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Title: Effect of Diet on Larval Development, Adult Emergence and Fecundity of the Cinnabar Moth, Tyria jacobaeae (L.) (Lepidoptera: Arctiidae)

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The cinnabar moth, Tyria jacobaeae (L.), is a biological control agent introduced in Oregon against the weed tansy ragwort, Senecio jacobaea L. Goals of this research were: to determine if there exists significant variation in progeny of different cinnabar moth egg masses; to determine if (and how) rearing on different ragwort diets affects larval growth and subsequent adult emergence and fecundity; to investigate adult mating behavior; and to refine rearing techniques.

Tansy ragwort diets used in this research include: leaves from first-year rosettes, leaves from second-year flowering plants, leaves from shade-grown plants, floral parts, and a mixture of the four preceeding diets. All larvae were reared in a growth chamber with photoperiod 12/12 and temperature 23.9° C. Mating pairs and ovipositing females were housed at photoperiod 14/10 and thermoperiod 23.9°/10° C.

Female and male pupae reared on the floral diet are significantly larger than those reared on the shade-leaf diet. Adults reared on the floral diet emerge significantly later than those reared on the other four diets. Female moths reared on the floral, mixture and second-year leaf diets lay significantly more eggs than those reared on the shade-leaf diet. Ranking diets by decreasing fecundity yields: floral, second-year leaves, mixture, first-year leaves, and shade-grown leaves.

Eggs laid by floral-diet females generally have a higher percent hatch with smaller variance than those laid by females reared on the other four diets.

Size of original egg mass was not correlated with any aspect of developmental size or emergence timing. Sex ratio of an egg mass was highly correlated with female pupal weight and length, correlated with male pupal length but not correlated with male pupal weight.

Female moths lay large egg masses ($\bar{x}$ = 66.0 eggs) on the first day of oviposition; batch size decreases steadily through an oviposition period lasting 5-11 days ($\bar{x}$ = 7.2). Fifty percent of the eggs are laid by the third day and 90% by the sixth day. Percent egg hatch increases with increasing batch size up to 70 eggs. Delay in beginning oviposition (presumably a delay in mating) results in fewer eggs being laid over a shortened oviposition period and increased hatch failure.

The peak of the male mating flight and attraction to the female is around sunrise. Relative attractiveness of virgin females is highly variable (0 to at least 40 males per female trap per day). A small percentage of females (perhaps 10%) probably fails to attract a male for mating. Size of female was not correlated with attractiveness, but attractiveness may decrease with increasing age.

Rearing techniques (for laboratory and insectary) are reported that yield 90-95% survival from egg hatch to adult emergence. Use of paper straws as pupation sites increases pupation success and ease in handling pupae.

Effect of Diet on Larval Development, Adult Emergence and Fecundity of the Cinnabar Moth, *Tyria jacobaeae* (L.) (Lepidoptera: Arctiidae)

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INTRODUCTION

Tansy ragwort, Senecio jacobaea L., is a weed in western United States, accidentally introduced from Europe. It is poisonous to cattle, horses and other livestock (somewhat less toxic to sheep) and competes with more desirable cover and grazing plants, especially in undeveloped or marginal pastures. It is also a common roadside plant, and is an early invader of the logged areas so prevalent in the northwest regions of the United States.

The cinnabar moth, Tyria jacobaeae (L.)[1/], is an arctiid moth whose larval stages feed primarily on tansy ragwort and the related plant, groundsel, Senecio vulgaris L. Originally European, the cinnabar moth has been introduced as an attempted biological control agent for ragwort in New Zealand (Miller 1929, Cameron 1935), Australia (Bornemissza 1966), Canada (Harris 1964), and the western United States (Frick and Holloway 1964, Ritcher 1966). Early Oregon releases were not closely monitered (Isaacson 1972).

