with a vacuum net (Echo model No. ES-1000, Echo Incorporated, Lake Zurich, IL), and released at a point equidistant from all blocks about 800, 850, and 1500 beetles (Fig. 7) on 27 November, 5 November, and 14

December 1992 respectively. On the first two releases I marked about 100 beetles with a yellow dye in order to evaluate the contribution of the artificial releases to the number of beetles in experimental patches.

Measuring insect responses

Changes in beetle density within and between generations. The response of the beetles to treatments was evaluated as changes in beetle density from adults in one generation to larvae and adults in the next. I then used these measurements to derive estimates of insect colonization and multiplication rates. Censusing methods differed between generations: first generation adults were censused with replacement (beetles were aspirated and re-released into their original patch to avoid counting beetles more than once on a single census date) in open plots, while second generation adults were censused without replacement in closed plots. Thus the same individual could be counted on more than one date in the first generation but not in the second generation.

Colonization of adults of the first generation. Colonization of adults in the first generation was estimated by counting the total number of adults within a patch on 10 dates spaced at approximately 1-4 week time intervals (15, 22, 27 November; 5, 14, 23 December 1992; 22, 29 January; 6, 27 February 1993). Colonization curves were obtained by plotting mean beetle density per patch as a function of time separately for each level of host density. I used beetle-days as an integrative measure reflecting both beetle intensity and duration. Beetle-days were estimated for each exposed plot by integrating the area under the colonization curve using the formula $\Sigma\ 0.5\ (N_{t+1} + N_t)\ (D_{t+1} - D_t)$, where N_t is density at time t, and D_{t+1} is density at next sample date. For colonization by the first generation, the relationship between beetle density and host density was

summarized by plotting mean adult-beetle-days per patch, per plant, and per unit of host biomass as a function of host density.

Reproduction. Reproduction by beetles in the first generation was represented by numbers of larvae in the second generation, estimated by destructively sampling 3 replicates (1 from each block) of each of 8 (2 Beetle x 4 Host Density) treatment combinations on a single date 25 March 1993. Estimates of larval density allowed me to assess (1) efficiency of exclusion procedure for the second part of the experiment, (2) changes in beetle density within generations from adults to larvae to adults.

Larvae were extracted using the heat-extraction protocol of James et al. (1992). Plants within each plot were placed in a Tulgren funnel with a 25 watt light bulb for 7 days or until the plants were dry. Larvae were collected in jars containing 70% alcohol and then counted in each sample or subsample. Subsampling using the protocol of Southwood (1978) and James et al. (1992) was used when larvae were numerous (usually > 20 larvae per sample). A petri dish containing larvae and alcohol was agitated to achieve complete mixing and then allowed to settle. The petri dish was placed over a counting disc and those larvae within the clear areas of the disc were counted and they represented a fraction of the total depending on the disc used. The relationship between beetle larvae density and host density was summarized by plotting the total number of larvae as a function of host density.

Emergence by adults of the second generation. Emergence of adults in the second generation was estimated by collecting and counting emerging adult beetles on 8 dates spaced at approximately weekly intervals except for the penultimate census (14, 23 July; 4, 15, 24, 31 August; 3, 10 September 1993). To trap emerging beetles, mesh bags on the sham cages (exposed patches) were pulled down and held firmly to the ground with sand bags on 23 April 1993. Emerging beetles were removed each time after a census so that number of beetles emerging as a function of time could be evaluated. Beetles removed

from cages were later sexed to determine sex ratio of second generation beetles at the time of emergence. Emergence curves were constructed by plotting mean density as a function of time separately for each level of host density. The relationship between beetle density and host density was summarized by plotting the cumulative number of emerging adults per patch, plant, unit host biomass as a function of host density. Sex ratio (proportion of males) was plotted as a function of host density.

Population growth. The observed multiplication rate (R_o) of the beetle population was calculated using two methods: (1) peak number of second generation beetles was divided by peak number of first generation beetles for each host density; (2) total number of second generation beetles was divided by peak number of first generation beetles for each host density. The first method assumes synchronous colonization and synchronous emergence, that is, it was assumed that all beetles throughout fall and summer periods were in adult stage. Synchronous emergence is clearly not true since beetles emerged over a two month period. This method will likely lead to underestimation of the size of the second generation and underestimation of the rate of multiplication. The second method assumes synchronous colonization and asynchronous emergence. This assumption is more realistic, however it does not take into account all beetle mortality, that is beetle mortality that would have occurred had I let all beetles stay in the field until September (I collected beetles from patches as they emerged). Therefore, this method will likely lead to a slight overestimation of beetle reproductive rates. The true reproductive rate is likely lie somewhere in between the two estimates.

Data analysis

The relationship between timing of colonization and distance from beetle source was analyzed using a MANOVA repeated-measures model. The data for December 5 (low beetle counts associated with cold weather) was considered an outlier and removed

for the repeated-measures analysis. The Michaelis-Menten equation ($Y = x / (a + bx)$) was used to fit the colonization curve represented by beetle-days plotted as a function of host density. The number of beetle-days and cumulative beetles per patch as a function of host density was analyzed by regressing $\log_e$ number of beetle-days on $\log_e$ host density. Log-transformation helped to linearize the relationship and stabilize the variance. All statistical analyses were performed using SYSTAT for windows, version 5.02 (SYSTAT, Inc. 1992). The significance level used was 5%.

RESULTS

Behavioral response to an increase in host density. The timing of colonization varied across levels of host density and across blocks (Fig. 8B). The number of colonizing beetles in most cases increased to a maximum in 5-10 weeks and then decreased. Block x time and patch size x time interactions indicate that colonization of blocks and patches was not consistent across time (Table 2A). The time-delay in beetle colonization roughly increased with distance from the roadside source. Only a single marked beetle out a total of 200 was found on 14 December 1992 from the artificial release. A sharp decline in beetle numbers on 5 December 1992 was associated with unusually cold weather (Fig. 8A). The maximum temperature (recorded at the Otis weather station) on December 5 was 3°C, whereas the maximum temperature for the other sampling days ranged between 8°C and 14°C.

The number of colonizing beetles integrated over time, represented by number of beetle-days per patch, increased in a convex asymptotic fashion (less than linear) as host density increased (Fig. 9). The slope of the relationship decreases most sharply in the transition from 1 to 4 plant patches. Levels of colonization represented by number of

Table 2. Analysis of host density effects on flea beetle L. jacobaeae colonization, emergence and sex ratio for the 1992-1993 experiment conducted at CHSRA. (A) Repeated measures MANOVA on colonization of patches and blocks as a function of time. ANOVA on (B) colonization rate integrated over time ($\log_e$ beetle-days per patch) in relation to host density ($\log_e$); (C) emergence rate ($\log_e$ number of beetles per patch) in relation to host density ($\log_e$); (D) sex ratio (Sqrtarcsin proportion of males) in relation to host density.

(A) Colonization as a function of time

Repeated-measures analysis (Pillai Trace)	df	MS	F	P
Time x Host density	24	57	2.712	0.001
Time x Block	16	36	2.581	0.009

(B) Colonization rate

Source of variation	df	MS	F	P
Host density (H)	3	5.918	29.248	< 0.001
Block (B)	2	2.897	14.316	< 0.001
H x B	6	0.076	0.378	0.886
Error	24	0.202		

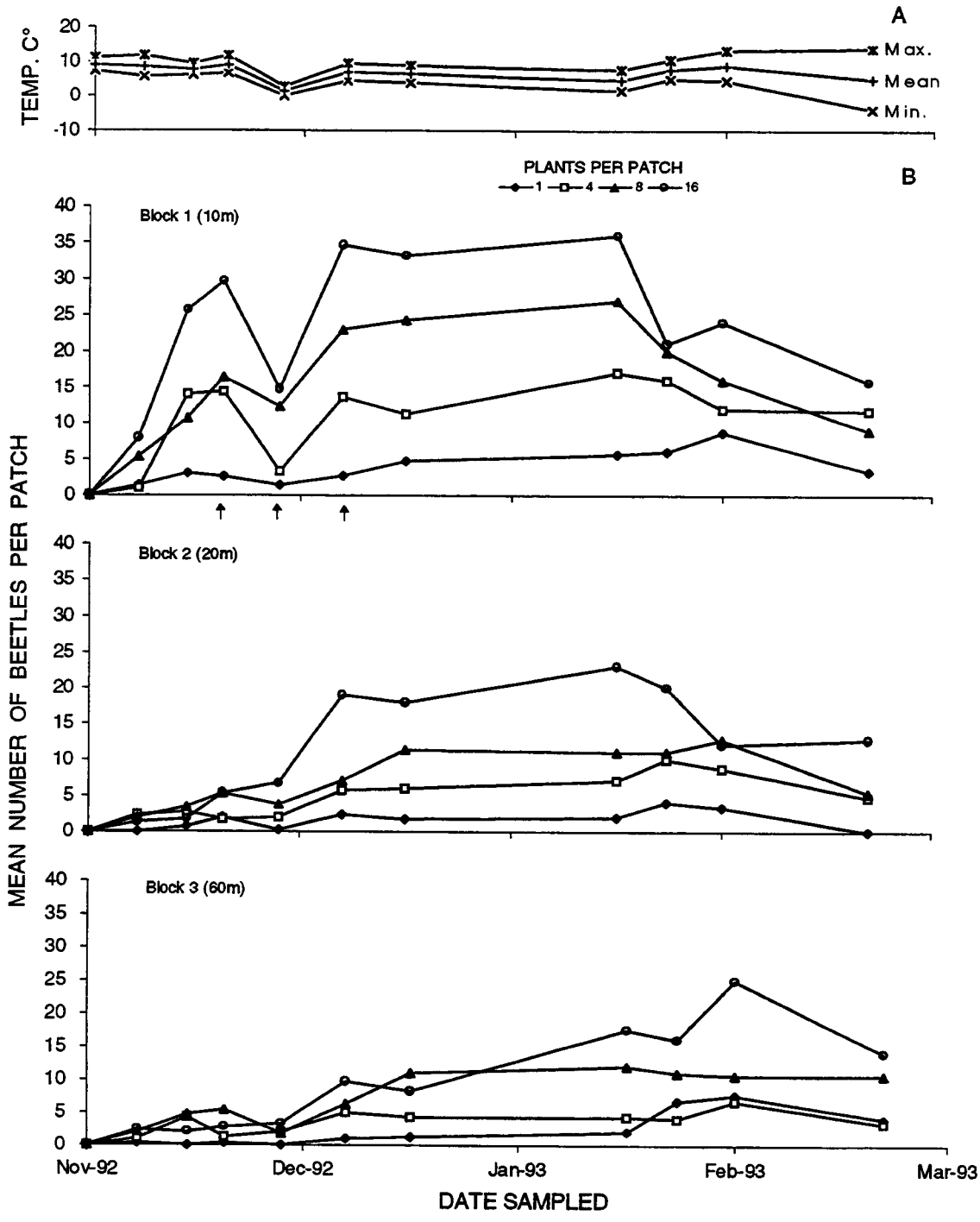
(C) Emergence rate

Source of variation	df	MS	F	P
Host density (H)	3	3.399	12.758	< 0.001
Block (B)	2	0.408	1.532	0.256
H x B	6	0.165	0.622	0.710
Error	12	0.266		

(D) Sex ratio of emerging beetles

Source of variation	df	MS	F	P
Host density (H)	3	0.024	12.149	0.001
Block (B)	2	0.001	0.427	0.662
H x B	6	0.004	2.243	0.110
Error	12	0.002		

Figure 8. Flea beetle L. jacobaeae colonization as a function of temperature and host density during the 1992-1993 experiment conducted at CHSRA. (A) Daily maximum, minimum, and mean temperatures as a function of time for 3 Rocks south pasture. (B) Mean number of adult flea beetles per patch colonizing in the first generation from outside the experimental array as a function of time for each block and level of host density. Arrows below block 1 indicate dates when beetles were artificially released and distance from roadside beetle source is shown in parenthesis next to the label indicating block number.


A
TEMP. C°
20
10
0
-10
Max.
Mean
Min.
PLANTS PER PATCH
1
4
8
16
B
Block 1 (10m)
Block 2 (20m)
Block 3 (60m)
MEAN NUMBER OF BEETLES PER PATCH
40
35
30
25
20
15
10
5
0
Nov-92
Dec-92
Jan-93
Feb-93
Mar-93
DATE SAMPLED

beetle-days ($\log_e$) as a function of host density ($\log_e$) varied across blocks and host density (Table 2B). The density of beetles (mean beetle-days per patch) declined non-linearly with the distance of each block from the roadside source, from 1647 beetles in Block 1 (10 m from roadside), 809 beetles in Block 2 (20 m from roadside), and 732 beetles in Block 3 (60 m from roadside) (Fig. 7). The absence of a Block x Host density interaction indicate that the relationship between insect density and plant density was consistent across all blocks (Table 2B).

