

AN ABSTRACT OF THE THESIS OF

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Title: The Relative Impact of Single vs. Multiple Agents
On the Biological Control of Tansy Ragwort (Senecio
jacobaeae)

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Field experiments were done to test the relative effectiveness of single and multiple biological control agents in reducing weed populations using two insects, the cinnabar moth, Tyria jacobaeae (L.), and the ragwort flea beetle, Longitarsus jacobaeae (Waterhouse). These two insects were released for biological control of the biennial weed tansy ragwort. Together, they had a greater impact on ragwort, at the experimental site, than either alone. Artificial defoliation simulated the cinnabar moth feeding behavior of defoliating and deflorating bolting plants in the early summer. Flea beetle larvae and adults were sampled to determine their seasonality and the plant parts and stages they most frequently fed on. Larvae fed

internally in the leaf petioles and roots from October to July. Adults fed on leaves year round with their greatest activity occurring in late summer and fall. Flea beetles alone reduced vegetative plant densities by about 95%, but only with simulated cinnabar feeding did they affect flowering plants, reducing regrowth of leaves and flowers. Cinnabar moths alone had an effect only on flower and seed production. This combination of herbivores with little nicheoverlap, spread out attack over time, plant stages, and parts, and yielded stronger host population depression than either insect yielded alone.

The Relative Impact of Single vs. Multiple
Agents on the Biological Control
of Tansy Ragwort (Senecio jacobaea)

by

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Typed by Rosalind James

I dedicate this thesis to my brother

Sage Russell Turner

who first kindled my interest in the biological sciences

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THE RELATIVE IMPACT OF SINGLE VS. MULTIPLE AGENTS ON THE
BIOLOGICAL CONTROL OF TANSY RAGWORT (SENECIO JACOBAEA)

INTRODUCTION

Single versus Multiple Introductions

An old debate in biological control is whether to introduce one or several control agents. When introducing species for biological control of a pest, two conditions are sought after; one is to bring the pest population to levels low enough not to cause economic damage and the other is to maintain the pest at these low levels. The first must be obtained before the second can be and it is this first aspect that has been investigated here. If biological weed control agents are selected such that they feed on different plant parts, at different times of the year, or in different geographical regions, they may cause a greater depression in the weed population than either one acting alone. In this field experiment, the complementary action of two insects introduced for the biological control of the noxious weed tansy ragwort, Senecio jacobaea L. (Asteraceae), was found to cause a much greater decline in weed abundance than either insect could cause when acting

alone. These two insects do most of their feeding on different plant parts and at different times of the year.

Practitioners often introduce several agents to improve chances of success, be it from the combined effect of all the insects (Harris 1985) or the increased chance of getting that one species which is sufficient for control (Myers 1985). However, relatively few of these insects become established and fewer still give adequate control. The uses and meanings of the word "control" vary widely but will be used here only to mean the reduction of pest populations and the continued maintenance of these lower populations. Use of the term "biological control" is restricted here to include only human introductions of biological agents to control a pest.

Turnbull & Chant (1961) found that well over half of the biological control projects of insects in Canada (up to the time of their paper) failed to satisfactorily restrict damage caused by the target pest. Worldwide, only 36% of the biological weed control projects involving the introduction of exotic control agents have consistently reduced target weed populations (Julien et al. 1984). Turnbull & Chant (1961), Turnbull (1967), and Ehler & Hall (1982) suggest that competitive exclusion between agents may have caused some or most of the failures in biocontrol of insect pests--by causing failure of establishment or by inhibiting population growth of the agents.

Correlations suggesting that multiple species introductions reduce success in control are inevitably clouded by other factors which reduce success. Poor release techniques (some people are just better at it than others), weather, and incorrect identification of agents before release may be more important in the failures of many projects than is competition between agents (Van den Bosch 1968). The activities of predators, parasitoids, and pathogens also influence the success of biological weed control projects (Goeden & Louda 1976). Ehler & Hall (1982) found correlations indicating that the rates of establishment of agents decline with increases in both the number of species released at one time and place, and the number of species already released. Keller (1984) contends that these correlations are not evidence for the occurrence of competitive exclusion because of biases in the data. For example, the first few species released in a program are probably deliberately selected as those most likely to establish, independent of competition. Also, large projects involving the release of several agents are more likely to be published when they fail than are projects involving the introduction of only one species.

Multiple vs. single introductions has also been debated among theoreticians. May and Hassell (1981) support multiple introductions of parasitoids (parasitoids are parasitic insects that feed on and eventually kill only one

animal in their life span). The multiple parasitoid model their conclusions are based on assumes that each parasitoid species attacks independently of the other and so the occurrence of superparasitism (the parasitization of a host by more parasites than it can sustain) increases as parasitoid densities increase (Takehashi et al. 1984). Takehashi et al. (1984) demonstrate that when this assumption is removed and there is complete niche overlap, introducing the better competitor alone yields the lowest host populations, assuming the better competitor is also the more effective natural enemy (this is an important assumption because in biocontrol, competition becomes the greatest problem when a less effective agent out competes a more effective agent and reduces its population). However, Takehashi et al. (1984) found that multiple introductions yield the lowest and most stable host populations if there is little niche overlap between parasitoids (perhaps one searches different areas than the other, or they attack different life stages).

Most of the evidence against multiple introductions comes from studies of parasitoids, not herbivores, and parasitoids differ from herbivores. Theories and methods for biocontrol of weeds may be similar to that for insect pests, because for both the goal is to use natural enemies to reduce pest populations below levels where they cause economic damage. However, the differences between herbi-

vores and predators and between plants and insects should always be kept in mind when comparing strategies. For example, predators and parasitoids usually kill their prey directly. Plants tend to be more resistant to herbivory and it may take large numbers of herbivores (particularly if the herbivores are small, as insects are) to kill a plant.

Insect pests and weeds are similar in that they may have refuges from enemies, be it a spatial or stage related refuge. However, weeds have one refuge that may be quite different from anything insects have and that is the seed bank. Large numbers of seeds may stay dormant in the soil for many years and not germinate until conditions are right. So the weed may reoccur even after the plants have been absent for years.

Kakehashi et al. (1984) suggest that "the most important problem left to us is, not to continue the endless debate about multiple v. single introduction, but to seek practical methods for deciding which strategy is to be undertaken." One strategy may be the introduction of multiple species which attack the pest at different times, different geographical regions, and or different plant parts. This strategy spreads out the attack on the plant and allows fewer refuges for it and could provide a means to more rapidly reduce the pest population. The tansy ragwort biocontrol project studied here provides a means

for testing this idea since the two major control agents feed at different times of the year and on different host stages and parts.

Biological Control of Ragwort

An experimental approach

Experiments were conducted to test the hypothesis that multiple weed control agents acting together can be more effective in depressing weed populations than are single control agents. Also, observational data were collected to see how life-history patterns of the agents might influence their interaction with the weed. The role of herbivores in the depression of host plants is generally inferred from observations of weed abundance before and after the introduction of biological control agents (Dodd 1940, Huffaker & Kennett 1959, Hawkes & Johnson 1976, Cullen 1978, and McEvoy 1985). Another approach is to experimentally disturb the insect/plant system by artificially increasing plant densities and selectively excluding herbivores from these populations. Increasing the host plant to artificially high levels and exposing them to herbivory allows one to determine whether or not herbivores are capable of depressing dense populations. The exclusion of herbivores from some of these populations is required as a control since other factors may play a role in depression.

The system tested was the biennial weed tansy ragwort and two insects introduced to the western United States for its control: the cinnabar moth Tyria jacobaeae L. (Lepidoptera: Arctiidae) and the ragwort flea beetle Longitarsus jacobaeae (Waterhouse) (Coleoptera: Chrysomelidae). These two insects differ in feeding behavior, both in the times of year they feed and in the plant parts and stages they feed on.

Ragwort

Ragwort is a plant native to Europe which was accidentally introduced to North America in the early 1900's. It now occurs as a noxious weed throughout much of the maritime climate regions of North America: Nova Scotia, Maine, and Rhode Island on the east coast and British Columbia, Washington, western Oregon, and northern California on the west coast. It often occupies pastures and clear cuts in these regions. Pyrrolizidine alkaloids in ragwort can cause fatal liver dysfunction in cattle (Duby 1979, Johnson 1979, Bedell 1984), so high populations of this weed can greatly decrease the value of pasture or make it completely unsuitable for grazing. Hawkes et al. (1985) label ragwort as "probably Oregon's most serious weed problem, as it infests millions of acres of both private and public range and pasture lands in the western part of the

state."

Ragwort is a biennial but may become a short lived perennial when damaged by cutting or defoliation. It usually reproduces by seed but can reproduce vegetatively by root and crown buds (Harris et al. 1978, Dempster 1982). The plant is a rosette the first year and usually bolts the second year producing a stem 2-10 dm in height. One to several stems may be produced per plant and each stem bears numerous flower heads which are produced from July to September. Plants usually senesce and die after flowering.

The achenes produced are dimorphic with differing dispersal and germination properties (McEvoy 1984). The "disk" achenes are located centrally on the flower, are numerous, and have a pappus and trichomes which facilitate wind and animal dispersal. These seeds germinate readily. The "ray" seeds are located around the periphery of the flower, are less numerous, lack dispersal structures, and are heavier due to a thicker seed coat. These seeds often have delayed germination. McEvoy (1985) found an average of approximately 35000 ragwort seeds m^{-2} in the soil when the weed population was high. If the plants are destroyed for some reason, this seed reserve can act as a source for replenishing the population.

Biological control of ragwort was attempted because of the limitations of chemical control methods. Three chemicals are registered for ragwort control in pastures: 2,4-D,

dicamba, and picloram. Appleby (1979) recommends the use of 2,4-D because it is less likely to adversely affect clover (which is important forage) and is very effective if applied in March or April (killing 93-100% of the plants). Timing of application is important because 2,4-D does not kill ragwort once it bolts. Herbicide use can be fast and effective but requires application every year or two. Ragwort typically infests low-value lands such as dry pastures and clear cuts (Isaacson & Schrupf 1979) for which such an investment is not economical. Many areas also have too rough a terrain for application equipment.

Sheep have been used for controlling ragwort in pastures. Sheep usually do not show symptoms of ragwort poisoning and, using grazing management, they can learn to preferentially feed on the weed (Mosher 1979). They eat both foliage and flowers. It is not known how long plants can survive grazing since regrowth is possible and it may cause plants to become perennial.

Cinnabar moth

The cinnabar moth is a herbivore of ragwort in Europe and was introduced to the United States as a potential biocontrol agent. Initially, it was released near Fort Bragg, California, in 1959 and redistributed from this site. The moth was introduced into Oregon in 1960 (Frick and Holloway 1964) and has since become well established.

Adult moths are active in May and early June, ovipositing directly on ragwort, generally on the undersides of leaves. Large bolting plants are usually selected for oviposition (Dempster 1982). Larvae develop through five instars. The tiny first-instar larvae usually feed by skeletonizing the leaves on which they hatched, but as larval development progresses, movement on the plant and between plants increases. Larvae preferentially feed on the developing flower buds and then move down the plant feeding on the leaves. In England, Dempster (1982) found the moth completely defoliated all plants in a 19 ha study area in five of the eight years studied. Isaacson (1973) found the same phenomenon to occur at several sites in Oregon. Along the Oregon coast, this intensive activity by the larvae usually occurs in June, July and early August and pupation occurs by September.

In Europe, the density of the moth does not appear to affect the density of ragwort populations, instead the density of ragwort has a great effect on the density of the moth (Dempster 1982). van der Meijden (1979) also found that localized populations of ragwort experience extinctions and reinvasions; however, he found both ragwort and the moth persisted when observed on a larger scale, which included a number of populations.