Isaacson (1972) began population studies of the cinnabar moth at the Silbernagel site in Linn Co., Oregon in 1970. This became the basis of long-term ecological studies at that site conducted by W. P. Nagel, in addition to yearly density surveys of tansy ragwort and the cinnabar moth at sites in Coos Co. (Nagel and Isaacson 1974). Stimac's (1977) model of the cinnabar moth-tansy ragwort system was an outgrowth of this data base. The Oregon State Department of Agriculture began a program

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1/ The cinnabar moth is also found under the generic names Callimorpha, Euchelia and Hypocrita.

initiated by Isaacson, of collection and redistribution of cinnabar larvae throughout western Oregon in 1973.

My involvement as laboratory and field help for Isaacson's research and in the redistribution program resulted in good understanding of the general biology of the cinnabar moth and practical experience in the field and laboratory rearing of all life stages. Curiosity about adult oviposition and larval food preferences and concern over poor laboratory rearing successes reported in the literature led to the following research objectives.

Research Objectives

The objectives of this research program were:

1. To refine laboratory rearing techniques for all life stages to enhance survival.
2. To elucidate adult mating behavior in the field that relates to rearing methods.
3. To determine if significant variability exists between progeny of different egg masses relative to larval and pupal weight and resultant adult fecundity; larval and pupal survival; and/or success and timing of adult emergence.
4. To determine if larval diet (ie. parts of ragwort plants such as flowers versus leaves, and shade-grown versus open-grown plant leaves) affects aspects of the life cycle listed above.

These objectives are explained in greater detail after the following summary of cinnabar moth life history and literature review.

Cinnabar Moth Life History

Tansy ragwort is basically a biennial plant, flowering and setting

seed in the second year of growth after a first year spent as a rosette. Mechanical defoliation (mowing, animal disturbance or feeding effects, etc.) will often disrupt the typical growth cycle and cause ragwort to become a semi-perennial. Nagel and Isaacson (1974) show that ragwort biomass appears to be decreasing and plant dispersion (clumping) increasing in western Oregon due to the cinnabar moth. Details of plant anatomy, growth, habitat and tolerances are given in Cameron (1935), Harper and Wood (1957), Harper (1958), and Meijden (1974).

Feeding tests conducted prior to cinnabar moth importation into New Zealand, Canada and the United States indicated that cinnabar larvae would not feed on any economic crops (Miller 1929, Cameron 1935, Parker 1960, Bucher and Harris 1961). Bucher and Harris (1961) suggest that cinnabar larvae feed on several genera of plants in the tribe Senecioneae due to the presence of feeding stimuli (presumably chemical) but that only plants of the genus Senecio and closely related genera can sustain development. Hawkes (1973) reports larvae congregating on dead, dry ragwort plants in the field, presumably attracted to some feeding stimulant.

Adult cinnabar moths usually emerge from early May through July, but they can be found as early as April and late as September depending upon year, elevation and locality. Adults mate soon after emergence, and females commence ovipostion within a day or two of mating. Oviposition lasts one to two weeks for each female. A female lays approximately 100-400 yellow eggs in batches averaging 30-45 eggs. However, females are capable of laying egg masses ranging from 1 to 150 or more eggs. Eggs are laid on the underside of ragwort leaves. Eggs hatch in 5-21 days in the field, dependent on temperature and perhaps, age of female. (Eggs laid last by each female tend to hatch more quickly at the same temperature than earlier laid eggs in the laboratory, personal observation.)

The first-instar larvae feed gregariously on the outer leaf layers,

usually on the underside of the leaf from which they hatch. There are five instars, and gregariousness generally decreases with increasing stadium (Bornemissza 1966). Larvae move about the plants and between plants from the 2nd stadium onward. Larvae tend to feed on the floral parts at the top of the plants (Miller 1929, Bornemissza 1966). Green (1974) showed that larvae only recognized plants when within about 3.5 cm of them. Larval development occupies four to seven weeks in the field.

Fifth-instar larvae seek pupation sites in ground litter, under bark, or in cracks and insect galleries in fallen logs, stumps and wood debris. The prepupa usually spins a bit of silk to seal off a tunnel or draw material about itself prior to pupation. The prepupal stage lasts one to four days. Cinnabar populations overwinter as pupae.