Although number of beetle-days ($\log_e$) per patch increased ($P < 0.001$, $R^2 = .61$; Fig. 10A) with host density ($\log_e$), number of beetle-days ($\log_e$) per plant (Fig. 10C) and

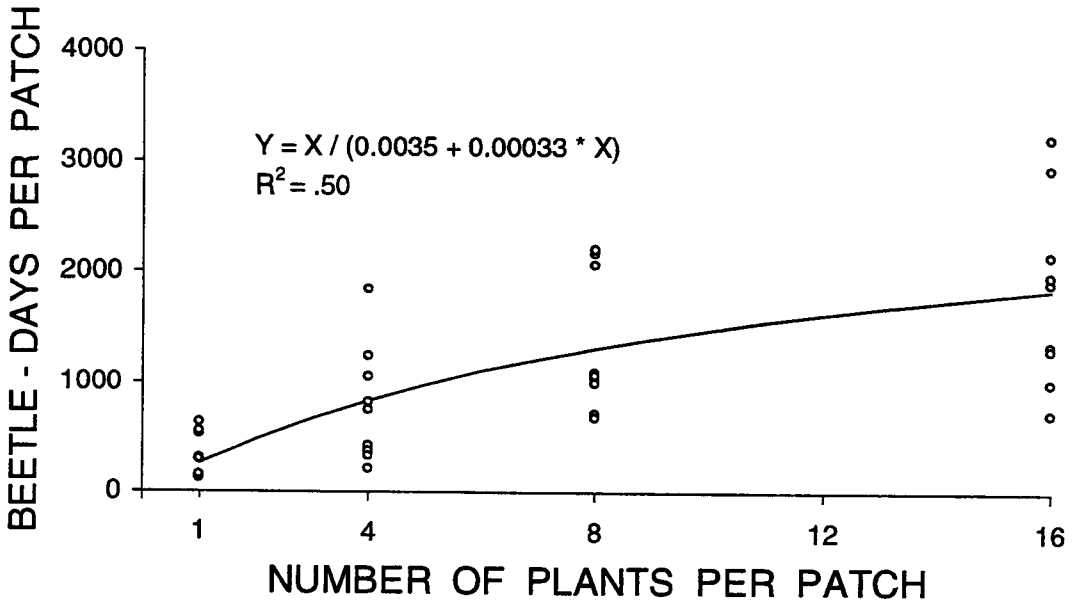


Figure 9. Flea beetle L. jacobaeae colonization as a function of host density during the 1992-1993 experiment conducted at CHSRA. Numbers of adult flea beetles (beetle-days) colonizing in the first generation from outside the experimental array integrated over time as a function of host density. Michaelis-Menten equation ($Y = x / (a + bx)$) was used to fit the colonization curve.

per dry mass of host (Fig. 10E) decreased with host density. First generation beetles per-plant and per-dry mass of host as a function of host density showed the same pattern, indicating that even when differences in plant size were taken into account the number of beetles declined with increasing host density.

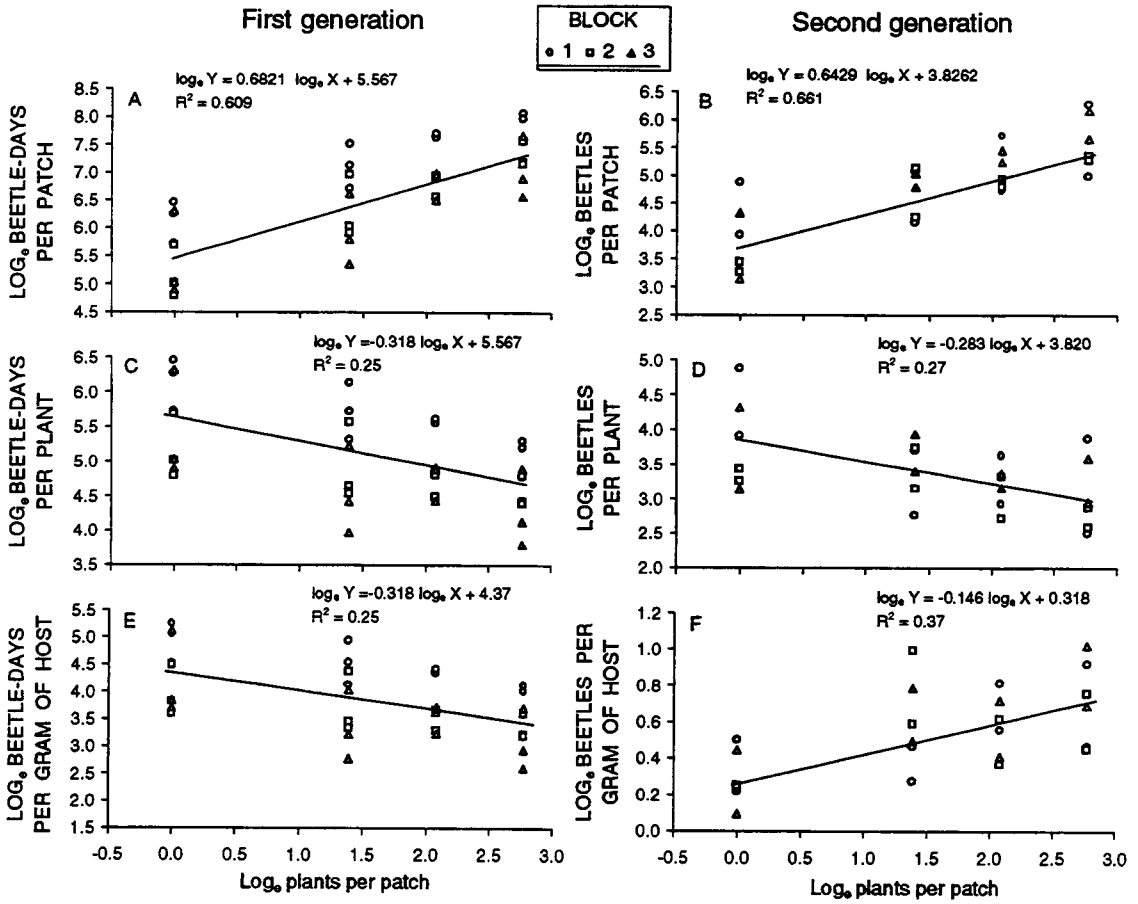


Figure 10. Flea beetle L. jacobaeae colonization and emergence as a function of host density during the 1992-1993 experiment conducted at CHSRA. Numbers of flea beetle adults ($\log_e$ beetle-days) colonizing in the first generation from outside the experimental array integrated over time per (A) patch (C) plant (E) dry gram of ragwort as a function of host density ($\log_e$); Numbers of flea beetle adults ($\log_e$) emerging in the second generation per (B) patch (D) plant (F) dry gram of ragwort as a function of host density.

Reproduction. The number of larvae varied erratically with number of hosts: the number of larvae increased with the number of hosts for the range of 4-16 hosts, but the number of larvae in 1-plant patches was inexplicably high (Fig. 11). Beetle larvae in exposed plots were 76% first and 24% second instars.

Emergence by adults of the second generation. The timing of emergence was similar across levels of host density and across blocks (Fig. 12). The number of beetles in the second generation began to increase on 23 July 1993, rose to a maximum between the dates of 4 August 1993 and 15 August 1993 and declined thereafter. The period of beetle emergence lasted about 111 days with average number of beetles per patch declining to about 20 beetles or less by 10 September 1993. The total number of adult beetles emerging per patch (Fig. 10B, Table 2C) and per dry mass of host (Fig. 10F) for the

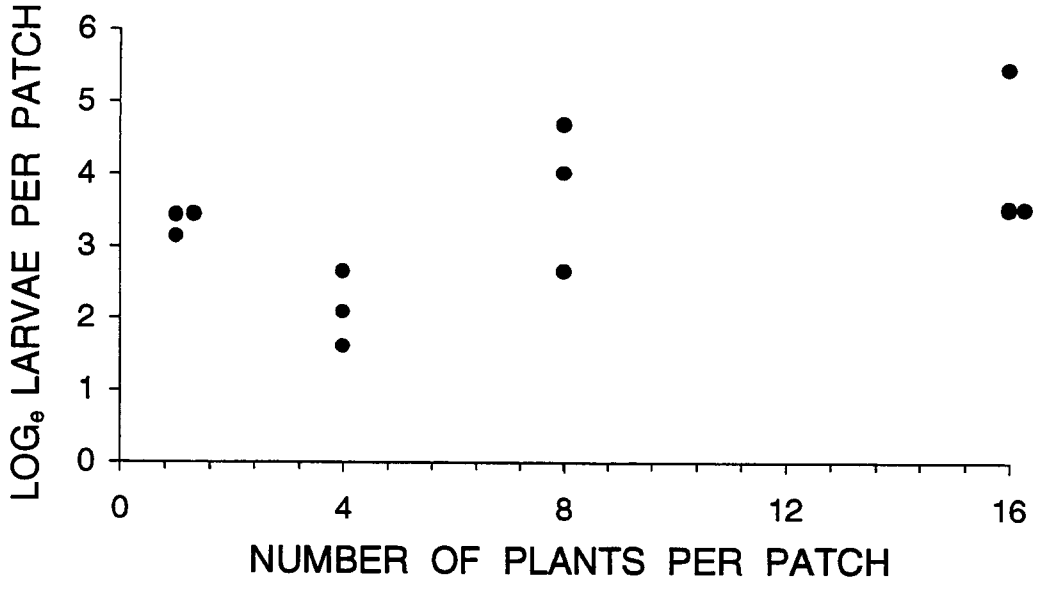


Figure 11. Number of flea beetle L. jacobaeae larvae (loge number of larvae per patch) as a function of host density. Larvae were sampled on 25 March 1993 at CHSRA

second generation increased with increasing host density, while the number of beetles per plant declined with increasing host density (Fig. 10D). Adult beetle density per patch increased with increasing host plant density in both fall and summer beetle generations. A block effect existed for fall colonization but there was no block effect for summer emergence. Thus initial differences in the relationship between beetles and hosts among blocks due to vagaries of colonization disappeared after one beetle generation.

Population growth. There was a 5 to 10.4-fold increase in beetle population as estimated by multiplication rate 1 and 2 respectively (Fig. 13). By visual inspection, it

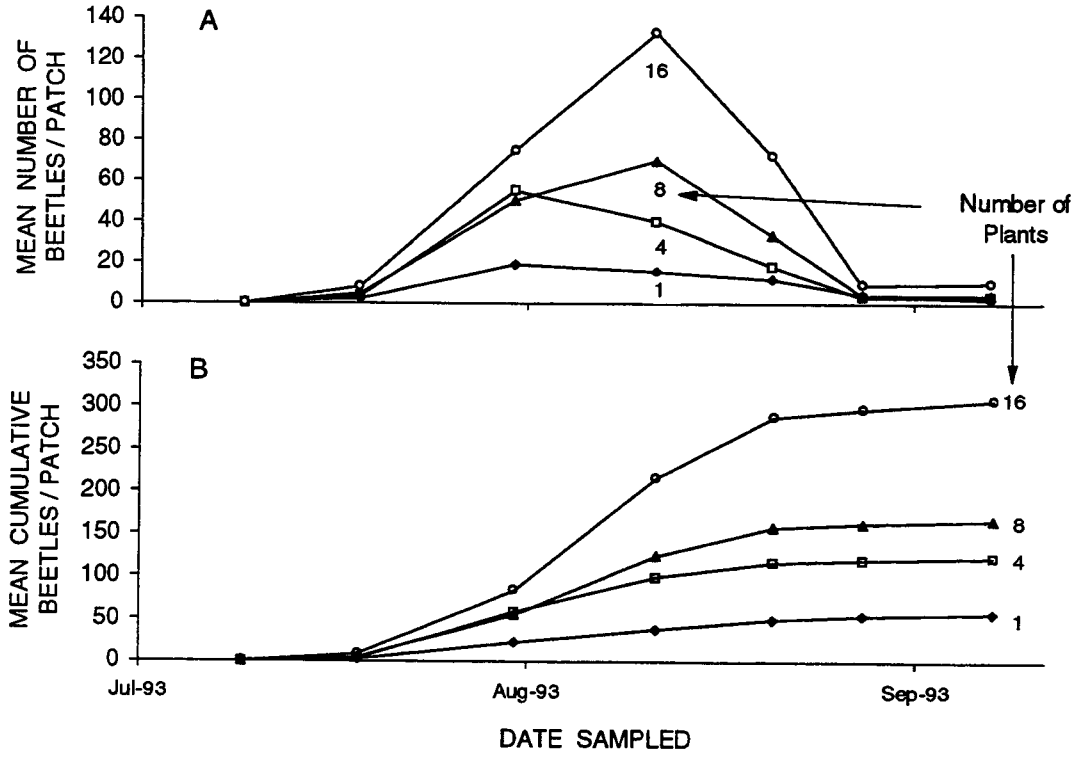


Figure 12. Flea beetle L. jacobaeae emergence as a function of time during the summer of 1993 at CHSRA. (A) Mean number of adult flea beetles emerging per patch in the second generation as a function of time for each level of host density. (B) Mean number of cumulative adult flea beetles emerging in the second generation as a function of time for each level of host density.

appears that beetle reproduction for single plant patches was inexplicably low and then increased and leveled off for 4, 8, and 16 plant patches. The sex ratio (Sqrtarsin) for beetles emerging in the second generation decreased with increasing host density (Fig. 14;

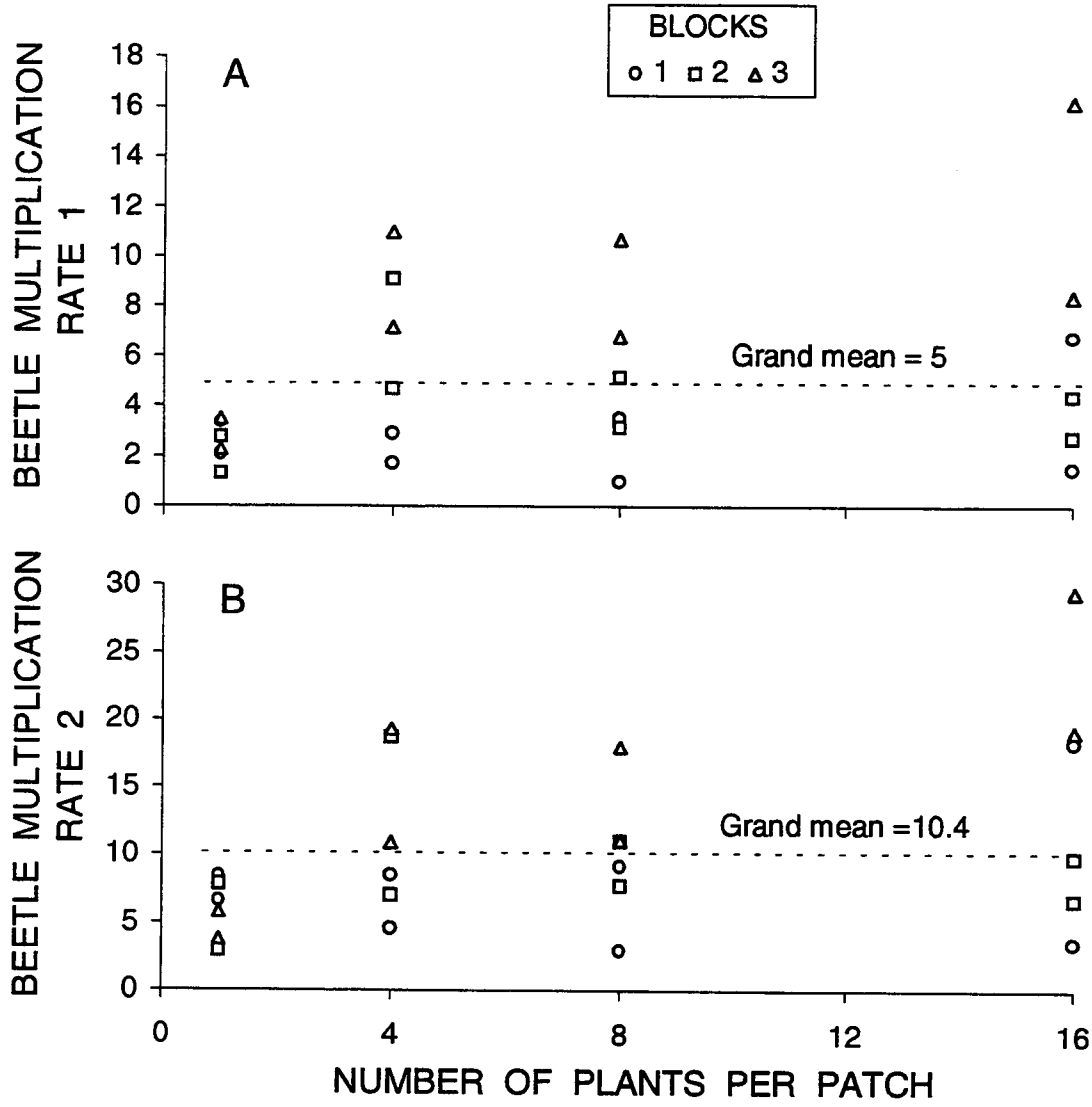


Figure 13. Multiplication rates of the ragwort flea beetle L. jacobaeae for the 1992-1993 experiment conducted at CHSRA. (A) multiplication rate 1 and (B) multiplication rate 2 of the beetle population as a function of host density.