In general, the introduction of the moth into North America has resulted in reduced populations of ragwort. At

Fort Bragg populations were reduced from 18 to 2.5 flowering stems m^{-2} (Hawkes & Johnson 1978) and a similar reduction was found in British Columbia (Harris et al. 1978). Considering the toxicity of the plant, this is not a satisfactory reduction in the weed. In the wet mild climates found on the west coast, ragwort plants are able to recover from defoliation. After defoliation, plants generally have shorter stems and reduced seed production but vegetative regeneration can result in increased rosette densities (Hawkes & Johnson 1978).

In areas of more marginal growth for the plant, such as where periods of frost follow defoliation or where water is limiting, the plant's ability to regenerate is greatly reduced and control by the cinnabar moth is more effective (Harris et al. 1978, Cox 1982). Harris et al. (1978) suggest that "the only biological control strategy likely to be effective in climates without severe stress is to attack the plant over the whole growing season." Practitioners felt this could be achieved by the introduction of additional agents, such as the ragwort flea beetle.

Flea beetle

The ragwort flea beetle was introduced to the Fort Bragg area in 1969 after the cinnabar moth had become well established. Two biotypes of this beetle are described by

Frick (1970), but only the biotype indigenous to Italy has been introduced to Oregon because its life cycle seems most suited to the area. Adults emerge in the spring but go through a summer aestivation which delays adult feeding and egg laying until the fall. Females oviposit approximately 83 days after they emerge (Frick 1970) and the eggs hatch two to three weeks later (Frick and Johnson 1972). There are three instars and pupation occurs in May in the soil adjacent to the plant.

Adults feed on ragwort by rasping through the leaves, leaving behind small circular holes or shot holes. Larvae bore into the roots and petioles of ragwort and feed on plant tissues. Potentially, the larvae could deplete root carbohydrate stores and damage plant vascular structures.

The ragwort flea beetle has been little studied in comparison to the cinnabar moth and some aspects of its life history are still uncertain. For example, McEvoy (unpublished) found the time of peak larval impact on ragwort and the plant sizes and parts fed on differed in Oregon from that described by Frick (1970). McEvoy (unpublished) found larval development to be slow during the winter months and the maximum number of larvae per gram of plant did not occur until March. The maximum concentration of larvae was in the petioles and leaves rather than in the root crown and roots as described by Frick (1970); therefore, the beetle may not be directly depleting root re-

serves during the winter months as much as disrupting the plant's vascular transport system or causing leaves to senesce prematurely. The flea beetle is studied much more extensively than the cinnabar moth in this thesis to determine the beetle's feeding patterns and seasonality on the Oregon coast and in the experimental plots. This information is already relatively well documented for the cinnabar moth.

The seasonal distribution of flea beetle larvae may be influenced by the timing and extent of adult summer aestivation and by the number of fecund adult females which overwinter. The occurrence or length of aestivation in Oregon is uncertain. The timing of aestivation is important in determining the temporal distribution of eggs and adult feeding. Females that overwinter may also affect the seasonal distribution of eggs by extending the period of oviposition at both ends of the season. Frick and Johnson (1973) found some females capable of living and ovipositing for as long as two years under artificial conditions. Beck (1980) states that in insects with an aestival diapause, the females which emerge in the spring usually do not lay any eggs until the following fall since the ovarioles remain undeveloped until after diapause. Females that overwinter may or may not oviposit during the winter but are likely to lay eggs the next spring before newly emerging females have completed ovarial development.

Co-occurrence of the two agents

Hawkes and Johnson (1978) found that the introduction of flea beetles to Fort Bragg further reduced ragwort populations to levels well below that achieved by the cinnabar moth alone. McEvoy (1985) measured the decline of ragwort populations after the introduction of both insects to a site on the central Oregon coast. After the introduction of the insects, total ragwort biomass declined 97%. Plant density also declined but fluctuated at low levels due to a temporary increase in the density of small plants. The seed bank was also affected by the herbivores, showing a decline of 86.7% between 1981 and 1985. The Oregon Department of Agriculture (ODA) made yearly analyses of ragwort densities at several sites in western Oregon where the beetle had been introduced. There was a decline in ragwort density to low or zero values at all but one of the sites. The moth was already widespread by the time of these introductions.

Beetles may complement cinnabar moth feeding in controlling ragwort since the timing of feeding and the plant parts fed on are different for the two insects. Beetles attack internal structures in the winter and spring and cinnabar moths attack foliage and flowerheads in the summer. There is some overlap in feeding behavior since adult

beetles have been found to damage the leaves and flower-heads of ragwort plants in England (Binns 1975) and re-growth of these parts in western Oregon (McEvoy, personal communication). Yet, if beetles attack all parts of the plant and throughout most of the year, they alone may be sufficient to control ragwort. The experiment done here compares the independent and joint effects these two insects can have on dense ragwort populations in a mild climate such as is found in western Oregon.

METHODS

Study Site

The study site is a 0.9 ha meadow in the Cascade Head Scenic Research Area on the central coast of Oregon. The ODA had previously released agents into the research area to control ragwort. Two thousand cinnabar larvae were released in 1978, 220 flea beetle adults in 1979, and 485 in 1980. In 1981, the average ragwort density was 157.2 plants m^{-2} with a population structure of 54% small vegetative plants, 32% large vegetative plants, and 14% flowering plants (McEvoy 1985). It took until 1983 for insect populations to become established. In that year, insect populations increased dramatically and ragwort declined to 3% of its former density.

Experimental Design

An exclusion experiment was conducted using cages and experimental ragwort populations to determine which of the following was more effective in depressing plant populations: the cinnabar moth, the beetle, or the combination

of the two agents. The timing of attack by the two insects varies such that selective exclusion can be obtained by timing the opening and closing of cages.

The experiment was a randomized complete block design analyzed as a two-way ANOVA. The meadow was divided into four 0.225 ha blocks and each treatment was randomly allocated to 0.5 X 0.5 m plots within a 3 X 4 m array located randomly within the block. The treatments included two levels of exposure to cinnabar moths (exposed, protected), two levels of exposure to beetles (exposed, protected), and one additional control treatment. The five treatments were:

1. Neither insect--cages which were continuously closed to cinnabar moths and flea beetles (control);
2. Moth only--cages which excluded flea beetles but were opened for cinnabar moths;
3. Beetle only--cages which excluded cinnabar moths but were opened for flea beetle attack. Both larvae and adults damage ragwort plants;
4. Both insects--cages which were continuously open to both flea beetle and moth attack;
5. Open controls--open plots with no cages to determine any side affects of caging (these plots were not included in the two-way ANOVA but were analyzed separately).

Each treatment was replicated three times within the block to allow for two destructive samples and an on going census of plants within the third replicate. Thus the total number of plots was:

$$4 \text{ Blocks} \times 5 \text{ Treatments} \times 3 \text{ Replicates} = 60$$

Cages were frames constructed of 1.27 cm diameter plastic (PVC) tubes covered with fine mesh nylon screens held down to the ground with sand bags when closed and rolled up partially when open. The plots were all tilled and, in February 1986, planted with two-month old ragwort plants started in the greenhouse; some seeds were sown haphazardly in March and again in November to give a variety of age classes. The plots were weeded and thinned to eight large plants per plot during the summer and fall of that same year (creating a density of 32 of these plants m^{-2}). Assuming most of these plants flowered in 1987, this would recreate a stand of flowering plants of approximately the same density as when the insects were first introduced to the pasture (i.e. 22 flowering plants m^{-2} (McEvoy 1985)). All cages were closed to exclude the insects while the plants became established.

Control cages were kept closed throughout the experiment, moth only cages were kept closed until adult moths were seen laying eggs (June 1, 1987) and were closed following hand defoliation (July 27, 1987, see below for an explanation as to why hand defoliation was used). Beetle only cages remained open except during this same period.

Cages were not entirely effective in excluding flea beetles during the summer of 1986, so adult beetles were removed with aspirators. However, this method was not very effective either, and eggs were found in the beetle exclu-

sion plots after October 1. On February 5, 1987, a systemic carbamate insecticide (carbofuran at a rate equal to 2.8 kg active ingredient ha⁻¹), was sprayed in all beetle exclusion treatments (the cinnabar moth was not active at this time). Carbofuran was selected because it is very effective against Longitarsus ferrugineus (Foudras) (formerly Longitarsus waterhousei Kutschera) in mint (Mark Morris, personal communication) and it is considered nonphytotoxic (Kuhr & Dorough 1976).

Carbofuran has never been used to control insects on ragwort, so a small test was done to determine any immediate detrimental effects it might have on this weed. Eight large rosettes were transplanted from the Corvallis area to 10-inch diameter pots and grown in the greenhouse for three to four weeks. One week before the field application was to be done, four of the potted plants were sprayed with the field dose and the other four remained as controls. For the first week the plants were examined for any signs of damage as compared to the control plants. None was found. The plants were examined for another month and no effects were seen.

The number of moths (from here on, the cinnabar moth will be referred to as "the moth", regardless of the stage of the insect which may be relevant, defoliation of ragwort by this insect is done only by the larval stage) was insufficient to cause significant damage to experimental

plants, so reproductive plants were hand defoliated July 13-24 to simulate moth damage. All reproductive plants were defoliated to simulate typical moth defoliation behavior when their populations are high. Henneberger (1986) found that flowering plants defoliated/deflorated by hand yield similar patterns of survivorship, fecundity, and leaf production to plants defoliated/deflorated by cinnabar larvae.

The intention of the experiment was to recreate an outbreak of the weed, with ragwort densities and insect activities similar to that found on the site in 1981. By the time of the experiment, ragwort populations at the site had been low for several years and moth populations were correspondingly low. Moths preferentially oviposit and feed on flowering plants and their populations frequently occur in high enough densities to completely defoliate whole populations of flowering stems both in Oregon (Isaacson 1973, Henneberger 1986) and Europe (van der Meijden 1979, Dempster 1982) and this was also seen in one open plot where moth eggs were laid and the caterpillars were active. Harris et al. (1978) found moths defoliated rosettes in Canada but with little effect on vegetative plant density or leaf number, particularly in the Pacific Northwest where rosette densities sometimes increased when the moth was present. Defoliation of rosettes had a detrimental effect on survival only in areas that were drier and

had more severe winters than are seen in this region. For these reasons, artificial defoliation should not have greatly biased the results of this experiment.

See Table 1 for a calendar of the methods used to establish and sample treatments.

Measures of Plant Response

Cohort of marked plants

In February, 1987, two large rosettes from each treatment in a block were randomly selected and marked with metal tags attached to the base of the plant. This marked cohort was censused monthly during the winter and once every two weeks during spring and summer when plants were assumed to be growing more rapidly. Counts were made of surviving plants, their leaves, and capitula (by stage) to measure the effect insects had on survivorship, reproduction, and development. The number of shot holes was counted at each census to estimate the timing of adult beetle activity.

Harvested plots

A. March harvest

Plots were harvested in March, one month after insecticide treatment, both to assure initial similarity in plant

Table 1. Calendar of events to establish and sample treatments.