Mortality factors in the cinnabar moth include starvation, predation, parasitism and disease. Desiccation and/or heat stress can also cause eventual death in all life stages. Stimac (1977) summarizes the major arthropod predators and parasites of the cinnabar life stages as reported in the literature.[2/] Importance of a given mortality factor varies between life stages and different localities (Bornemissza 1966, Dempster 1971, Hawkes 1973, etc.). Starvation was an important larval mortality factor in the high density population that Isaacson (1972) studied in Oregon. Ants may restrict cinnabar larval populations in localized areas (Meijden 1973, Myers and Campbell 1976a).

Diseases reported in cinnabar life stages include microsporidial infections (Nosema sp.), nuclear polyhedroses and various fungal pathogens (Cameron 1935, Bucher and Harris 1961, Steinhaus and Marsh 1962, Dempster 1971). Insectary breeding has been difficult at times

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2/ To this list can be added that I have seen phalangids chase, capture and consume healthy adult females.

in certain places due to "bacterial wilt" (Currie and Fyfe 1938), and Miller (1929) links disease problems with high temperatures and humidities, and overcrowding in insectaries.

Adult moths are aposematically colored. Frazer and Rothschild (1962) found the cinnabar moth to be the most unacceptable moth to a number of vertebrate predators they tested. But they note that the relationships between warning coloration, histamine content and acceptability to predators are not clear. Indeed, vertebrate predators of cinnabar larvae, pupae and adults include small mammals, birds and perhaps, lizards (Miller 1929,Isaacson 1972, Hawkes 1973, Dempster 1975, present research).

Research Considerations

Refinement of Rearing Techniques

This thesis includes an extensive section on lab rearing methods for cinnabar moths that I have borrowed, refined and developed over the years. I have obtained 90-95% survival from hatching through to adult emergence in the laboratory and insectary, regardless of egg source, diet, etc. These methods are listed by life stage and include a general discussion and notes on larger scale rearing.

It is hoped that new researchers in the cinnabar moth field and those desiring to rear cinnabar moths will find these useful. It is also hoped that some present workers will note them and perhaps re-evaulate some of their rearing and laboratory handling procedures. High larval mortalities, and pupation and adult emergence failures of 30-70%, appear frequently in the literature. These are invariably blamed on "disease" conditions developing during research. Disease is unavoidable sometimes, but I feel that much mortality could be avoided with better handling procedures and care. General reliability of experimental

results is questionable in the presence of high mortality and emergence failure that are as likely due to poor rearing conditions as to experimental manipulations.

Cinnabar moth research is presently being conducted in Canada, The Netherlands, England and Oregon. A vast body of knowledge is being accumulated about the cinnabar moth-tansy ragwort system and will doubtless continue to grow and yield valuable ecological work. Some standardization amongst workers relative to laboratory handling methods (standardized experimental temperature and photoperiod regimes, larval rearing methods and densities, overwintering methods) would greatly enhance the comparative work being carried on in different localities where field methods are already somewhat standardized.

Mating Behavior

Little work has been performed on cinnabar moth mating behavior. It is reported that males are attracted to females in the field by a sex pheromone (field observation, Hawkes 1973). It is generally noted that the males have an early morning mating flight (Green 1974). Hawkes (1973) places the mating flight as beginning about 7:30 am and lasting 2 hours. I have occasionally seen adults mating in the field about 2-4 hours before sunset and have found mating pairs at sunrise.

Experiments were designed to look at the following relating to cinnabar moth mating behavior, as some of this information was needed to validate experimental procedures.

1. Description of field mating behavior.
2. Investigate aspects of female attractiveness in the field including time of day males are attracted and variability in relative attractiveness between females.
3. How many females can a male mate with, and do females only mate once?

Egg Mass Variability = Control Experiment

Philogène's (1975) work with the cinnabar moth reared on different temperature-photoperiod regimes is the only study to date that researched parental influences on progeny for the cinnabar moth. He reports (pg. 1416):

> The origin of cinnabar moth larvae seems to have no consequences on their fate. For instance, individuals emerging from eggs laid by parents which had been reared at 10/14 did not develop differently at any of the temperature-photoperiod combinations used than their counterparts from 18/6, 0/24, or field-reared parents.