Table 2D). This indicates that proportionally more female beetles emerged from areas of high host density. The relationship between beetle sex ratio (sqrtarcsin) and host density did not vary with block.

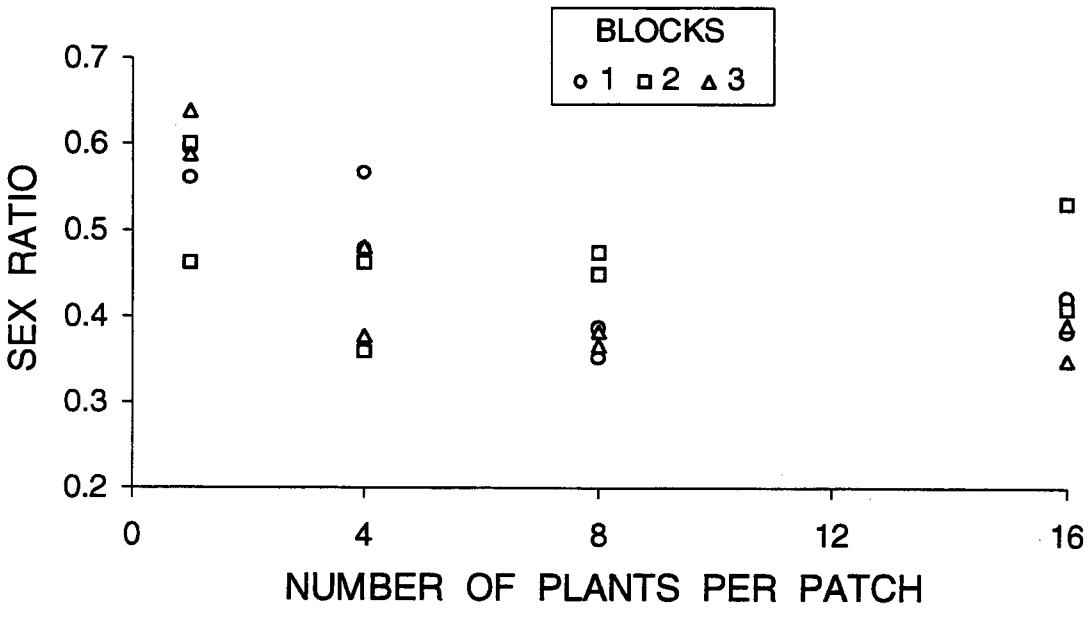


Figure 14. Sex ratio of second generation beetles L. jacobaeae per patch as a function of final host density during the summer of 1993 at CHSRA.

DISCUSSION

Behavioral response to an increase in host density

Beetle population increases in the first generation were due solely to immigration since plants were devoid of flea beetles when transplanted. Beetles readily colonized at all

plant densities (Fig. 10B). This supports prior results of McEvoy et al (1993) that suggests there is no minimum threshold host population size required for L. jacobaeae colonization. This contrasts with a finding by MacGarvin (1982) in which Altica sp. was not present on hosts within patches of less than $200m^2$. Similarly, Kareiva (1981) found both Phyllotreta cruciferae and P. striolata to be absent on single collards. It is desirable to have a biological control agent with the searching capacity to find and persist on single plant patches, for it reduces the delay and uncertainty in the arrival of natural enemies at the site of an incipient ragwort outbreak (McEvoy et al. 1993). The level of colonization increased with increasing host density (Fig. 8B). Looking at block 1, there was evidence of beetle presence in all patches (mean number of 1.3, 1, 5.3, 8 beetles for 1, 4, 8, 16-plant patches respectively) by 15 November the second week of the experiment. Beetles tended to colonize all host densities within a relatively short time.

Although the level of colonization increased with increasing host density, beetle density leveled off at the higher densities (Fig. 9). Two possible explanations for saturation in the response are the number of beetles colonizing a patch is limited by (1) the number of beetles in source populations outside the experiment, (2) the number of beetles that have already colonized a patch. If the source population outside the experiment was relatively low, then the number of hosts in experimental ragwort patches could have overwhelmed the beetle population. On the other hand, beetles that have already colonized a patch may compete with incoming beetles once the beetle population gets large enough. The leveling off of beetles at higher host densities was also indicated by beetle intensity (numbers of beetles per plant) and herbivore load (numbers of herbivores per unit of host biomass) declining with increasing number of hosts per patch (Fig. 10C, E).

I analyzed the data further by transforming the variables and performing linear regression. Statistical analysis showed that beetle-days ($\log_e$) per patch ($\log_e$) increased

with increasing host density (Fig. 10A). The theory of island biogeography predicts that immigration rates increase with increasing patch area, that is insects are more likely to find plants growing in large, dense patches. Zhang and McEvoy (in press) found that ragwort flea beetles, in lab wind tunnel experiments, orient towards upwind host plants over a distance of 60-300 cm, however the strength of the beetle orientation response to hosts did not vary with an increase in the number of hosts from one to six plants. This suggests that beetles do not increase with increasing patch size due to density dependent immigration. An alternative hypothesis, that emigration rates decline with increasing patch size, suggests that insects encountering suitable hosts may adjust local rates of movements to an "area restricted search" and settle more frequently where resources are more concentrated (Kareiva, 1983; Sheehan & Shelton, 1989; Morris & Kareiva, 1991). Area restricted search, which may be due to increases in turning frequency and reduced move lengths, has been reported for numerous phytophagous insects (Morris & Kareiva, 1991; Turchin, 1991). Increased emigration from smaller patches could also result from perimeter:area ratios increasing with decreasing host density and thus the border across which emigration occurs becomes disproportionately large (Kareiva, 1985). In other words small patches may be "easier to lose" than larger patches. The actual mechanisms contributing to more beetles in areas of higher host density in this system could be further tested by estimating beetle dispersal rates, that is immigration and emigration rates, for different host densities.

Influence of temperature on colonization. The level of colonization appeared to be affected by temperature. The drop in beetle counts on 5 December 1992 was probably due to unusually cold weather (maximum temperature was 3°C) (Fig. 8A). Field observations suggest beetles are likely to be found on the plants during warm sunny days and on the ground during cool wet days. Zhang & McEvoy (in press) investigated ragwort flea beetle response to temperature (0, 5, 10, 15, 20, 25, 30°C) in a lab

experiment and found that beetles left leaves at 0 and 5°C. The only sampling date where the maximum temperature fell below 5°C was 5 December 1992 and beetle counts dipped on that date. Although beetles may have been in the vicinity of the patch during sampling they were not detected because they probably had fallen from the plant and were hidden in litter or blended in with the soil background.

Reproductive response to an increase in host density

Reproduction. The larval data proved to be unreliable for estimating the total number of individuals passing through the larval stage (Fig. 11). The sample taken represented a single snap-shot in a moving picture, and larval density at one time may underestimate the total number of individuals passing through the larval stage. Problems included: (1) asynchronous development, 76% of the larvae were 1st instar, 24% were 2nd instar and 0% were 3rd instar, suggesting that some eggs may have not entered the larval stage and therefore were not sampled; (2) imprecision due to small sample sizes which arose because larval sampling required destructive sampling, and sacrificing more replicates for larval population estimates would have jeopardized subsequent estimates of beetle populations; (3) bias or underestimation because for generation 2 the number of emerging adults exceeded estimates of larvae.

Emergence by adults of the second generation. The reproductive response (second generation adults) to an increase in host density was due solely to local reproduction since patches were completely closed to immigration during summer. Beetles readily reproduced in all host densities: cumulative number of beetles emerging from 1, 4, 8, and 16 plant patches respectively was: 23-131, 63-168, 112-301, 145-528. This suggests there is no minimum threshold host population size required for beetle reproduction. This attribute is desirable because it means a better chance of establishment

in small patches, fewer augmentative releases of the biocontrol agent, and ultimately a better chance of successful control.

The timing and level of reproductive response did not vary with block (distance) as it did during the earlier colonization phase. While colonization may be expected to vary with distance, reproduction should be independent of distance and depend on environmental conditions.

Population growth. The observed multiplication rate (R_o) of the beetle population in this study was calculated using two methods. Under the first method, the mean observed multiplication rate of 5 probably underestimated the actual beetle population growth. Under the second method, the mean observed multiplication rate of 10.4 probably overestimated the actual beetle population growth. The true reproductive rate is likely to lie somewhere in between these two estimates. Bach (1980), using the same method that was used for calculating the first multiplication rate in my study found that the rate of increase (R_o) for the striped cucumber beetle Acalymma vittata (Coleoptera: Chrysomelidae) was (mean ± SD) 1.9 ± 1.7 for low density plots (77 cm spacing between all plants) and 2.2 ± 1.0 for high density plots (56 cm spacing between all plants). The relatively low reproductive rate for A. vittata was probably due to the presence of natural enemies. For comparison, it is interesting to look at the reproductive rate of an herbivorous insect raised under lab conditions. The net reproductive rate (R_o) of Trichobaris bridwelli (Coleoptera: Curculionidae), a potential agent for the biological control of Datura stramonium (Solanaceae), was estimated to be 43.35 at 24°C (Cuda and Burke 1991). The number of progeny produced per individual per generation for T. bridwelli was relatively high because the environmental conditions were ideal and there were no natural enemies.

Combining behavioral and numerical responses

Resource density can be viewed at different scales-- patch, plant, parts. It is useful to consider ways in which the description of insect responses might depend on the perspective of the observer. There is no single correct way to view these results, but examining the results from a variety of points of view might help assess the origins and consequences of insect responses. For colonization and emergence, increase in the number of plants per patch led to an increase in the number of beetles per patch, but a decrease in the number of beetles per plant. The change in pattern (from decreasing number of beetles with increasing host density to increasing number of beetles with increasing host density) found for beetles per gram of host can be explained. During colonization by first generation beetles, plant biomass varied in direct proportion to plant numbers, so beetles per plant and beetles per mass yielded similar relationships. By emergence of second generation beetles, plant biomass was nearly independent of plant numbers because growth rates decreased with increasing host density. The faster growth rate of plants at lower host density tended to "dilute" the number of beetles, leading to a decrease in herbivore load with increasing host density.

Behavioral and numerical responses to host density. Few studies have investigated the relative contribution of colonization and reproduction to increases in host density. Kareiva (1983) suggests that plant density and dispersion influences herbivore densities primarily by altering herbivore movement or searching and colonizing behavior rather than altering herbivore reproduction rates. I found that ragwort flea beetles responded to changes in host density primarily through changes in their behavioral (colonization) rather than reproductive (emergence) response: (1) the slope of the relationship between beetle density (per patch and per plant) and host density established in the colonization phase (reflecting a balance between immigration and emigration rates) was essentially unchanged after the emergence phase (reflecting a balance between birth

and death rates) (Fig. 10A, B); (2) the reproductive rate of the herbivore population appears to increase then level off for the 4, 8, and 16 plant patches (Fig. 13). This suggests that the increase in the level of emergence was a passive consequence of increase in the level of colonization with increasing host density.

The consistent pattern between generations of L. jacobaeae in response to host density in this study contrasts with a pattern Bach (1988a) found while investigating the effects of host patch size on the density of the spotted cucumber beetle over two generations; beetle densities per plant were greater in small patches for the first generation but became greater in large patches for the second generation.

Patterns deserving further study

Influence of beetle source. Immigration may have varied with distance from source in this study, but the evidence is circumstantial. The experiment had at least two sources for beetles, a large existing population at roadside (N_R) bordering the field, and a small release population that I introduced at the center of the field (N_C) (Fig. 7). Three lines of evidence suggest that the natural population at the roadside probably contributed more beetles to the experiment than the release population at the center of the field: (1) the number of beetles from N_C (800-1500) was only 2-5% of that estimated from N_R (33,000); (2) N_C made virtually no detectable contribution, since only one out of 200 individuals marked and released on 27 November and 5 December 1992 at the center was recovered in the experiment, and there was no visible change in beetle density within the experiment following the release outside the experiment. However, there was no parallel study to see whether individuals marked in the roadside population could be recovered from the experiment; (3) N_C population was equally distant from blocks whereas N_R population differed in distance from blocks. Since I found beetle density declined with

distance from N_R and beetle density showed no relationship with N_C it is likely that N_R is the major beetle source

Assuming that most beetles immigrated from the roadside source it is possible that the level and timing of colonization was affected by distance from the source. A block x time interaction for first generation beetles indicates the timing of the response varied among blocks. A possible explanation, is that the number of beetles were higher and peaked sooner for blocks closer in proximity to the beetle source (Fig. 7). For example, the time required to reach maximum level increased from 5 weeks at 10 m in Block 1, 11 weeks at 20 m in Block 2, and 13 weeks at 60 m in Block 3. The pattern of fewer beetles in more distant plots is consistent with the theory of Island Biogeography which predicts colonization rates reflect a balance between immigration and emigration, and immigration rates decline with distance from the source (MacArthur & Wilson 1967). An interesting path for further investigation would be to systematically investigate the influence of distance from the source.