<u>Event</u>	<u>Date</u>
1. Ragwort plants transplanted from the greenhouse to plots	Feb. 1986
2. Ragwort seeds sown in plots	March 1986
3. More ragwort seeds sown in plots	Nov. 1986
4. Insecticide applied to beetle exclusion plots	Feb. 1987
5. March harvest	March 18-25, 1987
6. Beetle only cages closed to exclude moths and moth only cages opened	June 1, 1987
7. Hand simulation of moth defoliation	July 13-24, 1987
8. Moth only cages closed to exclude beetles and beetle only cages opened	July 27, 1987
9. August harvest	Aug. 17-31, 1987

age structure, density, and biomass in all plots at the time when treatments were initiated and to determine the effectiveness of the beetle exclusions. To harvest treatments, all ragwort plants in a plot were dug up and separated into size classes. Plant size classes were defined by the following basal diameter measurements: 0.0-10.0 mm, 10.1-20.0 mm, and 20.1-40.0 mm. There were no plants as large as 40.0 mm. None of the plants had bolted or flowered. Rosettes over 10 mm looked like year old plants and so were expected to flower later in the year. Beetles were extracted from the plants as described later in the text and then all plants were dried and weighed.

B. August harvest.

Plots were harvested in August to measure the effect of treatments on plant survivorship, biomass, and reproduction, and to determine beetle larval activity. Survivorship was defined as the number of plants surviving to each size class (those living at the time of harvest). Vegetative plants were separated from flowering plants and divided into the same size classes as in the first harvest with the addition of size class 0-1 mm (seedlings). No rosettes 10.1-40 mm were found (presumably all had flowered). Plant biomass (dry weight) was also measured for each size category.

Reproduction was determined by the number of capitula per flowering stem, the number of seeds per flower, and seed viability. The number of capitula was counted directly but subsampling was required to determine seed number and viability. To subsample, two plants were randomly selected from each treatment in a block and three mature seed heads from each plant were selected haphazardly. Not all plants had three mature heads so the sample size was uneven. Achenes were sorted into disc and ray--10 ray and 10 disc achenes in each of the five treatments and each of the four blocks were individually weighed. From each treatment and each block, fifty achenes of each type were tested for germination using the methods described by McEvoy (1984). Thirty days were allowed for germination. Those achenes that failed to germinate were tested for viability by staining with tetrazolium. Seeds from each sample were pricked with a needle and placed in individual vials with 1% solution of tetrazolium and left for 24 hours (modification of Grabe 1970). Those embryos which stained red were considered viable--most seeds which did not germinate were empty.

C. Beetle extraction.

The intensity of infestation by beetle larvae was defined as the number of larvae found per gram of plant dry

mass. Beetle intensities were determined for each treatment and size category and all plants were separated into above and below ground parts to determine which parts were most heavily attacked by beetle larvae. Beetles were extracted by placing plants in Tulgren funnels. The number of larvae extracted by Tulgren funnels with 25 watt bulbs increases with time (see Fig. 1). After seven days (168 hours), the number of larvae extracted was still increasing because large plants had not completely dried out. For this reason, 40-watt bulbs were used for large plants and 25-watt bulbs for small plants and plants were left in the funnels for seven days. A higher watt bulb used for smaller plants would kill the insects before they could crawl from the plants (McEvoy unpublished). Once extraction was complete, plants were dried at 60 C for three days and weighed.

The number of beetles extracted by this method was compared to that extracted by dissection to give some indication of the efficiency of the Tulgren method. Experimental plots were randomly sampled and 4 large plants (20-40 mm basal diameter) and 2 plants from each of the other size categories (this sample was done in April, therefore, no flowering plants were available to sample) were dissected and compared to the beetle counts from Tulgren extractions done at the same time. It was thought that dissection would extract nearly 100% of the larvae present

and give a bases for determining the efficiency of the Tulgren method. However, the Tulgren method extracted twice the number of beetles per gram of plant as did dissection and required less labor. For this reason, it was the method used, and its actual efficiency is uncertain.

D. Statistical Analysis.

The experiment was designed such that the effects of the two insects could be statistically determined using a two-way analysis of variance (two-way ANOVA). When the data did not appear to fit an additive, linear model, transformations were used. A model was sought for that gave a normal distribution of variates and in which the variances were homogeneous (based on Hartley's F_{\max} test). Homogeneity of variances carried a greater weight in selecting an appropriate model because normality is often hard to determine with a sample size of four.

Flea Beetle Life History

Larval development

The stages of beetle larvae active at different times of the year, and the intensity of this activity, was determined from randomly sampled second-year plants (rosettes at least 10 mm in basal diameter, or bolting plants) taken at

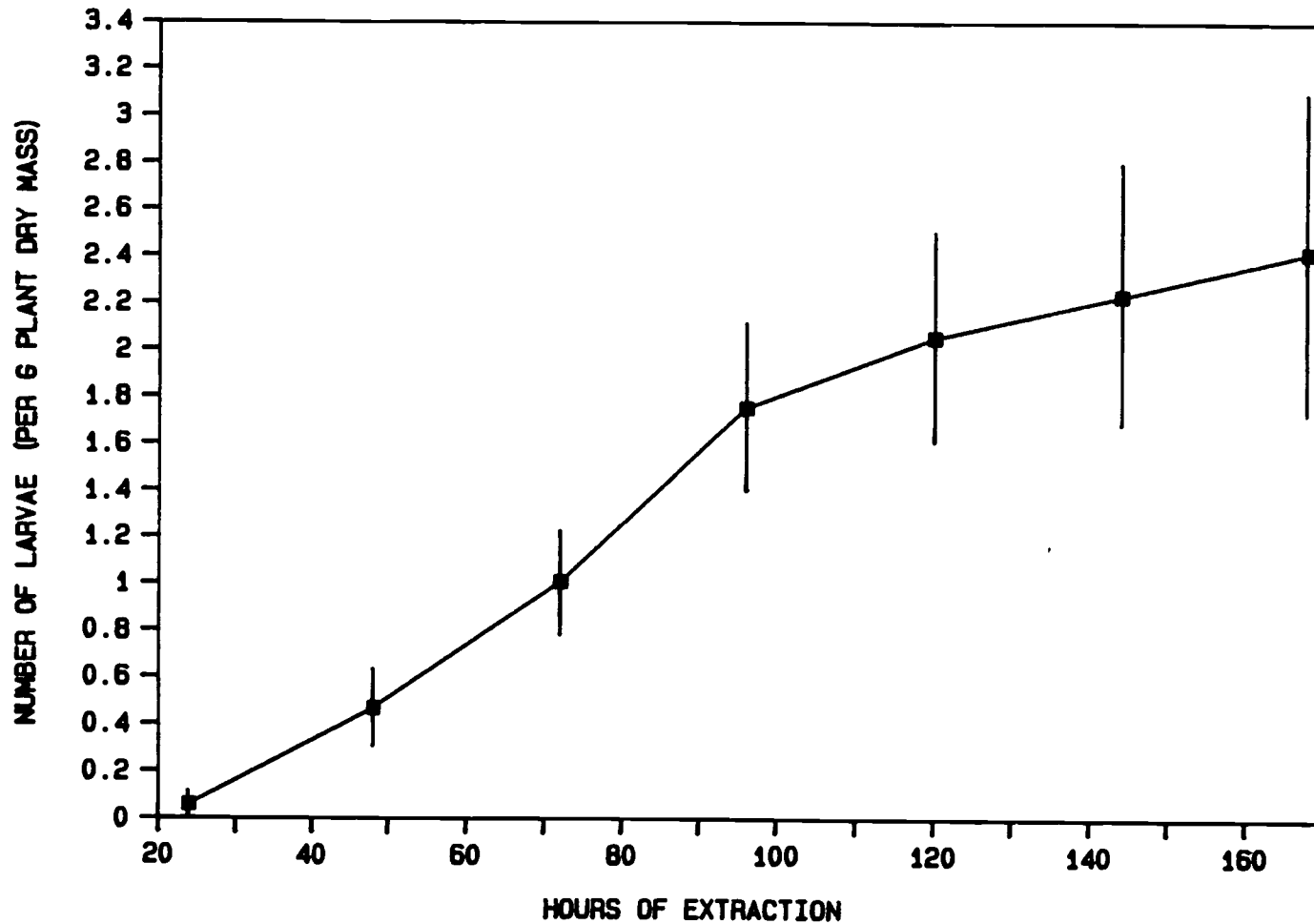


Figure 1. The relationship between the length of time plants are left in Tulgren funnels and the number of *Longitarsus jacobaeae* larvae extracted. Points are means (\pm SE) of larvae extracted from plants taken from beetle exposed plots. No beetles were found in plants from beetle protected plots.

one month intervals from Feb. 1987 to March 1988. From March 2 to September 25, 1987, plants were sampled from flea beetle exposed plots in this experiment and another similar experiment conducted simultaneously in the same pasture. However, once flowering and senescence had occurred in the second year plants, there were no second year plants to sample in flea beetle exposed plots so large rosettes were sampled from wild plants at the site and from another nearby similar site after September. These plants tended to be smaller and their age was unknown.

A. Instar determination.

Larval instar was determined by head capsule width, measured dorsally and at the widest point. Subsamples of the beetles were done by visually selecting from the above samples those which gave the whole range of larval sizes. Samples were selected from January, March, April, May, and June. All the head capsules in the samples selected were measured for a total of 527. This gave the distribution of head capsule sizes and provided the basis for determining the instar of any given larva.

B. Subsampling for the temporal distribution of larvae.

Instar distributions were counted in subsamples such that no more than 50 larvae per sample had to be measured.

The subsampling procedure was a modification of counting discs (Strickland 1954, Morgan et al. 1955, Southwood 1979). Sampling discs were constructed to split the sample to either 1/2, 1/3, or 1/6. The larval sample was placed in a petri dish with ethyl alcohol and swirled to spread the sample fairly even in the dish. The dish was then placed over the appropriate sampling disc and all of the larvae with 1/2 or more of the body falling within the dark areas were selected and the instar was determined.

Adults

Adult beetle densities were estimated by counting the number of adults found in flea beetle exposed plots during the monthly census of plant growth. Estimates of the time of oviposition and aestivation were made by collecting adult female beetles from the site at different times of the year and dissecting and examining them for oocytes. Adult feeding activity was measured in the monthly plant census as described earlier.

RESULTS

March Harvest

The March harvest was done to give some indication of the initial conditions of the experimental plots. It was done one month after pesticide was used to kill beetles in the beetle exclusion plots and before simulated moth defoliation of the moth treatments. The ragwort density and biomass was similar between treatments (Table 2) with no significant differences in main effects (Table 3). Plant density was also similar to that found in the pasture in 1981-1982 when there was an outbreak of the weed and the insects had been recently introduced for its control. In 1981-82 the density was 157.2 plants m^{-2} (McEvoy 1985) and in these experimental plots, the median density was 191.31 (SE=5.92) plants m^{-2} .

This harvest also demonstrated the effectiveness of beetle exclusions (Tables 2 and 3), with beetle protected plots having significantly and substantially fewer total larvae and fewer larvae per gram of plant dry mass.

Table 2. Ragwort plant density and biomass and flea beetle larval densities found in the March harvest. Values are means (with 95% confidence intervals) which have been back transformed when a transformation was used. See Table 3 for the transformation and statistical analysis. Plots were 0.25 m². Moth effects were simulated with artificial defoliation.