My research was desinged to look for variability amongst progeny of different cinnabar moth egg masses (hereafter referred to as egg mass variaibility as a shorthand notation). This is called the control experiment because it will establish a basis for future work. Egg mass variability is an experimental variable that is generally overlooked in designing experiments and therefore not properly controlled (or at least randomized) as an independent variable. Potential experimental bias in cinnabar moth laboratory work may be commonplace due to small sample sizes that include only a few egg masses.

Effects of Diet = Diet Experiment

Prior research on cinnabar larval rearing has mainly concentrated on larval development, pupation success and/or pupal weights as related to: rearing temperature (Isaacson 1972), temperature and photoperiod (Philogène 1975), quantity of food (Meijden 1971), and effects of crowding (Bornemissza 1966, Dempster 1971, Schmidl 1972). My research deals with effect of differing larval diet or food quality on quantifiable aspects of cinnabar life stages reared under otherwise equal conditions. No rearing on an artificial diet was attempted.[3/]

[3/] For work on artificial diet as related to insects feeding on tansy ragwort, see Singh and Mabbett (1976). They used an (undefined) artificial diet for the magpie moth (a New Zealand arctiid that also feeds on plants in the tribe Senecioneae) and compared growth on the artificial diet to growth on a ragwort leaf diet.

Female cinnabar moths tend to oviposit on second-year flowering plants as opposed to first-year rosettes. Green (1974) showed that this was possibly a reaction to plant size as well as choosing older plants over younger plants. He felt that the average-sized egg mass was basically adapted to the amount of biomass available as food in the average-sized ragwort plant. The female ovipositional preference for second-year plants was therefore adaptive in that it tended to insure enough food for a female's progeny. But might it also be adaptive to oviposit on second-year plants from the standpoint of some qualitative effect of this food source on the progeny? Phillips (1976) found that adult chrysomelid beetles feeding on young plant leaves were more fecund that beetles feeding on senescent leaves.

Cinnabar larvae in the later stadia prefer to eat floral plant parts as opposed to leaves. Does feeding on floral parts impart any selective advantage such as increased size (and hence, fecundity) or improved survival?

Tansy ragwort plants growing in the shade are usually the last plants to be utilized by larvae, even in areas with high density cinnabar populations (personal observation). Female moths prefer to oviposit in sunny locations (Frick and Holloway 1964, Harris et al. 1971). They avoid ovipositing on ragwort plants growing in the shade even when other nearby open-growing plants are overloaded with eggs or are partially defoliated. Removing the environmental variables associated with shade and sunlight, does a diet of sun-grown leaves affect growth differently than a diet of shade-grown leaves? Hence, would it appear adaptive to avoid feeding on or ovipositing on ragwort in the shade due to larval survival or pupal weight attained and influence on one's progeny?

Finally, in laboratory or insectary rearings, can one assume that different plant parts provided as food will not differentially affect cinnabar growth and fecundity?

The literature review for this research was completed in February 1975. Control and diet experiment larvae were reared in the summer of 1975 and oviposition work performed in the summer of 1976 with adults resulting from these rearings. In reviewing the 1975-1977 literature prior to writing this thesis, Meijden's (1976) diet rearing work came to my attention. We have performed similar research independently. Meijden performed an experiment rearing cinnabar larvae on two diets: ragwort leaves and leaves-plus-flowers. His results are similar and our interpretation of the effects of a diet containing floral parts on various aspects and "strategies" of cinnabar life history are similar. His work is referenced frequently in this thesis in comparison to my results.

Thesis Organization

The rearing techniques, the mating studies, and the control and diet experiments are presented as three separate sections in this thesis. The rearing techniques and mating studies are presented first because they form a logical background for the control and diet experiments. This organization is not strictly chronological; parts of all three sections were sometimes being investigated at the same time. Work in one area would often influence or suggest work to be performed in the other areas. Thus, some mention of the other subjects' results is unavoidable in each section of this thesis.