Sex ratio. I found that larger patches produced proportionally more female offspring than small patches (Fig. 14). This might lead to the prediction that a biological control agent that produces more female offspring in response to increased host density would have a higher degree of success at preventing pest outbreaks and therefore be a better biological control agent. A parallel study showed that when a constant number of female beetles were placed on varying host densities there was a rapid redistribution to an equilibrium state. So that although there appears to be a potential for a higher reproductive rates in areas of high host densities, in fact what may happen, is that emerging female beetles redistribute themselves and spread the reproductive potential equally among all host densities. To test this hypothesis another experiment controlling sex ratio and beetle density is needed.

CONCLUSION

The behavioral response was dependent on host population size: the numbers of colonizing beetles increased with increasing plant density, but leveled off at higher plant densities. In contrast, the numerical response, represented as observed multiplication rate, appears to increase then level off for the 4, 8, and 16 plant patches. Combining these results, the beetles apparently respond to spatial variation in the density of hosts primarily by changes in their movement behavior rather than by changes in their per capita reproductive rates. This was conjectured by earlier reviewers (Kareiva 1983), however, prior to this, few studies were of sufficiently long duration to measure and compare the relative contributions of the herbivore's behavioral (within generation) and numerical (between generation) responses to an increase in plant density (but see Bach 1980, 1988a, 1988b, Horton and Capinera 1987). This result highlights the importance of a natural enemy's colonizing behavior for controlling a sudden upsurge in host abundance and suggests that colonizing behavior should be integrated with other factors including host specificity, searching ability and other attributes to predict the effectiveness of a biological control agent. New experiments manipulating beetle density and sex ratio are needed in order to further examine the influence of host density as well as beetle density on beetle population dynamics.

IV. INTRASPECIFIC PLANT COMPETITION AND FLEA BEETLE LONGITARSUS JACOBAEAE (COLEOPTERA: CHRYSOMELIDAE) EFFECTS ON RAGWORT SENECIO JACOBAEA

INTRODUCTION

The purpose of this study was to examine the interplay between resource limitation and insect limitation on ragwort abundance (Fig. 15). I first fixed the carrying capacity of a patch by clearing and tilling a standard 0.5 x0.5 m area within the background vegetation. I then varied plant density by planting 1, 4, 8, or 16 plants within each patch and maintained the purity of plant stands by hand weeding. I varied insect density in a discrete, all-or-none way, by either protecting or exposing plant populations to the insects. However, I allowed continuous variation in insect density in exposed plots by allowing density to be determined by the natural processes of insect colonization and reproduction.

I examined the effects of plant density and herbivory on ragwort abundance by measuring four components of ragwort reproductive success: rates of growth, development, death, and birth (seed production and germination). In this investigation I attempted to answer two questions. First, how does plant density (competition between neighboring ragwort plants) affect ragwort performance? Antonovics and Levin (1980) describe three phases for populations responding to varying plant densities occurring over the course of time from seedlings to mature plants. These phases are: (1) low density phase — this level of density causes little competition for resources because at this time the plants are quite small. (2) Medium density phase — competition increases to a level that can lead to a reduction in growth rate and a subsequent decrease in size and reproduction. This can be seen as a decrease in dry weight per plant with increasing

density. Yield per unit area rapidly approaches a constant value. (3) High-density phase — this level of density causes severe competition leading to an increase in mortality, described as 'self-thinning'. Yoda et al. (1963) first described self-thinning as a phenomenon where plants under severe density stress tend to exhibit density-dependent mortality. The relationship between log mean weight and log density of surviving plants shows a close similarity to the -3/2 power law.

In crowded conditions, the outcome of competition may result either in death of plants (mortality effects) or reduction in growth rate of individuals (plastic effect) or both (Bazzaz and Harper 1975). In this system as plant density increases I expected a decrease

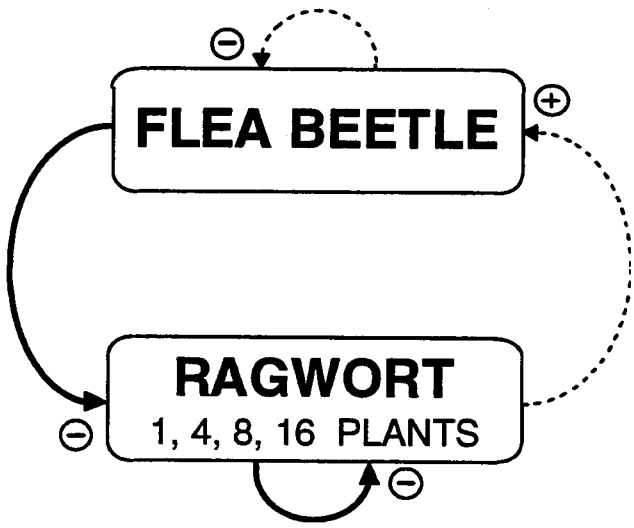


Figure 15. Signed diagraph representing the feedbacks in the ragwort-flea beetle L. jacobaeae interaction for part 2 of the second experiment. This study is represented by a solid line. The expectations are that an increase in plant density causes an increase in insect density, which in turn leads to a decrease in plant density. Increase in each population also has a negative feedback on itself.

in the supply of resources available to the plant population with subsequent declines in growth, delays in development, increase in mortality, and a decrease in reproduction.

The second question was, how does the beetle affect ragwort performance? It was expected that an increase in plant density would lead to an increase in the insect population, which in turn should lead to a decline in rates of plant growth, development, survivorship, and reproduction. The impact of herbivore feeding on plant growth, development, survivorship, and reproduction depends on such things as the parts of the plant fed upon and the timing of the attack relative to plant development (Crawley 1983). Herbivores that feed on roots, such as the ragwort flea beetle, may reduce rates of plant growth, development, survivorship, and reproduction by interfering with water and nutrient uptake and by reducing the carbohydrate supply for seed fill (Frick 1970a, James et al. 1992, McEvoy & Rudd 1993, Windig 1993).

In addition to the individual effects of plant density and insect density acting alone on ragwort performance it might be expected that plant density and insect density interact in their effects, such that the effect of one factor depends on the level of the other factor. Often herbivores introduced for biological control do not cause death of established perennial plants directly, but compromise the fitness of a plant to a point where attacked plants more readily succumb to competition and environmental factors. For example, recent experiments investigating the biological control of ragwort found that ragwort is regulated not by herbivory alone or plant competition alone, but by both (McEvoy 1993, McEvoy & Rudd 1993). Chrysolina beetles control Hypericum by reducing the roots ability to secure enough moisture to survive during the dry season (Clark 1953).

A key aspect of this experiment was following the fate of host plants from the time they were colonized by beetles until after damage had occurred. This allowed us to evaluate the relationship between both the distribution of attacks and the consequences of that distribution for plant performance. Reader (1992) notes that removing plant's

neighbors (equivalent to reduced density in this experiment) may reduce food and shelter for herbivores. As a consequence, a reduction in the level of herbivory, rather than a reduction in intraspecific competition may be responsible for increased plant performance. In a companion study investigating behavioral and numerical responses by the ragwort flea beetle to changes in density of ragwort, the number of first generation adults per patch increased with increasing host density, and herbivore intensities (numbers per plant) declined with increasing number of hosts. This led to a testable prediction that a partial refuge from beetle damage exists at the highest host densities. Studies have shown that the effects of host density on herbivore level of damage are variable. For example, Reader (1992) showed that herbivory was a confounding factor in a neighbor- removal experiment designed to measure competition among plants. Similarly, Segarra-Carmona and Barbosa (1990) noted that low-density plots of soybeans had a higher proportion of pods bored by Etiella zinckenella compared to high density plots. In contrast, the number of leaves eaten by larvae of Battus philenor was independent of host density (ranging from 0.33 plants/m^2 to 4.0 plants/m^2), with plant mortality increasing and plant growth rate decreasing by similar amounts at all host densities (Rausher and Feeny 1980).

METHODS

Study site

This is the second-part of a two-part field experiment manipulating insect and host densities. Part I investigates the effect of increasing plant density on insect density. Part II investigates the effects of increasing plant density and increasing insect density on plant performance (Fig. 15). The experiment took place in a 0.9 ha meadow (Three Rocks Road South, 45°2' 2" N, 123° 58' 42" E) at Cascade Head Scenic Research Area

(CHSRA) located in Lincoln county on the central coast of Oregon. The meadow, last grazed by cattle in 1977, is currently grazed by elk Cervus elaphus and deer Odocoileus spp. with some disturbance by fossorial rodents, mainly moles Neurotrichus or Scapanus spp. The vegetation consists mainly of perennial introduced grasses including Holcus lanatus L., Festuca arundinacea Shreb., Dactylis glomerata L., and Anthoxanthum odoratum L. with ragwort at very low density of 1-2 flowering plants per ha.

The experimental site has a maritime climate consisting of cool wet winters and warm dry summers. The following averages are based on normals for the period 1961-1990. The mean monthly temperatures range from 4.9°C in January to 20.3°C in August. The mean annual rainfall is 247 cm, with 69% of the rainfall occurring during the months of November through March (Oregon Climate Service 1994)

Experimental design and layout

The experiment was a randomized complete block design consisting of 3 blocks with 4 levels of ragwort density (1, 4, 8, 16, plants per 0.25 m^2 patch) and 2 levels of exposure to beetles (exposed, protected). The exposed treatment was replicated three times and the protected treatment replicated twice within each block across all ragwort densities. Thus the total number of patches was: 3 blocks x 4 levels of ragwort density (1, 4, 8, 16) x 2 levels of flea beetle (exposed and protected) x variable replication for each treatment combination (3 exposed, 2 protected within each block) = 60 patches. Results reported in this paper are from the exposed and protected level of the beetle treatment.

Plot size and spacing were as follows (Fig. 7). The experimental patches were arranged in 3 blocks. Blocks measured 6.5 x 5 m = 32.5 m^2 and were separated from each other by ~ 50 m. The patches measured 0.5 x 0.5 m = 0.25 m^2 and were separated from each other within blocks by 1 m. I varied the quantity of resources available per

plant by clearing and tilling a fixed area and planting 1, 4, 8, 16 plants per patch. Henceforth, the number of plants per $0.25m^2$ patch will be referred to as host density.

The spacing of plots was appropriate for both practical and scientific reasons: (1) it allowed use of prior experimental plots which were protected from disturbance by fossorial rodents using buried hardware screen (McEvoy, 1993); (2) it was close enough to allow a thorough mixing of the beetle population from which colonists were drawn. The area between patches was mowed twice, and patches were weeded 4-5 times throughout the experiment to exclude interspecific plant competition. Two other patches, referred to as sacrificial patches, were planted (without the buried rodent exclusion screen) in the vicinity of each of the three experimental blocks (Fig. 7). Sacrificial patches were harvested destructively prior to harvesting experimental patches to gauge the stage of larval development.

Manipulating plant and insect density

Manipulating plant density. Plant density was manipulated by planting ragwort in densities of 1, 4, 8, or 16 plants per 0.5 x 0.5 m plots in the field. Ragwort seeds collected from CHSRA were germinated in a greenhouse at Oregon State University, Corvallis, Oregon, during July and August 1992. Seedlings were later transplanted individually to pulp pots (14 cm square x 12.7 cm deep) containing a soil medium consisting of soil, peat moss, sand, and pumice (1:1:1:2 parts respectively). Plants were fertilized with 20% N, 20% P, 20% K (Grace-Sierra Horticultural Products Company, Milpitas, California), and watered and weeded as necessary. Upon reaching rosette stage (10-20 cm tall plants that have not bolted), plants were transplanted to the field and randomly assigned to blocks and treatments on 7 and 8 November 1992.

Manipulating herbivore levels. Investigating the effect of the beetle on ragwort required the use of cages to create two levels of beetle density (plants exposed and plants

protected). Plots were either protected from herbivores using closed cages or exposed to herbivores using open sham cages. The use of sham cages helped ensure any unwanted side effects of cages would be experienced equally by both exposed and protected patches. Cages and sham cages were similar except the screen walls of sham cages were rolled up from the ground about 15-25 cm (the rolled screen stayed in place by friction) to allow full beetle access. The cages measured 61 cm x 61 cm x 61 cm (outside dimension) and were constructed of 22 mm (OD) PVC pipe sections with couplers and covered with fine mesh screen "Leno weave" bags (open spaces in the mesh were 0.6 x 1.0 mm). To accommodate plant growth, cages were extended in height using PVC pipe sections and couplers. Frames were adjusted to 61 cm, 122 cm, or 183 cm high as needed depending on the height of the ragwort plants.

The efficiency of the exclusion procedure was evaluated by destructively harvesting 3 replicates of exposed and 3 replicates of protected ragwort patches for each level of host density on a single date 25 March 1993. Larvae were extracted using the heat-extraction protocol of James et al. (1992). Plants within each plot were placed in a Tulgren funnel with a 25 watt light bulb for 7 days or until the plants were dry. Larvae were collected in jars containing 70% alcohol and then counted in each sample or subsample. Subsampling using the protocol of Southwood (1978) and James et al. (1992) was used when larvae were numerous (usually > 20 larvae per sample). A petri dish containing larvae and alcohol was agitated to achieve complete mixing and then allowed to settle. The petri dish was placed over a counting disc and those larvae within the clear areas of the disc were counted and they represented a fraction of the total depending on the disc used. The relationship between beetle larvae density and host density was summarized by plotting the total number of larvae as a function of host density.

Beetles from the surrounding area were allowed to colonize the experimental patches assigned to the exposed level of the beetle treatment. A survey of the area to the

north of the study site showed various densities of ragwort and beetles depending on location. For example, the ~ 200 m^2 area along the roadside just north of the study site (Fig. 7) averaged 203g/m^2 (SE = 32.4) of ragwort and an average of 163 beetles/m^2 (SE = 35.1).