Measured Character	Treatment			
	Neither Insect	Moth Only	Beetle Only	Both Insects
Small plant density (# per plot)	44.60 (13.29-144.47)	53.60 (21.42-131.95)	36.34 (29.88-44.15)	59.95 (49.4-72.70)
Large plant density (# per plot)	7.50 (4.91-10.09)	7.50 (6.52-8.48)	7.00 (6.20-7.80)	7.75 (5.90-6.60)
Total plant density (# per plot)	55.26 (21.42-140.17)	62.43 (28.09-138.77)	42.38 (35.06-50.42)	67.03 (55.83-80.45)
Small plant biomass (# per plot)	15.26 (7.42-23.1)	25.23 (13.72-36.72)	14.57 (6.66-22.49)	30.34 (18.42-42.27)
Large plant biomass (# per plot)	150.00 (110.16-189.84)	124.46 (91.90-157.02)	192.87 (99.35-286.39)	136.68 (83.00-190.31)
Total plant biomass (g per plot)	165.26 (125.64-204.88)	149.68 (118.91-180.45)	207.45 (107.13-307.77)	167.00 (114.53-219.49)
Beetle larval densities (# per plot)	4.87 (0.01-33.12)	2.90 (-0.19-17.69)	322.76 (161.88-636.78)	251.14 (129.71-481.51)
Beetle larvae g ⁻¹ dry plant	0.09 (-0.03-0.21)	0.05 (-0.03-0.15)	1.56 (0.76-3.68)	1.47 (0.77-3.09)

Table 3. Statistical analysis of the March harvest to determine initial differences between herbivory treatments. Values are F ratios from two-way ANOVA (df=1,9) and stars denote significance levels. Moth effects are effects due to simulated moth defoliation.

Measured Character	Main Effects		Block Effect	Inter-action	Transform-ation used
	Moth	Beetle			
Small plants per plot	1.17	0.02	2.89	0.26	Log _e
Large plants per plot	0.19	0.02	1.07	0.19	---
Total plants per plot	1.17	0.13	2.74	0.40	Log _e
Small plant biomass (g/plot)	9.57**	0.28	3.00	0.48	---
Large plant biomass (g/plot)	1.56	0.71	0.46	0.22	---
Total plant biomass (g/plot)	0.64	0.73	0.27	0.13	---
Beetle larvae per plot	0.27	40.86***	1.12	0.02	Log _e
Beetle larvae g ⁻¹ dry plant	0.06	48.82***	0.15	0.01	Inverse

* P<0.05
 ** P<0.01
 *** P<0.001

Census and August Harvest

Plant survivorship

In the August harvest, the number of flowering plants at the time of harvest was not affected by either the beetle, the moth, or the combination of the two (Fig. 2). Furthermore, all the censused rosettes survived and flowered that summer. However, beetles caused a highly significant reduction in vegetative plant survivorship (Fig. 2 and Table 4). There was no significant moth effect on survivorship of these plants, as expected since none were artificially defoliated. The effect beetles had on plant survivorship reduced small and large vegetative plant densities to 7% and 2%, respectively, of the densities found in control plots. The demographic structure of the plant population in beetle exposed plots was 11% small vegetative plants, 5% large rosettes, and 76% flowering plants. In control plots the population structure was pyramidal--50% small vegetative plants, 30% large rosettes, and 12% flowering plants, which is very similar to the population structure found in the pasture by McEvoy (1985) when ragwort densities were very high.

Plant biomass

In the absence of the moth, beetles reduced small

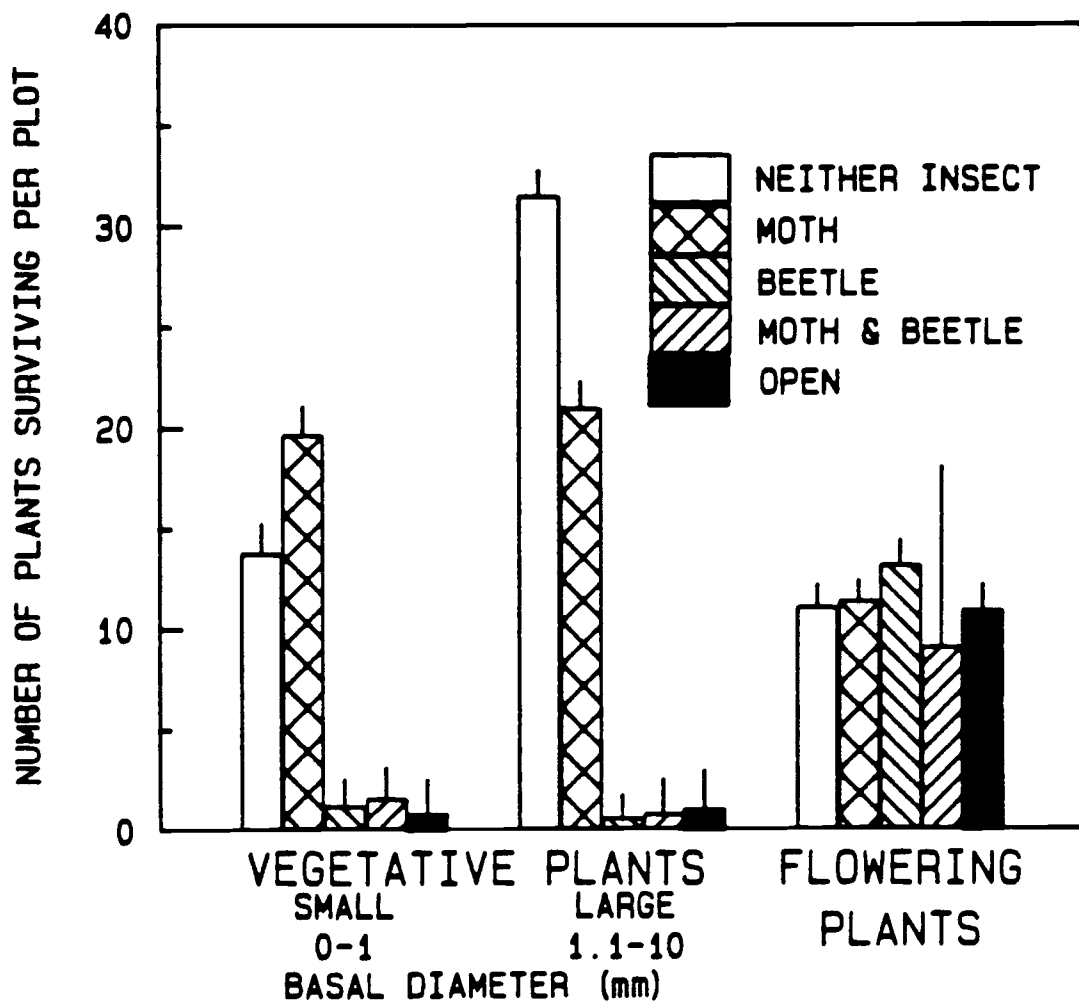


Figure 2. The effect of herbivory treatment on ragwort survivorship in each plant size category. Bars represent means (+SE) from the August harvest. See Table 4 for statistical analysis.

Table 4. Statistical analysis of the effects of insect activity on plant characteristics. Values are F ratios from two-way ANOVAs (df=1,9) and stars denote significance levels. Significance levels for the main effects are not reported when there are significant interactions. Moth effects are effects due to simulated moth defoliation.

Measured Character	Main Effects		Block Effect	Inter-action	Transformation used
	Moth	Beetle			
Small plants per plot	0.51	34.20***	2.44	0.07	Log _e
Large plants per plot	0.15	68.38***	0.60	1.11	Log _e
Flowering plants per plot	3.28	0.05	3.85	4.68	Log _e
Total plants per plot	0.39	48.18***	1.21	0.02	Log _e
Small plant biomass (g/plot)	0.29	24.82***	3.28	0.98	Inverse
Large plant biomass (g/plot)	5.22	144.56	1.16	5.73*	Log _e
Flowering plant biomass (g/plot)	<0.005	1.07	1.38	0.48	Log _e
Total plant biomass (g/plot)	0.01	0.68	1.34	0.58	Log _e
Leaves per bolted stem	7.56	2.15	1.37	7.06*	Log _e
Capitula per stem	42.41***	10.37*	2.13	0.54	Square root

* P<0.05
 ** P<0.01
 *** P<0.001

vegetative plant biomass by 81% and large vegetative plant biomass by 99% (as compared to controls). In the absence of beetles, moths had no indirect effect on small vegetative plants but reduced large vegetative plants by 60% (Fig. 3 and Table 4). The beetle was expected to show a much greater impact on vegetative plants than the moth since moth treatments included defoliation of flowering plants only. Any effects this had on vegetative plants must be indirect--such as by decreasing shade and allowing a greater drying of the soil.

As with flowering plant survivorship, neither insect, nor the combination of the two had an effect on flowering plant biomass.

Leaves

Acting independently, moth and beetle activity have little impact on the mean leaf number of flowering plants (Fig. 4). By one month after defoliation, plants are able to regenerate nearly all of their leaves. Beetles appear to have some impact on leaf number but their effect is greatly magnified by the presence of the moth (Fig. 4 and Table 4). This interaction may be evidence that the beetle reduces the plant's ability to regenerate leaves after defoliation. Evidence for this was also seen when changes in foliage production were followed over a period of time (Fig. 5). There was little difference in foliage produc-

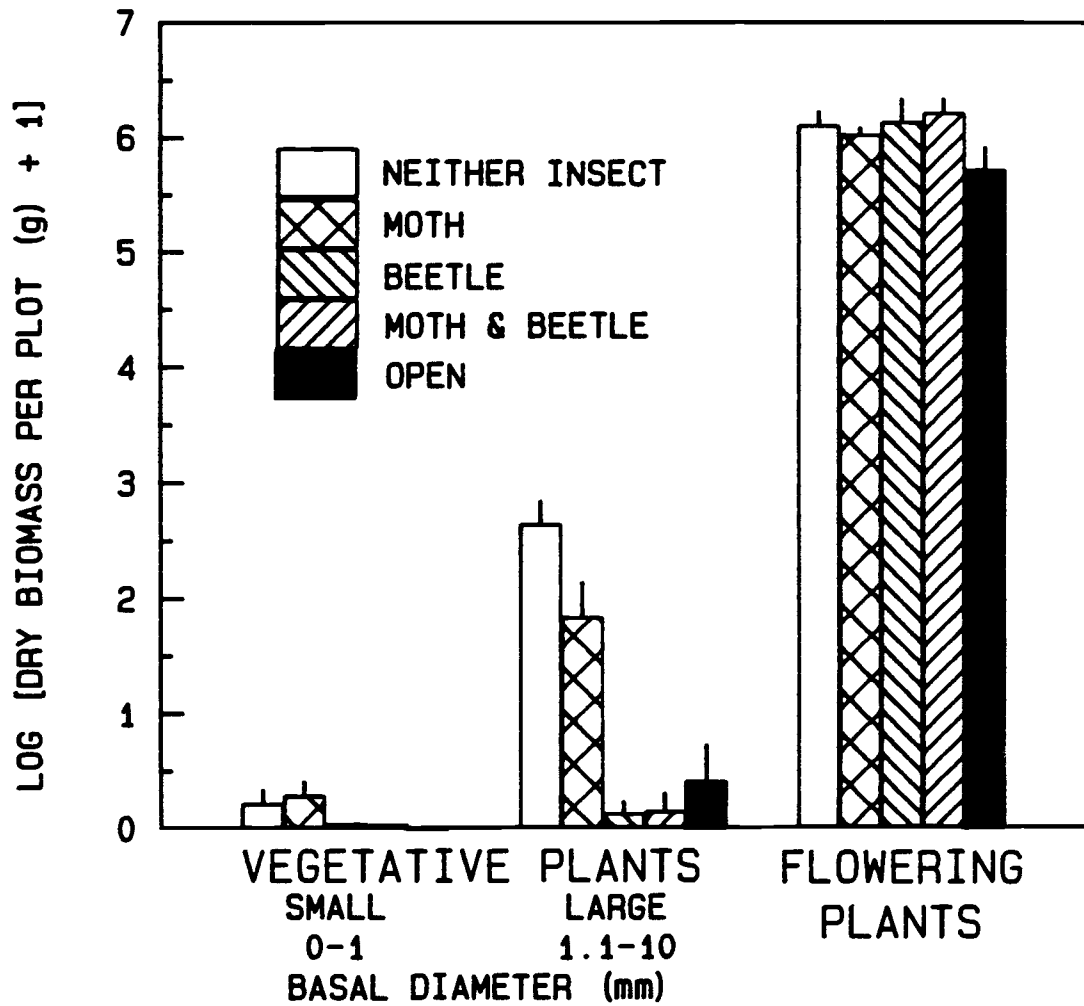


Figure 3. The effect of herbivory treatments on ragwort biomass in each plant size category. Bars represent means (+SE) of the August harvest. See Table 4 for statistical analysis.