REARING TECHNIQUES

The rearing methods presented in this section were borrowed, refined, and developed over several years rearing experience with all cinnabar moth life stages. These methods were originally designed for use with a growth chamber (without humidity control) and only limited materials. Some comments can be ignored if one has sophisticated rearing equipment (like a growth chamber with humidity control) and abundant supplies and laboratory help. But in their presence or absence, these rearing methods should give 90-95% or better survival from egg through adult emergence.

Avoidance of desiccation is perhaps the most recurrent theme that will be noted in the following sections. Exact humidities for optimal cinnabar moth rearing and overwintering are unknown, but artificial rearing conditions often ignore or inadequately maintain humidity. For example, growth chambers without good humidity controls often create dry conditions (from blowers connected with thermostats) that hinder larval development and yield low adult emergence from pupae. On the other hand, humid conditions stress larvae and activate latent diseases. The methods presented here are designed to maintain somewhat high humidity (perhaps 60-80%) within individual containers.

Eggs

Eggs should be incubated in covered containers (petri dishes, hard-plastic containers with snug but easily removable lids, etc.). Containers should be checked daily and two to three water drops added inside the container to prevent desiccation.

This incubation method works well for eggs on ragwort leaves (field

collected) or for eggs laid directly on the inside of a container. The eggs are moistened through the atmosphere; direct contact with water is avoided. Absorbant paper liners should not be used as they tend to encourage pathogen growth by allowing the leaf and eggs to be in direct contact with moisture. These liners also hasten leaf deterioration.

Incubation temperature determines the time necessary for egg hatch (Table 1). Figure 1 depicts days required to hatch regressed on temperature as a guide to laboratory incubation periods. A regression of the inverse of days-to-hatch on temperature indicated that the minimum threshold for egg development is about 6.5° C. Approximately 90-100 day-degrees must be accumulated above 6° C. for egg hatch, where day-degrees = sum of daily [((maximum temperature + minimum temperature)/ 2) - 6]. This compares to Stimac (1977) who listed 9.00 as the "egg hatching threshold in cumulative degree-days" but did not explain how this threshold was determined nor how the degree-days are accumulated.

Table 1. Number of days required for egg hatch at various temperatures for the cinnabar moth, Tyria jacobaeae (L.).

Temperature ° C.	Replications, n	Days to hatch $\bar{x} \pm$ S.E.	Days to hatch range	Cumulative day-degrees = Sum of mean daily temperatures above: 0° C. per day	Cumulative day-degrees = Sum of mean daily temperatures above: 6° C. per day
26.7	6	4.3 ± 0.2	4-5	114.8	89.0
23.9	5	5.6 ± 0.2	5-6	133.8	100.2
18.3	5	8.0 ± 0.3	7-9	146.4	98.4
10.0	6	23.5 ± 0.2	22-24	235.0	94.0
1.7	5	none hatched within 92 days[a/]			

a/ These egg masses were moved one at a time to 26.7° C. to determine effect of cold storage on incubation time. One egg mass was moved on days number: 16, 35, 48, 58 and 92. All egg masses hatched after 3 days at the higher temperature. Hatch was normal for all but the eggs moved at 58 and 92 days; these had a low hatch due to desiccation and possible effects of cold storage.