To ensure adequate pollination by insects, the roof for each cage was removed 2 July 1993. Most of the cinnabar moths by this time had laid their eggs and further colonization by adults would be unlikely. A pest glue (Seabright enterprises, Emeryville, California) was spread on duct tape which was applied around the top perimeter of the cages, and reapplied as needed, to reduce escape of crawling flea beetles and entry of cinnabar moth larvae. Since I did not see a beetle fly in the 3 months of field observations June through August 1993, it is likely that beetles moved among the experimental patches on foot rather than on the wing. I removed any cinnabar moth larvae found within the cages. Although the ragwort seed head fly was found visiting ragwort flowers it was assumed to have a negligible bias on estimates of the beetle effect. This assumption is based on results from a similar experiment that found the seed head fly infestation rate was low and independent of the level of the beetle density (McEvoy et al. 1993).

Measuring plant responses

Ragwort responses to herbivore and host density treatments were represented by plant damage by adult beetles and changes in growth, development, survivorship, and seed production. Ragwort change in growth, development, and survivorship were measured on: 24 April; 16, 31 May; 15 June; 1, 14, 23 July; 4, 15, 24, 31 August; 3, 10 September 1993.

Damage inflicted by adult flea beetles was estimated by counting the total number of 'shot holes' on 2 randomly selected plants from each of the exposed patches on 25

March 1993. The mean number of shot holes per plant were plotted as a function of host density and regression analysis was performed.

Ragwort growth. Ragwort growth was represented by a change in plant height and standing crop (initial, spring, and final biomass). Ragwort height was estimated by measuring length of longest leaf for juvenile plants (rosettes), and length of stem for adult plants. Growth curves were constructed by plotting mean stem height only (longest leaf measurements for rosettes were not included) for each treatment combination as a function of time beginning at the time when plants were bolting (May 31). Changes in standing crop was estimated by comparing initial, spring, and final biomass in protected patches of each ragwort density. Initial fall biomass was estimated by randomly selecting 50 plants out of the total plants transplanted; they were then cleaned, dried (at 60°C until dry constant mass was attained) and weighed. To estimate spring biomass I harvested one replicate of protected ragwort patches within each block on 25 March 1993. To estimate final summer biomass I harvested the remaining replicates on 13, 14, 15 September 1993; the plants were processed as in the earlier sample. Mean dry mass of plants for all patches was recorded. Fall, spring, and summer ragwort biomass ($\log_e$) was plotted as a function of host density ($\log_e$).

Ragwort development. Development rate was measured as the transition from juvenile to adult stages. Individuals were classified by one juvenile (rosettes) and two adult (bolted plants and flowering plants) stages, and the proportion of individuals passing form one stage to the next was estimated for each experiment unit. Development curves were constructed by plotting proportion of ragwort in each stage over time. Duration of the juvenile period was calculated by subtracting time of planting from time of when 75% of plants had bolted.

Ragwort survivorship. Survivorship was estimated by following the fate of individuals in each experimental cohort and recording the number of individuals living on

each sample date. Survivorship curves were constructed by plotting the number of living ragwort plants for each treatment combination as a function of time. I measured total ragwort mortality among treatments on August 24.

Ragwort reproduction. Ragwort reproduction was evaluated by estimating the quantity and quality of seeds produced. The quantity of seed produced was estimated by counting the number of mature capitula present on the flowering plants at the time of harvest, assuming 70 seeds per capitulum (McEvoy 1984). It is further assumed, based on the finding by James et al.(1992), that beetles have no effect on the number of achenes per seed-head. Quality of seed produced was estimated as percentage of seeds germinating under lab conditions for both disk and ray achene types.

The seed germination experiment was conducted using the following methods. All mature capitula were harvested from patches when at least 5% (mean ± SE = 6.52 ± 0.565) or more of the flowers were at mature stage. Seeds were harvested at this time to allow a more accurate measure of final biomass. If harvesting of seed-heads was delayed until all seed-heads were mature, the task of estimating ragwort biomass would have been much more difficult because many of the leaves of the ragwort plants would have already dropped. Harvesting only 5% of the seed-heads also greatly reduced the amount of time needed for sampling. The dates that mature heads were sampled included: 24, 31 August; 3, 7, 15 September 1993.

Ray and disk flower seeds were separated and placed in separate envelopes at the time of harvest. Ragwort flowers have two types of flowers, ray and disk, which produce achenes with different characteristics (McEvoy 1984). Achenes produced by disk flowers are equipped with pappus, are lighter (mean ± SE = 199 ± 5 μg), and more numerous (mean ± SE = 58 ± 0.6 achenes per head) than ray achenes. Ray achenes, produced by ray flowers, lack dispersal structures, are heavier (mean ± SE = 286 ± 7 μg), less numerous (virtually invariant at 13 achenes per head), exhibit reduced germination percentage (mean

for disk = 60%, ray = 40%) and reduced germination speed (mean for disk = 6 d, ray = 12 d) compared to disk achenes. Since the two types of achenes exhibit different germination percentages they were separated and germinated in different boxes to reduce variation. Seeds were kept in the envelopes at room temperature for 5 months prior to the experiment.

To insure that all achenes were removed from the seed heads and to make it easier to randomly select achenes with pappus, both disk and ray achenes were threshed and cleaned. To remove pappus from disk achenes and ray seeds from seed heads the achenes were placed in an air scarifier measuring 5.1 cm in diameter by 7.7 cm long (Hoffman model sc-50, Albany, Oregon) for 30 seconds at 4.92 kg/cm^2 air pressure and then sifted throughout two screens to clean the seed (the holes in the first and second screens measured 0.94mm and 0.36mm respectively). Both achene types were then put through a Gamet centrifugal divider (which divides seeds in a random manner) to reduce the number of achenes to 75-100 achenes per type, thus making it easier to do a final random selection of achenes. The batches of 75-100 achenes were then placed on a flat surface, mixed, and counted.

Fifty cleaned achenes of each of disk and ray representing all ragwort densities and beetle treatments were placed in moisture-proof boxes on moist filter paper. The filter paper was impregnated with a fungicide solution (Thiram®, 100 ppm, 65% active ingredient) to reduce fungal infection. The seed germination test was conducted at room temperature (20° C) with a light regime of 12h light and 12h dark. Protrusion of the radicle was the criterion for germination. All seeds were allowed to germinate over a 30 day period with seed boxes being checked every 2-3 days to count germinated seeds and to maintain moisture levels.

The pappus reduces the likelihood of germination in some species (Stevens et al. 1986). I tested the effect of the pappus on ragwort germination in a laboratory

experiment. Fifty uncleaned disk and ray achenes representing only single plant densities for both exposed (6 replicates) and protected (3 replicates) patches were randomly selected and placed in the moisture-proof boxes under the same conditions as the first experiment.

Data analysis

Ragwort biomass and capitula as a function of host density was analyzed by regressing biomass ($\log_e$) and capitula ($\log_e$) on host density ($\log_e$). Log-transformation helped to linearize the relationship and stabilize the variance. Difference in mortality rates among treatments were compared using the Kruskall-Wallis test. All statistical analyses were performed using SYSTAT for windows, version 5.02 (SYSTAT, Inc. 1992). The significance level used was 5%, except when multiple tests were performed using the same response variable. In these cases, to protect against type 1 error, or falsely rejecting the null hypothesis, I examined pairwise correlations between errors in the responses estimated for each experimental unit, and if errors were not independent, I adjusted the criterion for rejection of the null hypothesis using the sequential Bonnferoni technique of Rice (1990).

RESULTS

Levels of herbivory

Levels of shot-hole damage inflicted by first-generation adult beetles was independent of host density over the entire experimental range of 1-16 plants per patch (regression, P = .397; mean ± SE = 115 ± 25.9; Fig. 16). Levels of infestation by larvae of first generation adults was significantly different than protected levels of the beetle treatment ($F_{1,23}$ = 36.180, P < 0.001; Fig. 17).

Little damage was inflicted by other herbivores. An exception was the aphid Aphis

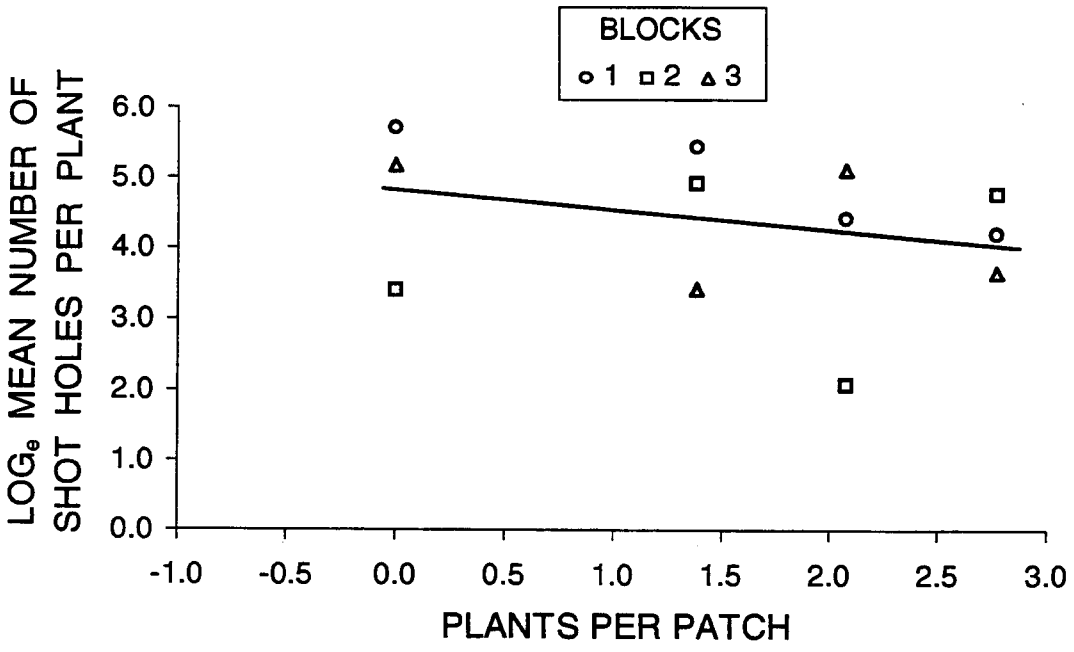


Figure 16. Adult ragwort damage L. jacobaeae ($\log_e$ mean number of shot holes per 2 plants) as a function of host density ($\log_e$). Data was taken on 25 March 1993 at CHSRA and adult beetle damage occurred from 8 November through 25 March

lugentis, which infested 1 protected and 4 exposed plots. The level of infestation was severe in only one patch, a 4-plant host density x exposed beetle treatment combination, and this patch was labeled as an outlier in the graphs and removed from the statistical analysis of ragwort responses.

Ragwort responses

Plant growth, represented by change in biomass, virtually ceased between fall and winter, then increased rapidly between spring and summer (Fig. 18). Initial mean ragwort

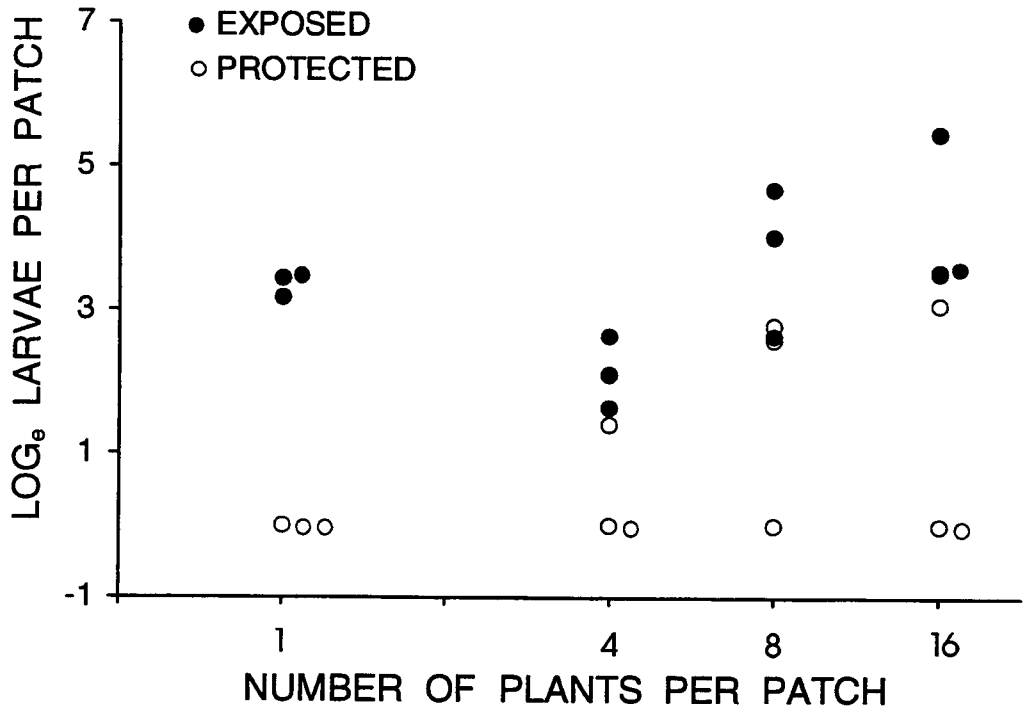


Figure 17. Number of flea beetle L. jacobaeae larvae ($\log_e$ number of larvae per patch) as a function of host density for each level of beetle density. Larvae were sampled on 25 March 1993 at CHSRA