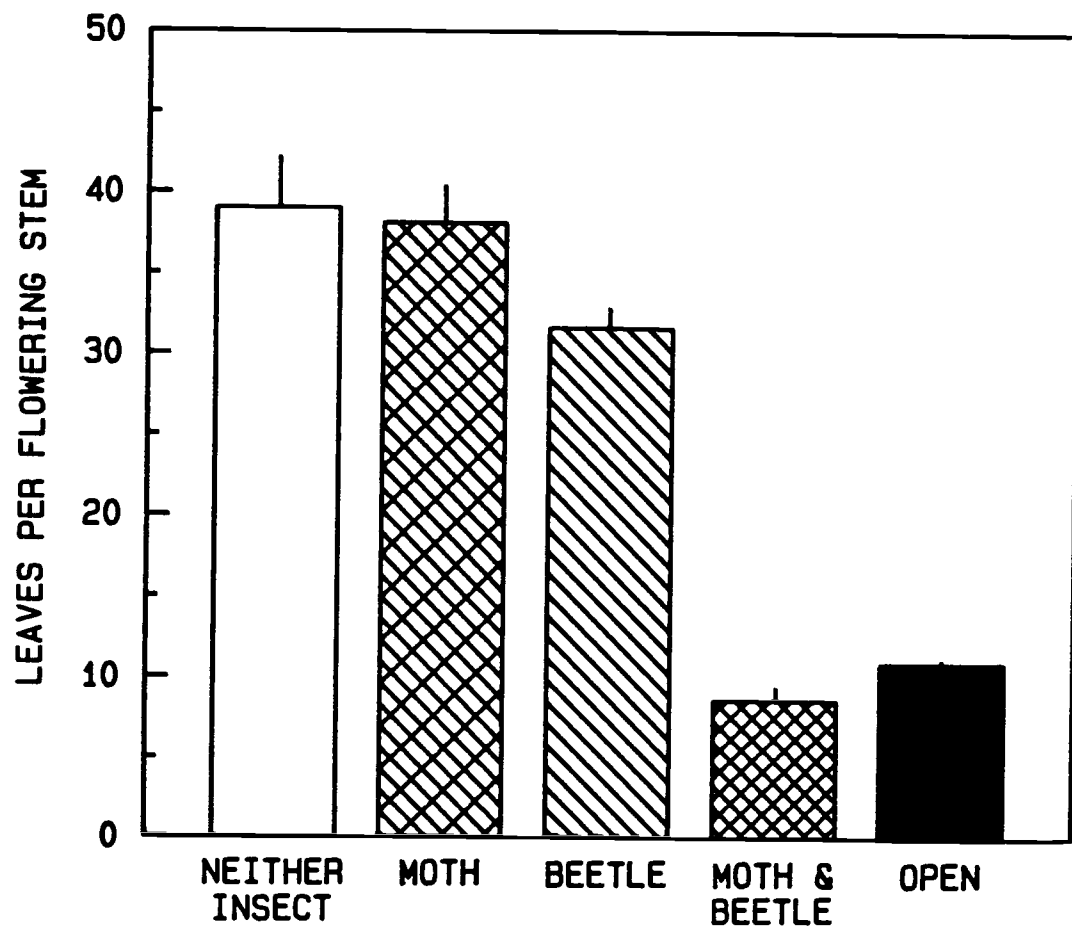


Figure 4. The effect of herbivory treatments on the number of leaves per stem on flowering plants. Bars represent means (+SE) of the August harvest. See Table 4 for statistical analysis.

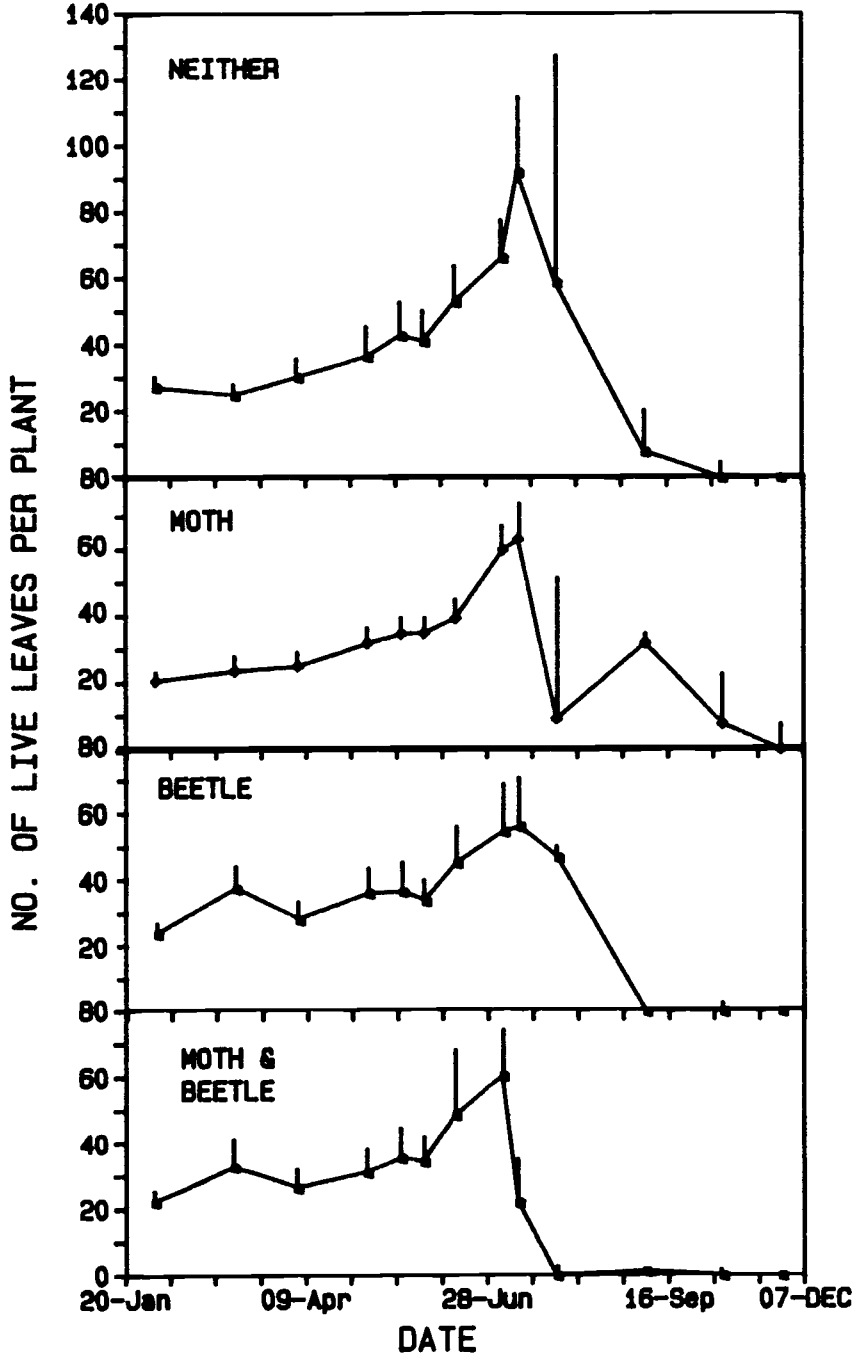


Figure 5. Ragwort foliage production throughout 1987 for each treatment. Points represent the mean (+SE) number of live leaves from a marked cohort of ragwort plants.

tion up until the time of defoliation. In early July, the number of leaves per plant began to naturally decline as plants matured. Simulated moth defoliation (July 13-24) caused an abrupt decline in leaf number and then regrowth occurred and senescence was delayed. When only beetles were present, senescence occurred slightly earlier than in controls. The plants' ability to regenerate defoliated leaves was greatly reduced when beetles were present.

Plant reproduction

The number of capitula present on flowering plants at the time of harvest differed greatly among treatments. Moths and beetles each caused a significant reduction in capitula production: moths reducing capitula number by 77% and beetles by 39% of control levels (Fig. 6 and Table 4). Together, insects reduced capitula number by 98%.

The census determined the effect of treatments on capitula production over the flowering season. Flower buds (primordia) were little affected by any treatment, yet the survivorship of these buds to flowering and fruiting was strongly reduced by herbivory (Fig. 7). Moths reduced and delayed flower and fruit production while beetles caused only a small reduction in flowers and fruits. When both insect occurred together, flower and fruit production were very low.

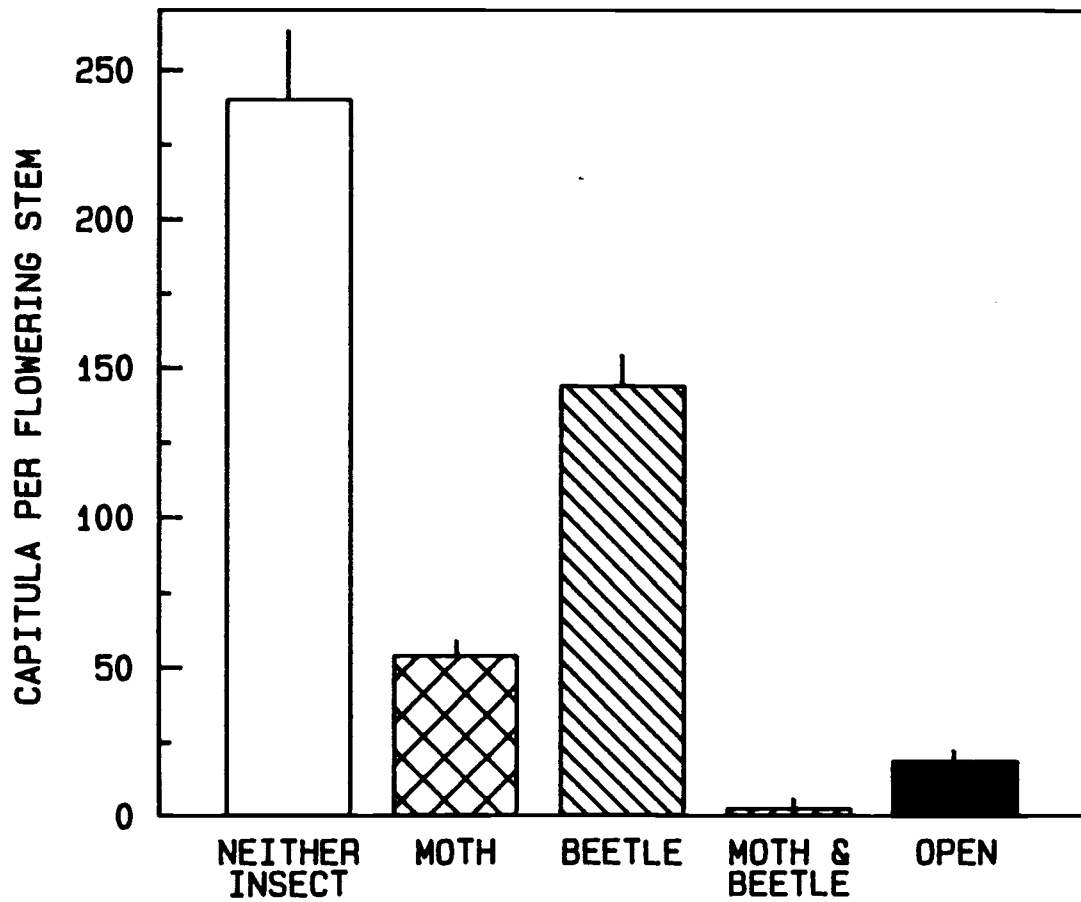


Figure 6. The effect of herbivory treatments on the number of capitula (including all stages of flower development) produced per stem on flowering ragwort plants. Bars represent means (+SE) of the August harvest. See Table 4 for statistical analysis.

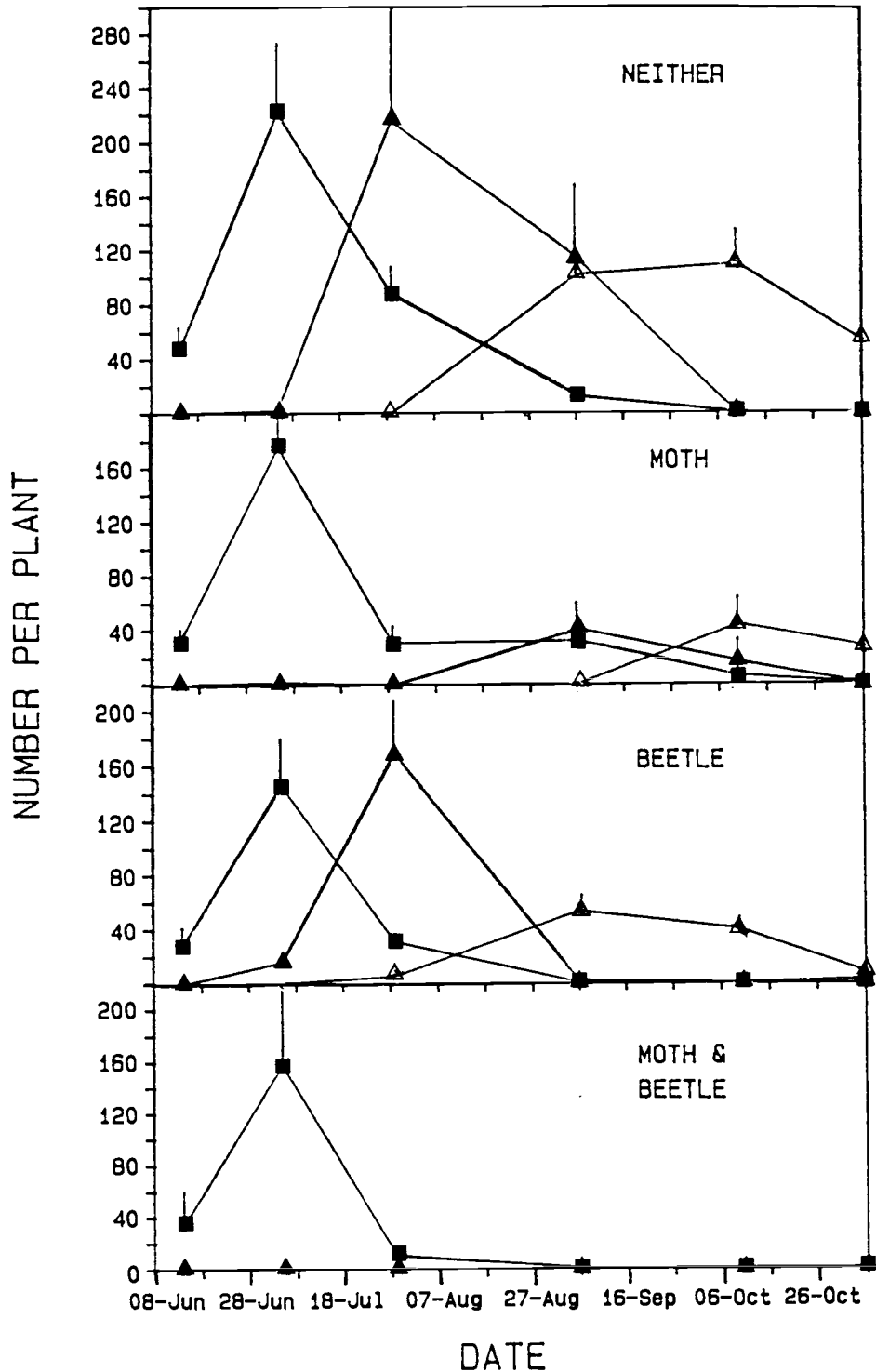


Figure 7. Ragwort capitula production throughout the flowering season and for each treatment in 1987. Points are means from a marked cohort of plants. Three stages of development are represented, ■ primordia (buds), ▲ flowers, and △ fruits (capitula whose achenes have a well developed pappus).

Seed number, mass, and viability also were measured to determine plant reproduction. Moths had the greater impact on these parameters (Table 5 and 6), reducing the number of achenes per head by 16%, seed weight by 44%, and seed viability by 65% when they occurred alone. The effect of the beetle alone appears to be small, however; when both herbivore activities are present, there is a synergistic effect causing a 40% reduction in the number of achenes per head, an 89% loss of individual achene weight, and 0% seed viability.

The effects of the insects on seed mass and viability were determined by comparing treatment plots to the control plots that were caged year round to exclude both insects. Cages may have affected flower pollination by bees and flies but any bias caused by this would tend to make estimates of the amount of reduction in seed quality conservative since moth and beetle exposed plots had the lower viability but were more open to pollinators than were the control plots. Further, bees and flies were seen feeding on flowers through the caging material.

Open plots

The open plots served as a control to monitor the effects of caging on the experiment. An analysis of variance was used to compare the means of open plots with

Table 5. The relationship between treatments and ragwort achene number, mass, and viability. Values are means (with 95% confidence intervals) backtransformed when transformations were used. See Table 6 for transformations and statistical analysis. Moth effects were simulated with artificial defoliation.

Measured Character	Treatment			
	Neither Insect	Moth Only	Beetle Only	Both Insects
Disk achenes per head	54.50 (42.95-66.05)	47.00 (40.65-53.34)	55.25 (47.00-63.5)	38.25 (-7.57-84.07)
Ray achenes per head	11.66 (9.42-12.60)	8.00 (5.88-10.12)	11.25 (9.57-12.93)	1.25 (-0.60-3.10)
Disk achene mass (mg)	0.178 (0.086-0.275)	0.093 (0.045-0.142)	0.176 (0.103-0.252)	0.019 (-0.027-29.62)
Ray achene mass (mg)	0.295 (0.173-0.419)	0.229 (0.104-0.360)	0.301 (0.227-0.376)	0.034 (-0.001-0.035)
% Viable disk achenes	31.5 (16.9-46.0)	10.5 (-0.7-21.7)	52.0 (35.6-68.4)	0.0 (0.0)
% Viable ray achenes	31.3 (13.8-68.7)	17.5 (1.61-130.6)	55.94 (47.4-65.4)	0.0 (0.0)

Table 6. Statistical analysis of the effects of insect activity on achene number, mass, and viability. Values are F ratios from two-way ANOVAs (df=1,9). Significance levels are not given for moth and beetle effects when there is a significant interaction between the two. There were no significant block effects.

<u>Character Measured</u>	<u>Main Moth</u>	<u>Effects Beetle</u>	<u>Inter-action</u>	<u>Transformation used</u>
Disk achenes per head	2.45	0.33	0.17	---
Ray achenes per head	47.16	11.79	13.67**	---
Disk achene mass (mg)	23.24	4.75	4.92*	Square root
Ray achene mass (mg)	20.35	8.98	10.37**	Square root
% Viable disk achenes	33.28	0.91	1.94*	---
% Viable ray achenes	11.44	2.34	1.93*	Log _e

* P<0.05
 ** P<0.01
 *** P<0.001

those of sham cages exposed to both herbivores. The variables compared were the total number of plants, the number of seedlings, rosettes, and flowering plants; the biomass of seedlings, rosettes, flowering plants, and all plants; the number of leaves per flowering stem; and the number of flowers. No means were significantly different except for the biomass of flowering plants (Table 7). Flowering plants in open plots were 63% of the biomass of those in the sham cages. In the March harvest, when treatments were first initiated, there was no significant difference in any of these parameters, nor was there any difference in beetle larval loads (Table 8).

Flea Beetle Life History.

Larval development

A. Head capsule measurements and instar determination.

The frequency distribution of larval head capsule widths yields three groups, each representing one of the three instars (see Fig. 8). Larval instars could be determined by the following criteria: those with head capsule widths of $\leq .21$ mm were considered first instars, of $>.21$ - $<.31$ mm were second instars, and of $\geq .31$ mm were third instars.

Table 7. The effects of cages on various ragwort characteristics at the time of the August harvest. "Sham-plots are cages open to both insects. "Open-plots" had no cages.

Measured Character	Means (SE)		F Ratio (df=1,9)
	Sham-Plots	Open-Plots	
Small vegetative plants per plot	3.25 (2.36)	1.50 (1.19)	1.62
Large vegetative plants per plot	2.25 (2.25)	2.25 (1.93)	<0.005
Flowering plants per plot	9.50 (1.44)	11.25 (1.18)	0.95
Small vegetative plant biomass (g)	0.03 (0.02)	0.04 (0.02)	0.40
Large vegetative plant biomass (g)	0.20 (0.20)	0.83 (0.72)	1.47
Flowering plant biomass (g)	484.83 (53.13)	291.92 (49.78)	72.69**
Leaves per stem on flowering plants	9.75 (2.59)	11.00 (3.49)	0.10
Capitula per stem on flowering plants	4.25 (2.52)	28.00 (17.62)	1.35

* P<0.05

** P<0.01

Table 8. The effects of cages on ragwort plant density and biomass and flea beetle larval number at the time of the March harvest. "Sham-plots" are cages open to both insects. "Opens-plots" had no cages.

Measured Character	Means (SE)		F Ratio ¹ (df=1,3)	Data Transformation used
	Sham-plots	Open-plots		
Plants per plot	68.75 (7.77)	60.00 (11.47)	1.66	---
Plant biomass (g)	167.02 (33.44)	210.16 (38.34)	0.51	---
Flea beetle larvae per plot in small plants	6.46 (2.62)	1.80 (0.27)	3.76	Log _e
Flea beetle larvae per plot in large plants	1.09 (0.49)	0.90 (0.61)	0.02	---

¹F ratios are from an ANOVA. None are significant.

B. Temporal distribution of instars within plants.

The beetle intensities in plants varied at different times of the year (Fig. 9), probably showing the effects of both the seasonality of the insect and the increasing size of plants from February to May and from September to March (only the largest plants available were sampled at any one time). In August and September, few or no larvae were found. First instars predominated in the early winter and fall and third instars were not seen until early spring. A peak in larval intensity occurred in May of the first season, another much larger peak occurred in early December of the second season, a new increase in larval numbers followed a sharp decline. The difference in numbers between March 1987 and March 1988 may be due to either temporal differences (i.e. differences in weather between years) or spatial differences because the later plants were sampled from outside the experiment.

The greatest loads (and the greatest total number) of larvae were found in the shoots. However, larvae do occur in the roots, particularly during the third instar. Beetles tended to move into the roots during the last instar and prepupa and pupa were found in the soil near the plant.