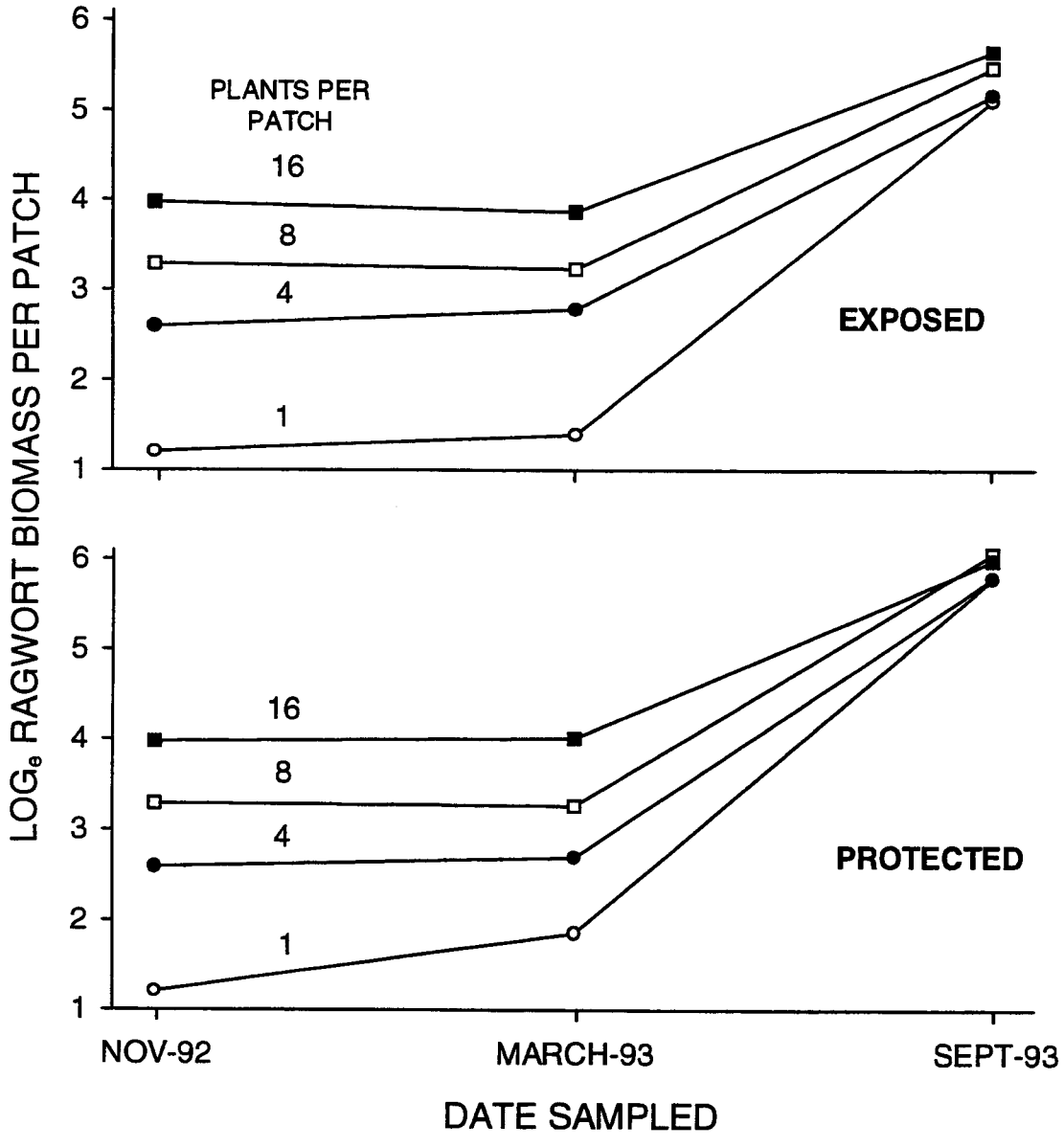


Figure 18. Ragwort S. jacobaea biomass sampled during the 1992-1993 experiment at CHSRA. Ragwort biomass ($\log_e$ mean dry ragwort biomass per patch for roots and shoots) for each level of beetle density and plant density for three time intervals: November 1992, March 1993, and September 1993.

biomass was estimated to be 3.32 (± 1.02 SD) grams per plant for both exposed and protected plots. Stem length of ragwort (averaged across treatments) increased at a mean rate of 11 cm per week from May 31 to July 22 (Fig. 19).

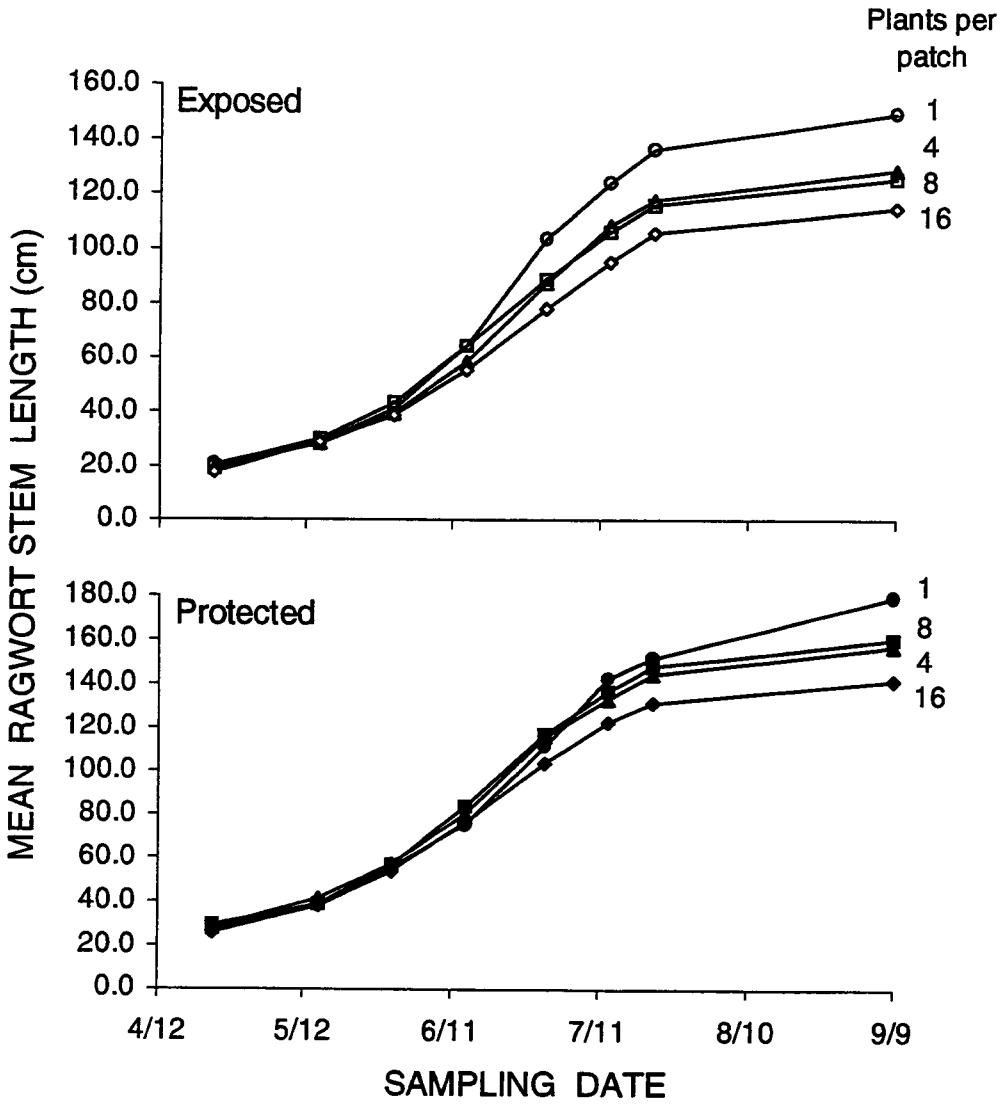
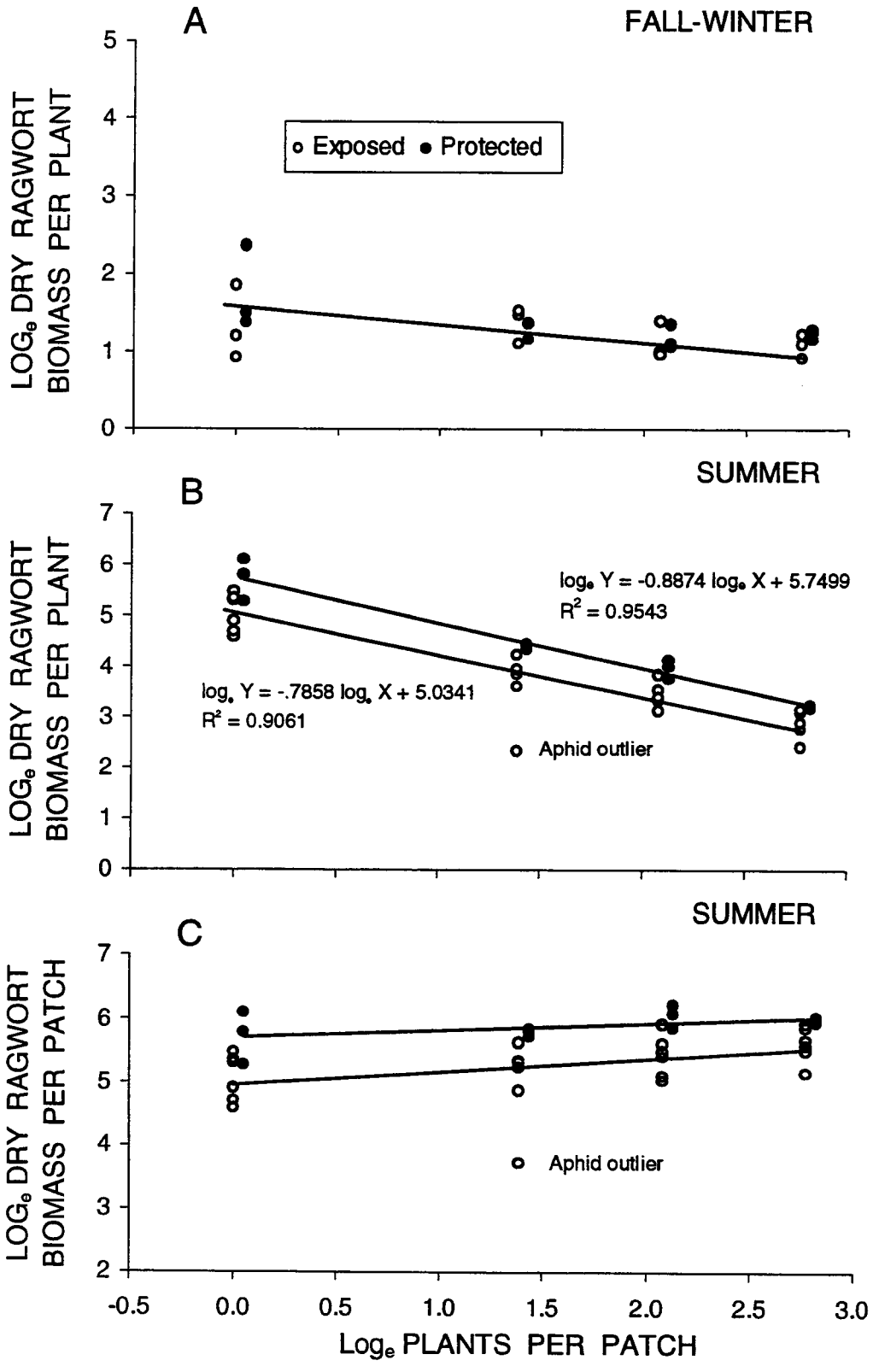


Figure 19. Mean ragwort S. jacobaea stem length as a function of sampling date for each level of beetle density and plant density during the 1992-1993 experiment conducted at CHSRA.

There was only a slight decline in spring ragwort biomass (roots and shoots) per plant with increasing host density indicating that plants were still of nearly equal size across all host densities. Ragwort biomass (for both protected and exposed patches) ranged from (mean ± S.E. = 5.2 ± 1.2 g) per plant for a single plant patch to (mean ± S.E. = 3.2 ± 0.16 g) for a 16 plant patch (Fig. 20A). Spring ragwort biomass in exposed patches was 15% lower compared to protected patches. There was a beetle effect on $\log_e$ ragwort biomass by summer harvest (Fig. 20B, Table 3A). The yield, in terms of dry biomass per plant, was 48% lower in exposed patches compared to protected patches. Summer ragwort biomass ($\log_e$) per plant declined with increasing host density ($\log_e$) (Fig. 20B, Table 3A). Plants in protected single-plant patches were, on average, 12 times larger than plants in 16-plant patches. Yield per unit area was nearly constant across host densities (Fig. 20C). The effects of host density and beetle on roots and shoots separately showed a similar pattern as was found for total ragwort biomass. There was no significant interaction between host density and beetle effects (Table 3A).

Development curves for all treatment combinations show that, in general, ragwort plants remained rosettes from November through May (total juvenile period of ragwort included June-October in the greenhouse and November-May in the field), bolted early June through late July, and flowered from early July through harvest (Fig. 21). Only two plants remained rosettes and did not flower, both were in 16 plant patches, one in exposed and one in protected plots. The duration of the juvenile period (time of bolting-time of planting) averaged (mean ± S.E.) 205.94 ± 1.186 days. A decline in the number of plants flowering in a patch towards the end of the sampling period was due to mortality. The effect of host density on survivorship varied with the level of herbivore density treatment (Fig. 22). Plant mortality was higher in exposed compared to protected levels of the beetle treatment. A total of 25 out of 26 ragwort plants died in the exposed patches.

Figure 20. Spring and summer ragwort S. jacobaea biomass as a function of host density for each level of beetle density for the 1992-1993 experiment conducted at CHSRA. (A) Spring ragwort biomass ($\log_e$ mean dry ragwort biomass per plant for roots and shoots) as a function of host density ($\log_e$) for each level of beetle density. (B) Summer ragwort biomass ($\log_e$ mean dry ragwort biomass per plant for roots and shoots) as a function of host density ($\log_e$) for each level of beetle density. (C) Summer ragwort biomass ($\log_e$ mean dry ragwort biomass per patch for roots and shoots) as a function of host density ($\log_e$) for each level of beetle density.