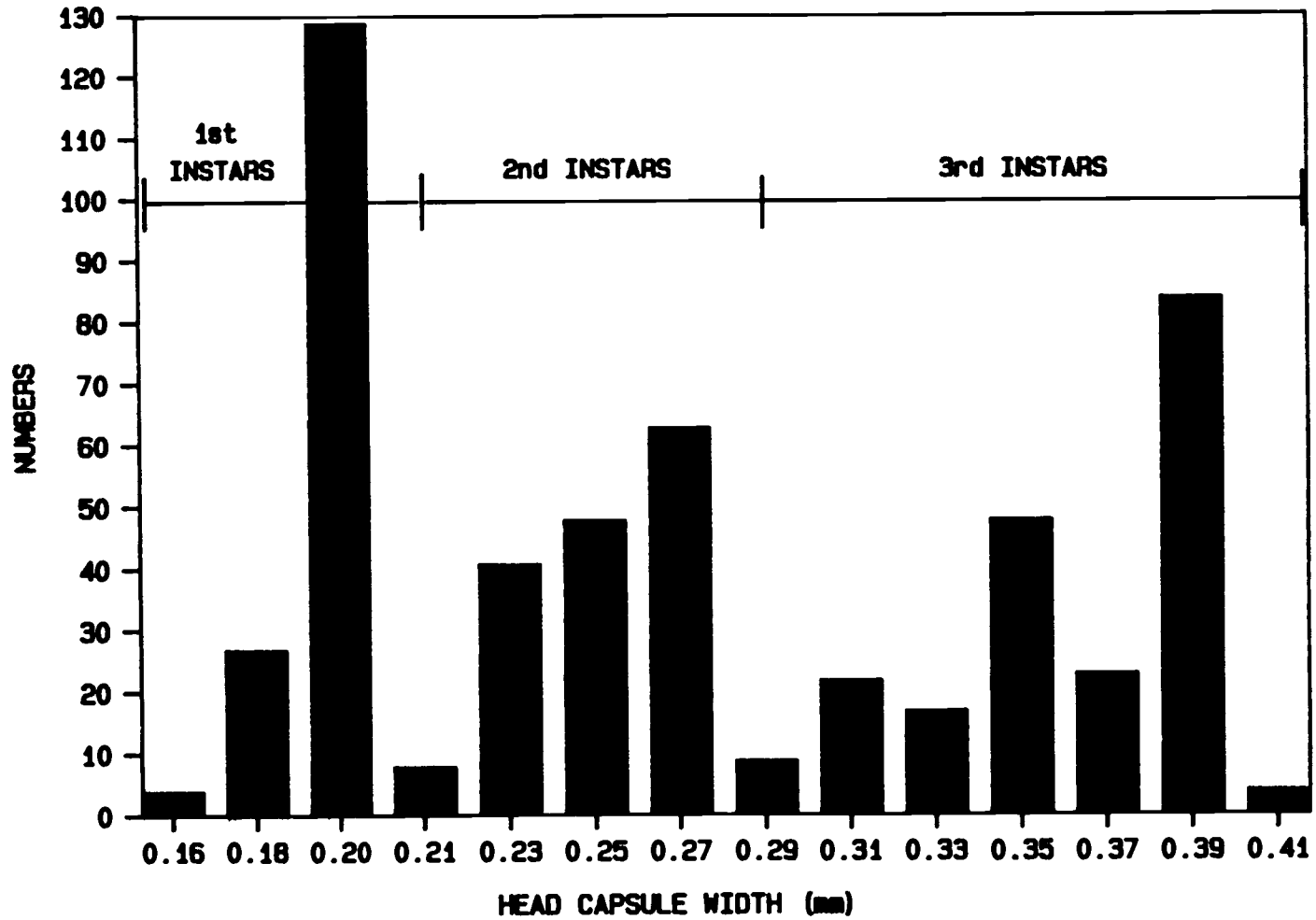


Figure 8. Relative frequency of head capsule widths in Longitarsus jacobaeae larvae.

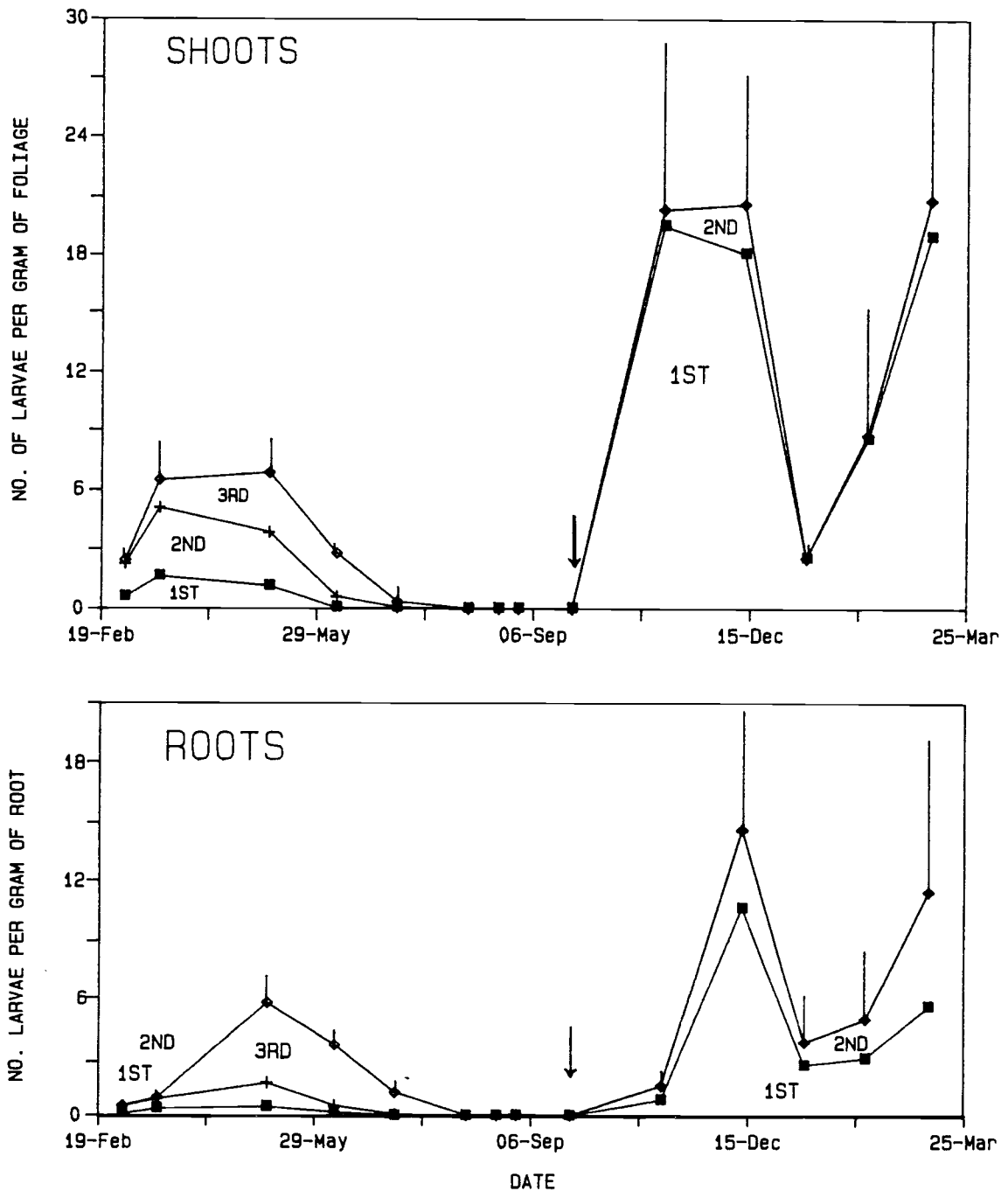


Figure 9. The number of larvae per gram of (A.) dry foliage and (B.) dry root. The figures are stacked line graphs where the top lines represent the total number of larvae found and the area between curves represents the number in each instar indicated. Points represent means (+SE) of monthly samples (semimonthly in August). The arrow indicates the time when experimental plants were no longer available to sample so wild plants were sampled at all later dates.

Adults

A. Adult beetle population and damage levels.

The number of active adult beetles seen was low (less than one per plot) during the winter and spring and declined to very low levels in May (Fig. 10). In early summer, adult populations began to increase at the same time the larvae from the previous winter and spring completed development (May to August). These populations continued to increase to the end of the summer. The sampling for adult beetles by visual observation may not be very effective, because they are small and sometimes hard to find and their activity varied with the time of day. They were less active in the mornings when it was colder. The sampling method was probably adequate to act as an index of activity and seasonal timing when trends in activity are observed. The actual sampling efficiency is unknown.

Like adult beetle activity, the amount of damage adult beetles cause to ragwort plants is also lower during the winter and spring. The amount of damage present at any one time is a function of both the activity of adults and the rate at which damaged leaves senesce. Low levels of damage (and presumably, feeding) continue for another month beyond the time when adult populations begin increasing again.

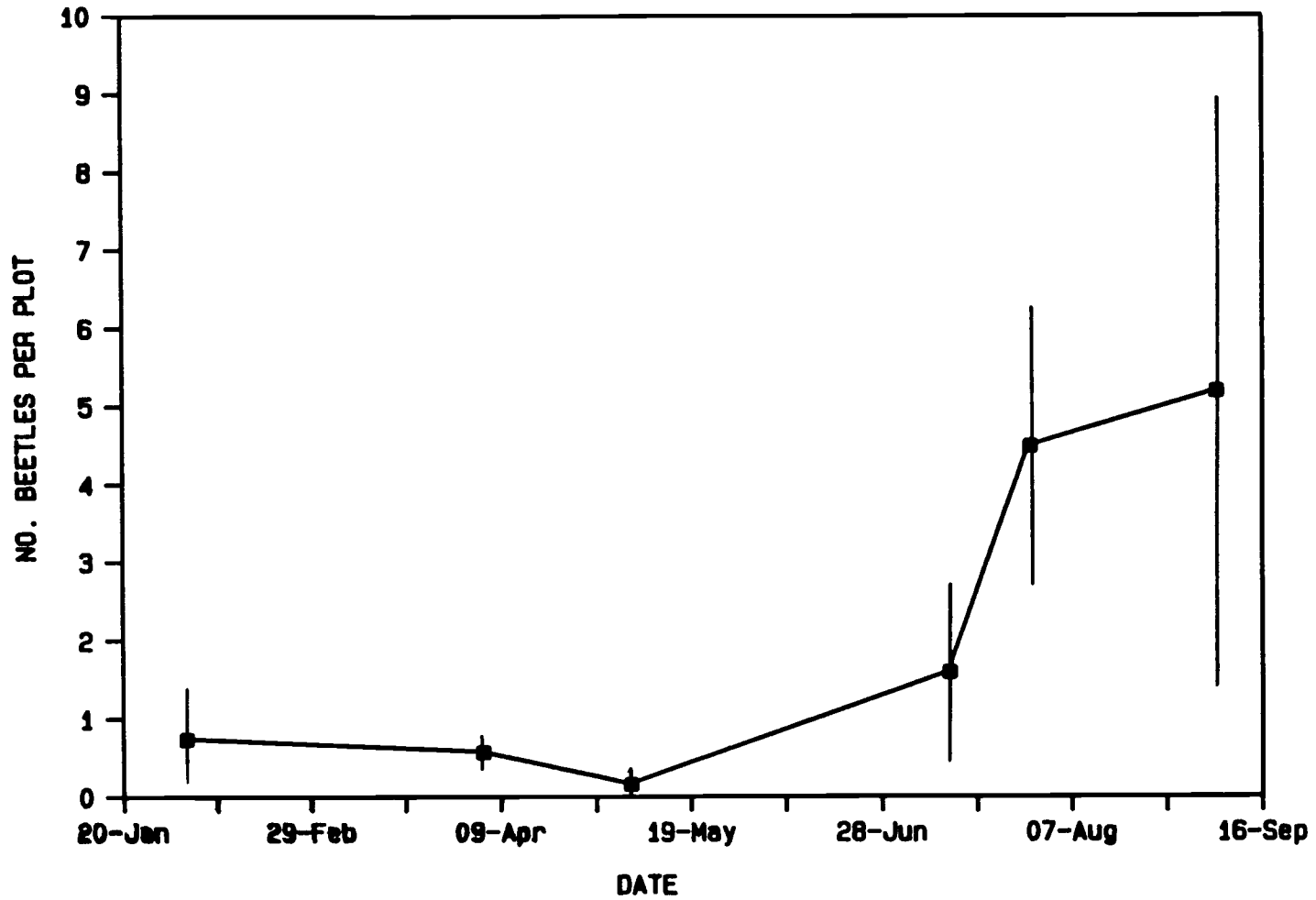


Figure 10. Mean (+SE) number of adult *Longitarsus jacobaeae* sampled from experimental plots in 1987.