A
FALL-WINTER
LOG$_e$ DRY RAGWORT BIOMASS PER PLANT
o Exposed • Protected
B
SUMMER
log$_e$ Y = -0.8874 log$_e$ X + 5.7499
R^2 = 0.9543
log$_e$ Y = -.7858 log$_e$ X + 5.0341
R^2 = 0.9061
o Aphid outlier
C
SUMMER
LOG$_e$ DRY RAGWORT BIOMASS PER PATCH
o Aphid outlier
Log$_e$ PLANTS PER PATCH

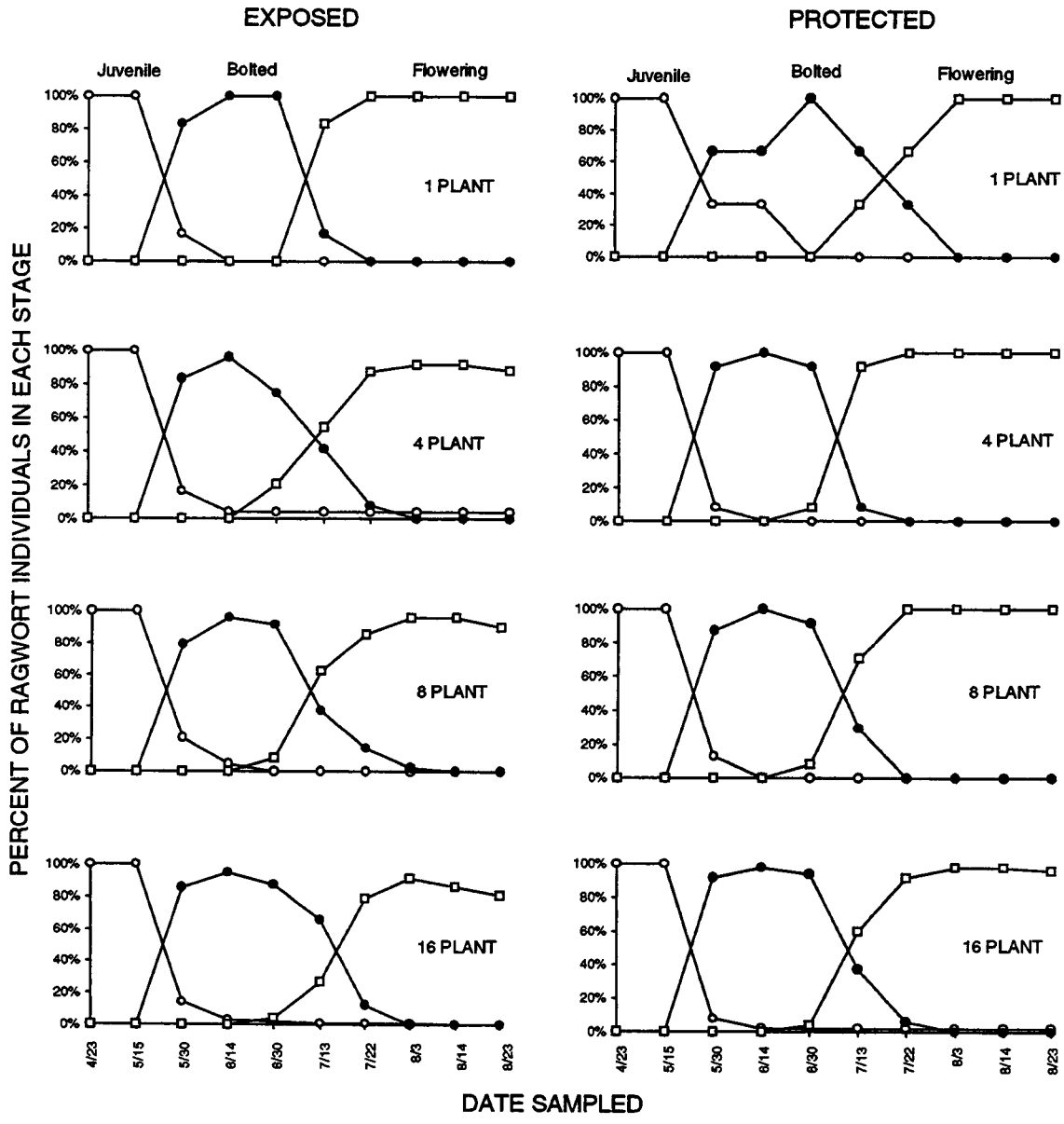


Figure 21. Changes in the percentage (mean percentage over all host densities) of ragwort S. jacobaea individuals in each development stage as a function of time during the 1992-1993 experiment conducted at CHSRA. Developmental stages of ragwort were recorded as juvenile (vegetative state), bolted, and flowering (reproductive state).

Most ragwort mortality (88%) occurred post-reproduction (after 75% of plants were in flower) and was most evident in the exposed x 16-plant treatment combination.

For both protected and exposed treatments, a regression of $\log_e$ seed-heads

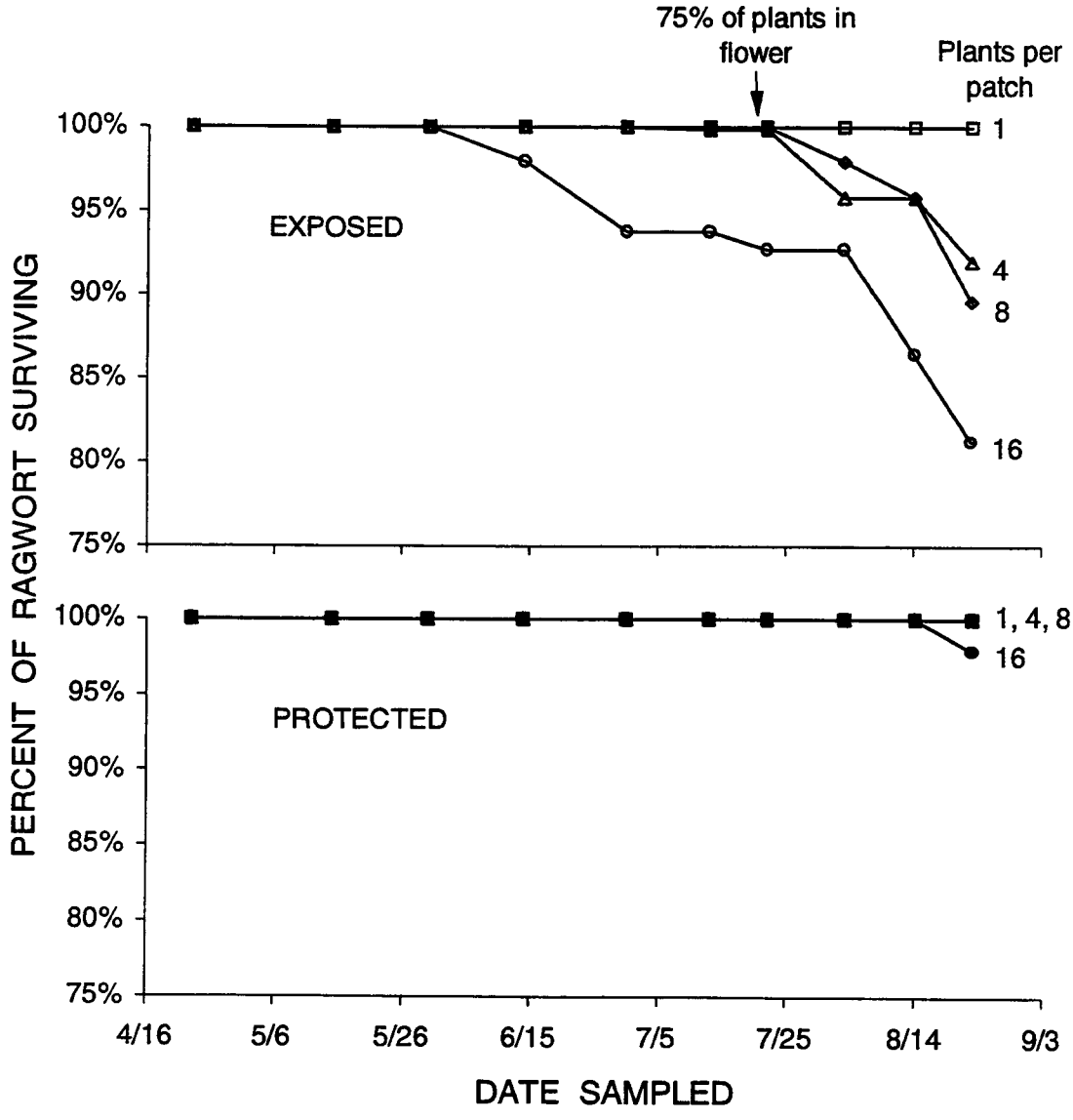


Figure 22. Percent ragwort S. jacobaea survivorship as a function of time for each level of beetle density and plant density during the 1992-1993 experiment conducted at CHSRA.

(capitula) produced per plant on $\log_e$ dry final host biomass was significant ($F_{1,34}$ = 247.58; $P < 0.001$; Fig. 23). Mean time to reach flowering stage (time of flowering - time of planting) ranged from 251d (SE = 2.45) in 1-plant patches and 256 d (SE = 0) in 16-plant patches. Two components of plant reproduction, seed quantity and seed quality, differed in their response to beetle and host density treatments. The number of seed-heads produced per plant declined with increasing host density treatment (Fig. 24A): a 16-fold increase in host density (in protected patches) led to a 11-fold decrease in the number of capitula per plant. However, the number of seed-heads per patch was nearly constant

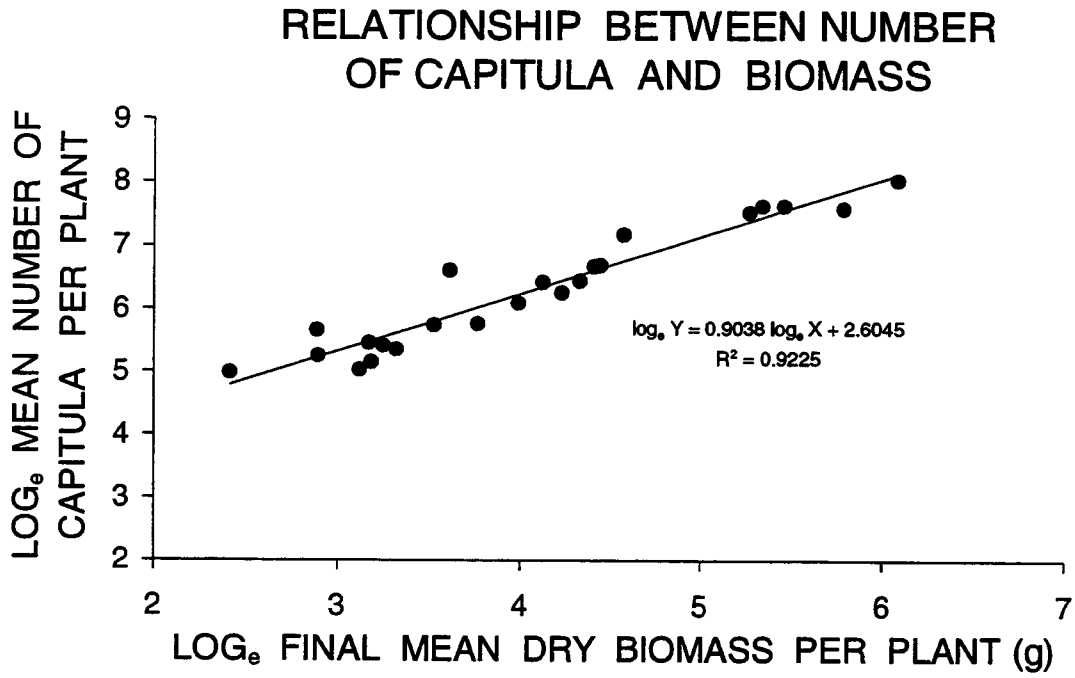


Figure 23. Ragwort S. jacobaea capitula as a function of ragwort biomass for the 1992-1993 experiment conducted at CHSRA. Number of ragwort capitula ($\log_e$ mean number of capitula per plant) as a function of ragwort biomass ($\log_e$ final mean dry biomass per plant) for both beetle densities.

across host densities. For example, single plant patches produced (mean ± S. E.) 2017 ± 231.9 seed heads per patch while 16-plant patches produced (mean ± S. E.) 2829 ± 301.9

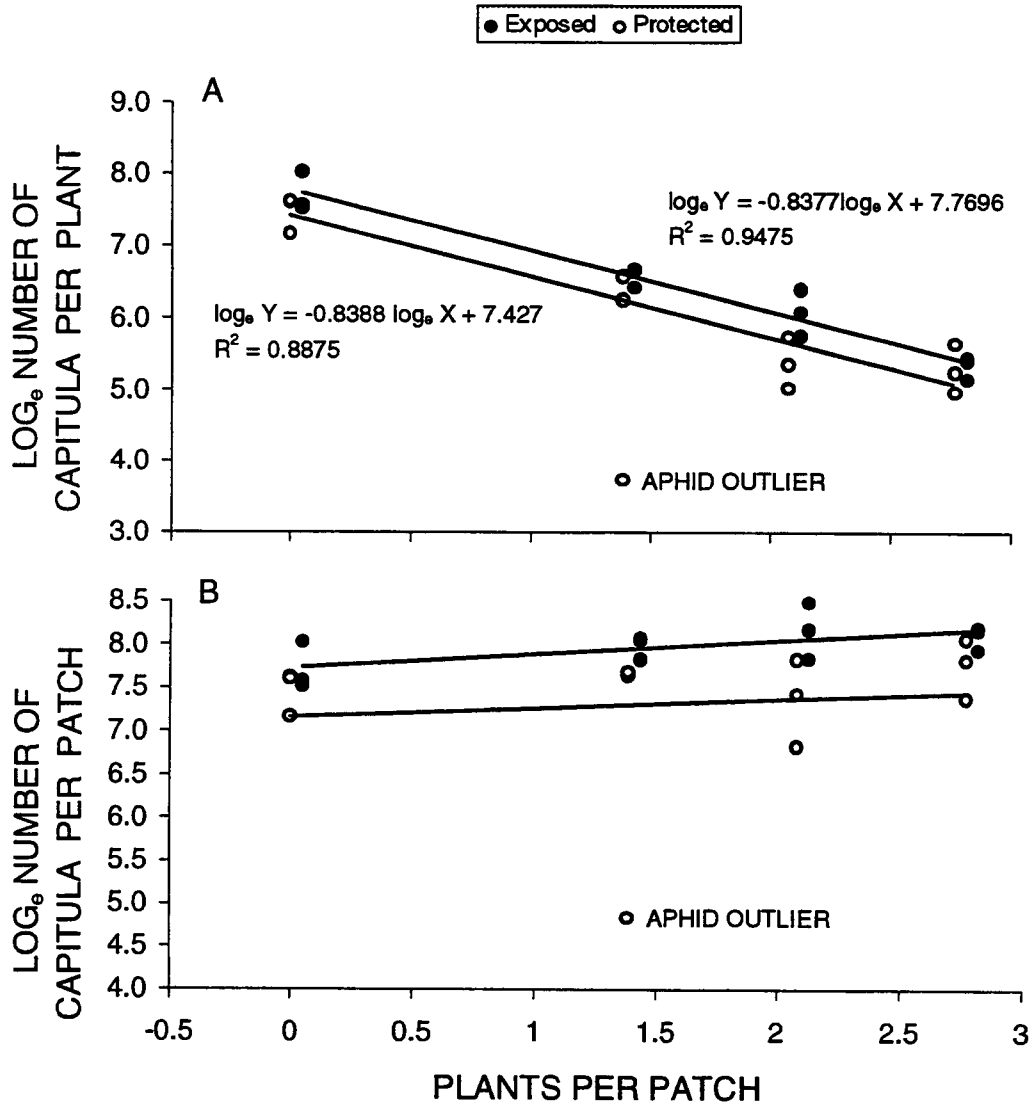


Figure 24. Number of ragwort S. jacobaea capitula as a function of host density during the 1992-1993 experiment conducted at CHSRA. (A) Number of ragwort capitula ($\log_e$ mean number of capitula per plant) as a function of host density ($\log_e$) for each level of beetle density. (B) Number of ragwort capitula ($\log_e$ mean number of capitula per patch) as a function of host density ($\log_e$) for each level of beetle density.

Table 3. Analysis of beetle L. jacobaeae effects on ragwort S. jacobaea summer biomass and ragwort seed germination for the 1992-1993 experiment conducted at CHSRA. (A) ANCOVA on summer ragwort biomass (log_e mean dry mass of ragwort per plant) as a function of host density (log_e); (B) MANOVA on log-odds germination of disk and ray achenes showing Wilks' Lambda

(A) Ragwort summer biomass

Source of variation	df	MS	F	P
Host density	1	21.643	366.960	< 0.001
Block	2	0.014	0.237	0.791
Beetle	1	1.146	19.435	< 0.001
Host density x Beetle	1	0.210	3.566	0.069
Error	29	0.059		

(B) Ragwort seed germination

Source of variation	Value	F	Numerator df	Denominator df	P
Block	0.995	0.077	2	29	0.926
Host density	0.914	1.366	2	29	0.271
Beetle	0.912	1.397	2	29	0.264
Host density x Beetle	0.884	1.906	2	29	0.167

seed heads per patch (Fig. 24B). The number of seed-heads per plant was lower in exposed compared to protected levels of the beetle treatment (Fig 24A). Exposed plots averaged 18% fewer capitula per plant compared to protected plots. There was no significant difference in germination rate for exposed and protected patches (Table 3B). Germination rate was higher for disk compared to ray achenes: odds of germinating for ray flowers was 25-87% of that for disk.

DISCUSSION

I measured the effects of host density and herbivory on four components of reproductive success: growth rate, development rate, reproduction (seed production, germination), and annual survivorship. I found that ragwort growth, reproduction (number of capitula), and survivorship all declined in the presence of the beetle. While ragwort growth, timing of seed production, and reproduction declined with increasing host density. The effect of host density on survivorship varied with the level of the herbivore density treatment. Survivorship was independent of host density in protected patches but decreased with increasing host density in exposed patches.

Levels of herbivory

I found that shot-hole damage was independent of host density. A similar result was found by Windig (1993) where number of holes did not vary with ragwort density and proportionally the same amount of leaf was eaten from small and large plants.

Effect of plant density on ragwort performance

Growth. In crowded conditions, the outcome of competition may result either in death of plants (mortality effects) or reduction in growth rate of individuals (plastic effect) or both (Bazzaz and Harper 1975). In this study, ragwort's 'plastic response' to increased crowding through a reduction in growth and reproduction was greater than the mortality response. A decline in ragwort growth suggests self-limitation by ragwort: a 16-fold increase in host density led to a 10-fold decrease in biomass but no detectable increase in mortality. As ragwort increases in size, resources such as light, water, and nutrients may

become limited (Harper 1977). It is difficult to say what resources limited plant growth in this experiment.

Reproduction. A second component of reproductive success is the ability to produce an adequate number of viable seeds. I found that seed quality (seed germination) did not vary with host density but seed quantity (number of seed-heads) did. The number of seed-heads per plant decreased with increasing host density, while the number of seed-heads per patch showed constant yield across all host densities (Fig. 23B). Harper and Gajic (1961) found that Agrostemm agithago responded to an increase in host density by reducing the number of capsules per plant while maintaining constant seed yield per unit area. Likewise, in another study cucumber yield (fruit) per plant in high density plots (289 plants / $100m^2$) was lower than in low density plots (144 plants / $100m^2$) (Bach 1981). In contrast, Segarra-Carmona (1990) found that host density (densities ranged from 1 plant / linear m to 5 plants / linear m) had no effect on soybean pod production under conditions with ample moisture.

The reduction of seed-heads per plant with increasing host density was probably due to plants being smaller. This is suggested by the strong correlation between the $\log_e$ number of seed-heads produced per plant and $\log_e$ dry final host biomass (Fig. 22; R^2 = .92); the number of seed-heads produced per plant increased as host biomass per plant increased. Thus plants in less crowded plots, being larger, should produce more seed-heads than plants in more dense plots. A similar finding was noted by Rausher and Feeny (1980) where large Aristolochia reticulata plants produced more seeds than small plants.

Survivorship. Mortality may be more of a factor at different stages of ragwort's life cycle or it may depend on the degree of crowding. Forbes (1977) notes that ragwort mortality rates decline with each stage of ragwort. It is estimated that more than half of ragwort plants (57%) die as seedlings when crowding can be intense. Since I put plants out as rosettes most of the mortality probably had already occurred. Plant density in this

experiment most likely conformed to a medium-density phase as described by Antonovics and Levin (1980). As a result, plants did not experience intense crowding and compensated for crowding mainly through variations in amount of growth and reproduction rather than in mortality. The lack of density dependent mortality or self-thinning and greater growth and reproduction of plants grown at low densities agrees with other similar studies (Donald 1951, Harper and Gajic 1961, Watkinson and Harper 1978, Bach 1981). In contrast, Segarra-Carmona and Barbosa (1990) found that soybeans had higher mortality in high-density patches. The only evidence of self-thinning appears to be associated with exposed 16-plant patches where the combination of crowding and beetle effects were sufficiently intense.

Effect of herbivore density on ragwort performance

Larval infestation was lower in protected plots compared to exposed plots indicating that the exclusion procedure (caging) was effective. Using methods similar to those in this study, James et al. (1992) also found beetle-protected plots had significantly fewer larvae than in beetle-protected plots. The contamination of some plots in this study was probably due to beetles entering the cages while being censused. The fact that I found beetles in protected patches probably means that beetle effect on ragwort performance was underestimated. If protected patches had been absolutely clean of beetles then ragwort performance would have been greater in those patches than was estimated and therefore the difference in ragwort performance for exposed and protected patches would have been greater.

Plant growth. It was not until late summer that ragwort showed a reduction in growth due to beetle effect. Herbivores often do not respond immediately to a perturbation in host density, but with time lags (May 1973, Antonovics & Levin 1980) resulting from delays in the herbivore's behavioral and reproductive responses'. Ragwort

flea beetles increased due to colonization and reproduction in fall and winter, whereas ragwort plants increased due to growth in the spring. As a consequence, adjustments in insect and plant populations to changes in each others abundance are always slightly out of phase. An important feature of this system is that the time lag associated with self-limitation is short compared to the beetle effect. Self-limitation arrests ragwort population growth, allowing the process of colonization and reproduction by the beetles to continue until eventually beetle populations reach such severe levels that plant populations are driven locally extinct (McEvoy et al. 1993). Were it not for resource-limitation in the plant population, weed control would be more difficult and the oscillations in population sizes in plant-herbivore interaction would be much greater.

Plant reproduction. I found that seed quality did not vary with beetle treatment, which is consistent with a prior study by James et al. (1992) that found beetle had no effect on seed viability. However, there was an effect of beetle on seed quantity, such that the number of seed-heads per plant decreased in patches exposed to the beetle. Beetles feeding internally in the root and crown areas could have a indirect effect on seed production both by interfering with water and nutrient uptake and by reducing transport of carbohydrates critical for seed fill (Crawley 1983). A reduction in water and nutrient uptake could lead to a reduction in size of plants. Since there was a positive correlation between ragwort biomass and seed production, any reduction in plant size could reduce the number of seed-heads produced. A reduction in carbohydrate transport could also reduce seed production, but at this time there is no indication which of these explanations should be favored.

Due to the fact that a little more than 5% of the seed-heads were harvested it is difficult to judge whether the sample used to estimate of seed quality was truly representative. For example, if ragwort plants that experience stress such as herbivory or

crowding produce more viable seeds early in the reproductive cycle then the treatment effects may have been underestimated in this study.

Plant survivorship. Survivorship rate was constant through much of the experiment and became variable towards the end. More than half of ragwort mortality (58%) occurred post-reproductive, that is, after ragwort had already flowered. The remaining 42% of the ragwort plants died before flowering. Since most of the mortality occurred after flowering, it may be of little or no consequence for ragwort reproductive success or population dynamics. It should be noted that ragwort plants prior to being transplanted into the ground were grown in a greenhouse for 5 months with no exposure to beetles. If ragwort plants would have been exposed to beetle attack from the beginning there may have been an increase in pre-reproductive ragwort mortality. Many studies, including studies on effects of biocontrol agents on pests, suggest that it is unusual for insect herbivores to be the direct cause of death for established plants (Crawley 1989). In fact, insect feeding is much more likely to increase the death of a plant when plants are subject to inter- or intraspecific competition. This appears to be the case in this study, where I found the highest mortality in the exposed 16-plant patches.

Consequence of herbivore attack

In a prior report of this experiment I found that the per capita rate of infestation decreased with increasing plant density. For example, a 16-fold increase in host density led to a 2.4-fold decrease in herbivore intensity (insects per plant) and in herbivore load (insects per unit of plant dry mass). This leads to the prediction that the magnitude of the herbivore effect (difference in ragwort performance between protected and exposed levels of the insect density treatment) might also vary with the level of the host density treatment. Evidence from this study falsifies this prediction. I found no significant interaction between host density and beetle effects, which suggests that at this local spatial

scale, the magnitude of the beetle effect is similar for all host densities. This contrasts with a study by Segarra-Carmona and Barbosa (1990) which looked at the effects of patch plant density (1 and 5 plants per linear meter) on herbivory levels by Etiella zinckenella and found that greater herbivore intensity (larvae/raceme) in low-density patches led to an increase in pod damage (% pods bored) in low-density patches. On the other hand, in study investigating the effect of Battus philenor on Aristolochia reticulata it was found that low density and high density patches (0.33 plants/m^2, and 4.0 plants/m^2 respectively) had equal chance of being oviposited on and that subsequent herbivore damage (represented by change in growth rate, seed production, and survivorship) was independent of density treatment (Rausher and Feeny 1980). Thus, it appears that an unequal distribution of foragers does not necessarily translate into unequal distribution of ill-effects.

CONCLUSIONS

The resource-limitation effect increased with plant density, while the herbivore effect was independent of plant density. The resource limitation effect appeared to be stronger than the herbivore effect, however, this may be due to plants having a herbivore-free period (isolated in the greenhouse) during their first 5 months of existence. If beetles had colonized the ragwort plants from the beginning there might have been a stronger herbivore effect because the beetles would have inflicted damage sooner and for a longer period of time (McEvoy et al 1993).

Evidence from comparing protected and exposed plots suggest that herbivore effects are independent of host density: a 16-fold increase in plant density led to no detectable change in magnitude of the herbivore effect. This suggests there is no

density-dependent refuge for ragwort operating at these local scales. This is consistent with prior studies, conducted on local spatial scales over longer time scales (McEvoy et al. 1993, McEvoy & Rudd 1993), which show that all ragwort plants, regardless of density, are eventually destroyed.

V. SUMMARY

In the first experiment I found that ragwort flea beetles were highly sensitive to local distribution of their food plants. When beetle populations were manipulated by establishing equal numbers of beetles in patches with unequal number of hosts, the beetles rapidly re-distributed themselves, and the number of beetles was strongly and positively correlated with the number of hosts per patch. The perturbation-response approach effectively isolated the effects of host density on the flea beetle's spatial distribution.

In the second experiment I found that the behavioral response was dependent on host population size: the numbers of colonizing beetles increased with increasing plant density, but leveled off at higher plant densities (the number of beetle-days ranged from 261 for 1-plant patches to 1822 for 16-plant patches). Whereas the reproductive response, represented as observed multiplication rate, appears to increase then level off for the 4, 8, and 16 plant patches. Combining these results, the beetles apparently respond to spatial variation in the density of hosts primarily by changes in their movement behavior rather than by changes in their per capita reproductive rates. These results highlight the importance of a natural enemy's colonizing behavior for controlling a sudden upsurge in pest abundance. They suggest that movement behavior should be integrated with other factors including host specificity, intrinsic rates of increase and other attributes to predict the effectiveness of a biological control agent.

In the second experiment I also found that both insect herbivory and intraspecific competition had an effect on ragwort performance. For example, over approximately one year, ragwort's rate of biomass accumulation was 48% lower, and seed-head production 18% lower in exposed compared to protected plots, while intra-specific competition reduced ragwort's rate of biomass accumulation and seed-head production, such that a 16-fold increase in host density (in protected patches) led to a 12-fold decrease in biomass

per plant and a 11-fold decrease in the number of seed-heads per plant. Evidence from comparing protected and exposed plots suggest that herbivore effects are independent of host density. This suggests there is no density-dependent refuge operating at these local scales. That is, all ragwort plants regardless of density are eventually destroyed.

Taken together these results confirm the superior searching efficiency of the ragwort flea beetle and highlight the importance of a natural enemy's colonizing behavior for controlling a sudden upsurge in pest abundance. The superior searching and colonizing behavior of the ragwort flea beetle may contribute to its superiority as a regulator of ragwort abundance. The results also suggest that colonizing behavior should be integrated with other factors including host specificity, searching ability and other attributes to predict the effectiveness of a biological control agent.

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