Adults begin emerging and/or diapausing adults begin to become active again in late June-early July (Fig. 10) at which time there is a sudden increase in feeding activity (Fig. 11). Feeding activity after July is not recorded in Fig. 11 because the marked plants began senescing at this time and so damage counts would not be representative of actual beetle activity since beetles feed only on live leaves.

B. Timing of egg production.

Dissections of female beetles revealed seasonality in egg production, and a fairly clear pattern in egg development can be seen (Table 9). Very few or no eggs are laid until late September and early October. This corresponds with general observations in the field (Frick 1973, and personal observation). However, egg production seems to remain high throughout the winter and early spring. These early spring females probably all emerged as adults the previous summer and are not new adults since no third instar larvae (the last instar) were seen until this same time (Fig. 10) and no pupae were observed until June. Furthermore, in June and July, several (19) new adult females extracted from root samples in the Tulgren funnel were dissected and no egg development was seen. Females appear not to emerge with fully developed eggs.

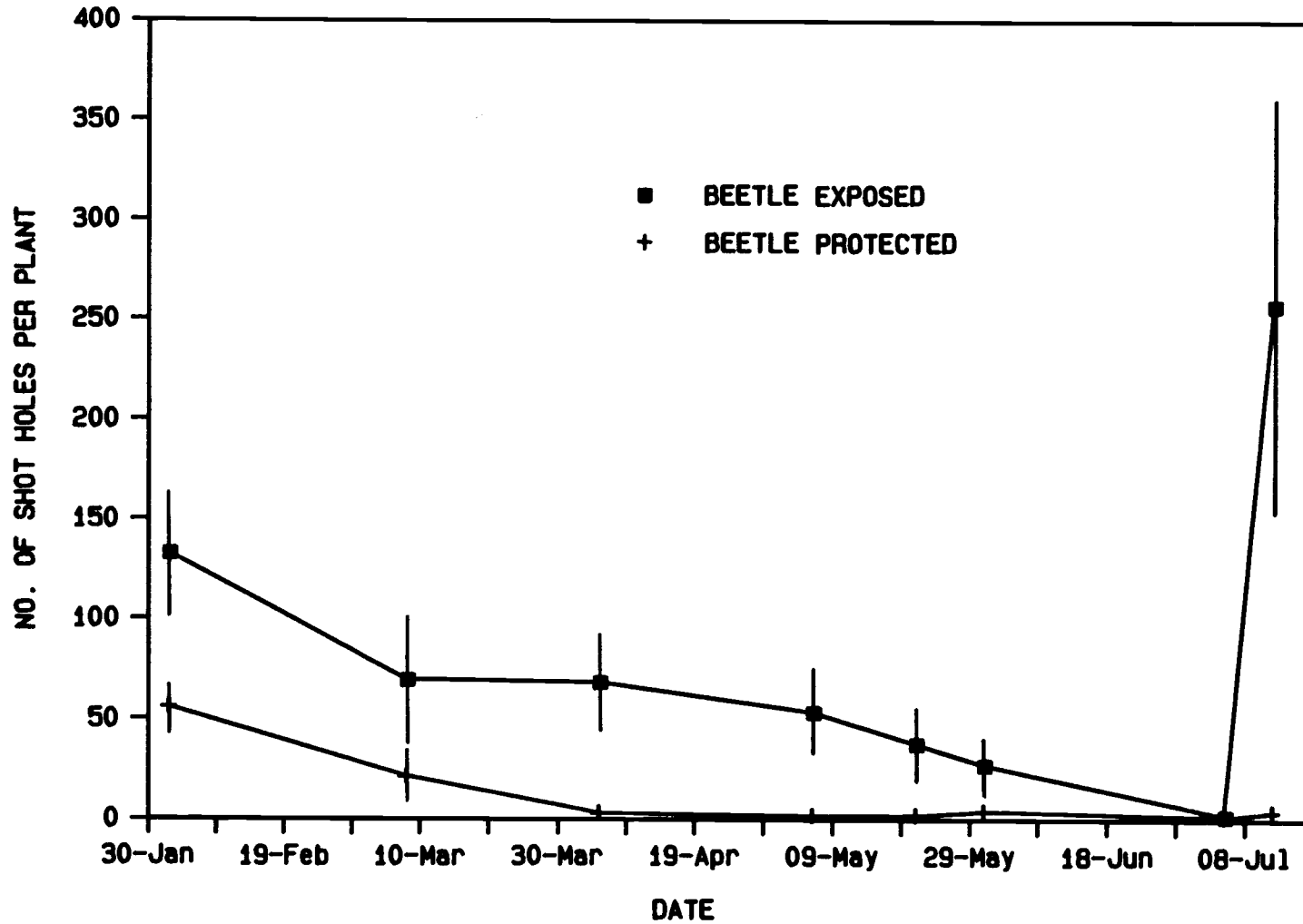


Figure 11. Mean (+SE) adult Longitarsus jacobaeae damage to a cohort of ragwort plants in the experimental plots.

The higher percentage of ovipositing females found in the winter does not necessarily mean a greater number of eggs are being laid at this time since the total population of adults is much greater in the fall than the winter (Fig. 11).

Table 9. Percentage of adult female Longitarsus jacobaeae with eggs present in the ovarioles.

<u>Date</u>	<u>No. females dissected</u>	<u>% with eggs</u>
6/22/87	5	0
7/27/87	15	0
8/02/87	6	0
8/31/87	12	0
9/07/87	8	0
9/29/87	9	22.2
10/11/87	22	45.5
11/06/87	9	44.4
12/12/87	12	75.0
1/15/88	12	41.7
2/08/88	10	80.0
3/10/88	6	83.3

CONCLUSIONS & DISCUSSION

The Ragwort System

The experimental plots recreated conditions of high weed density which were selectively subjected to two different kinds of herbivory. The results of this experiment support the hypothesis that two insects together, feeding on different pest plant parts and at different times of the year, can, at least in the short term, have a greater impact on host plant populations than either insect acting alone. The two insects spread out damage to the weed: the beetle was more effective than the moth in reducing plant density, biomass, and capitula number, and moths were more effective in reducing the number of seeds per head and seed mass and viability. Further, the two insects had an interactive effect which reduced the number and mass of seeds and reduced seed viability to zero. The monthly census of plants showed beetles reduced the regeneration of leaves and flowers that occurs after simulated moth defoliation.

Of the two insects, the beetle caused the greater reduction in plant populations. This is marked by its ability to increase plant mortality, reducing vegetative plant survivorship and biomass by 80-99%. The high mort-

ality of young plants caused by beetle activity undermines the pyramidal stage structure of the plant population, leaving fewer individuals to be recruited into the reproductive stage.

Beetles did not affect survivorship or biomass of second year plants. These plants were started in the greenhouse and transplanted to the field in late winter but differential beetle treatments were not initiated until a year later. The beetle appeared to have little impact on the survivorship of these plants since all plants in the marked cohort survived to flower. It appears that small vegetative stages of the plant are most sensitive to beetle attack and large plants with no history of herbivory may be invulnerable.

Simulated moth defoliation of flowering plants had an impact only on plant reproduction. Survivorship of these plants was not affected by defoliation, perhaps due to ragwort's ability to regenerate photosynthetic parts. The impact of the moth on ragwort may not be fully represented in these experiments because of the uses of artificial defoliation.

The side effects of cages were minimal as demonstrated by the open control plots. The greatest discernible effect of cages was to increase flowering plant biomass. This may be a result of etiolated growth caused by the lower light levels in the cages.

One reason why the beetle may be so much more effective than the moth in depressing ragwort populations is that feeding by one beetle stage or another is fairly continuous, where as feeding by the cinnabar moth is limited to early summer. Egg laying by adult beetles occurs in the early fall and the first larvae are seen soon afterwards. High mortality of larvae may occur during the winter but continued ovipositional activity of overwintering adults helps populations recover by early spring. The increases in larval populations occurring after February in both 1987 and 1988 must be due to either continued oviposition by adults during this period, late hatch of eggs laid earlier in the season, or a combination of both since the larvae themselves cannot migrate (even movement between neighboring plants is uncertain). Dissection of adult females revealed the presence of eggs from late September to at least March, and Frick and Johnson (1972) found eggs could survive at least six months at near-freezing temperatures.

Larval development continued until the end of July at which time the new adults were emerging. The emergence of new adults in the early summer was followed by increasing adult feeding damage, beginning in early July. Although not represented by experimental data, field observations (unpublished) indicate that the greatest amount of feeding by adults occurred during the summer and fall--when larval activity was lowest.

This study does not demonstrate the mechanism by which beetles affect the plants; however, the beetles were more abundant in the leaves and petioles than previously recognized and were actually more abundant (number per plant) there than in the roots. Eggs were laid at the base of petioles and larvae tunneling into these petioles may have caused leaves to senesce prematurely, as noted by Hawkes and Johnson (1978). Beetles could affect the amount of carbohydrates plants are able to store if they cause continuous early senescence of leaves. This may mean the beetle has more of an indirect effect on the root carbohydrate stores than a direct effect.

The impact of adult beetles is uncertain, but their defoliation activity may be more important than previously thought. Seedlings emerging in early summer may not be able to tolerate the high levels of adult feeding that occur in the summer and fall.

Considerations for Single vs. Multiple Agents

An important consideration in biological control is the persistence of pest-enemy interactions over the longer term. Ideally, control agents would reduce pest populations to low levels and maintain them there. However, if the biocontrol agents' populations drop below what is required to control the host plant, a resurgence of the pest

may occur, particularly if there is an intact seed bank. The persistence of the ragwort-moth-beetle system is uncertain. Why was there a decline in moth populations during the year of the experiment? Was it due to low ragwort populations the year before, competitive interference with the beetle, or other biotic and abiotic factors? Whether or not beetle numbers or densities have declined since the decline of ragwort on the site is not known due to the lack of an accurate estimate of previous populations. However, sufficient numbers of beetles were present on the site (or were able to migrate to the site) to cause a substantial decline in ragwort.

The main question of interest in biological weed control is what strategy gives the best results. The results of this experiment supports the strategy of selecting multiple species which feed on the weed in different ways, be it by attacking different plant parts or by attacking in different seasons or geographical regions. This method spreads out the attack on the plant and not only more rapidly reduces pest populations but may facilitate coexistence of natural enemies as well since it reduces niche overlap and thus competition. Such a strategy may also improve biocontrol if some of the control agents are more effective during times of high weed densities, such as when agents are first introduced, and other agents are more effective during times of low weed densities.

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