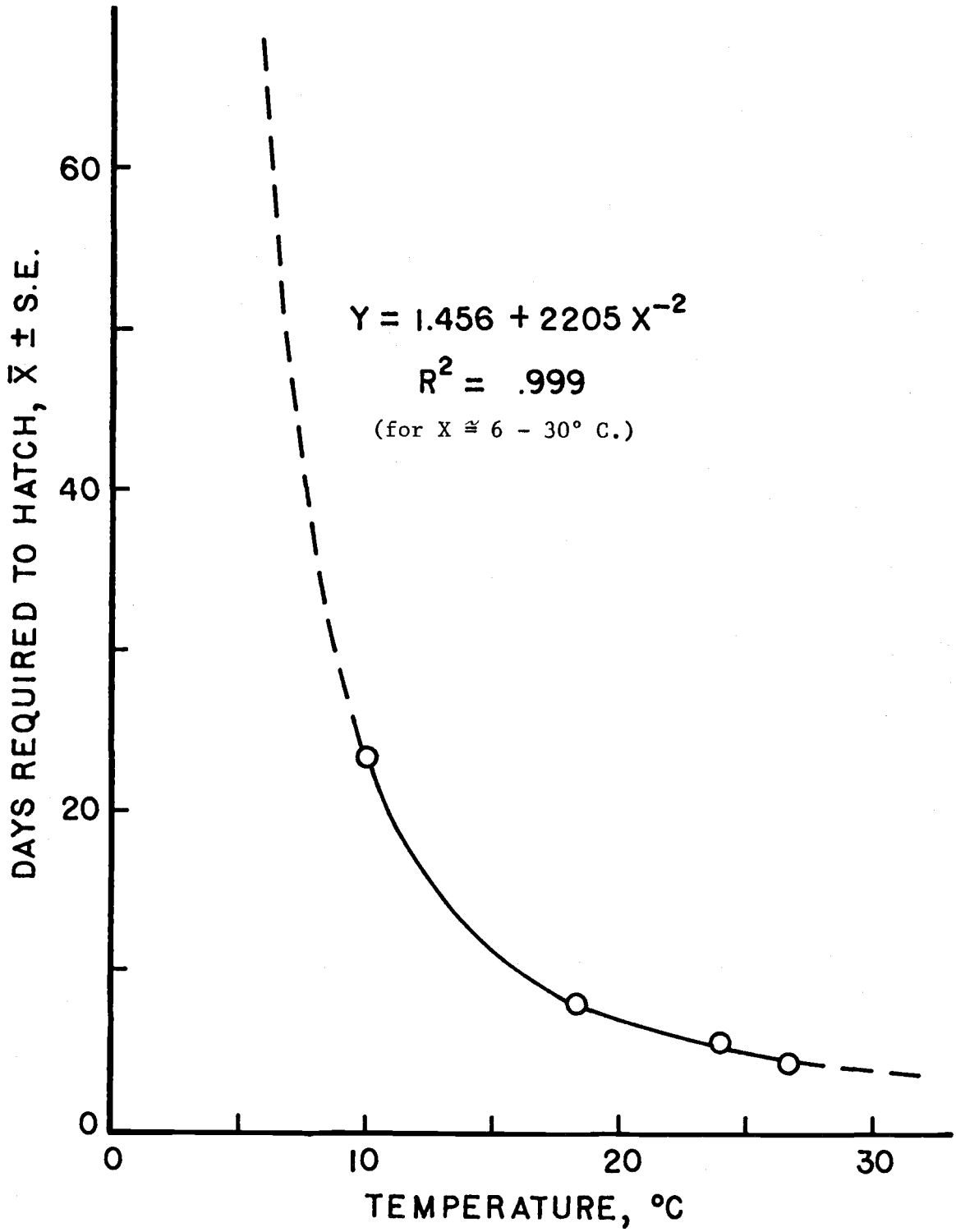

Figure 1. Days required to hatch regressed on laboratory temperature for eggs laid by field-collected female cinnabar moths, Tyria jacobaeae (L.).

Higher incubation temperatures such as 22-27° C. require daily watering, while 10-15° C. only require water every fourth day or so. Daily checking is still a good rearing practice, however.

Many field-collected egg masses can be incubated together in the same large container. Eggs laid on wax paper (or other non-absorbant material) can also be incubated in a common container. Ventilation of the incubation container is not necessary.

Larvae

Feeding is minimal in the early instars; the first three instars together only consume about 1-5% of the total wet weight of ragwort consumed during larval development (Isaacson 1972, Green 1974). The 4th instar consumes about 14-17% and the 5th instar about 80-85% of the total (Isaacson 1972, Green 1974). A single ragwort leaf will suffice daily as food for about 50 1st-instar larvae in a petri dish and about 10 3rd-instar larvae. A 5th instar can consume one leaf per day. Food should always be changed every day.

Tansy ragwort plants can be gathered in the field weekly and rinsed in water as the leaves or desired parts are stripped off. The plant parts are then stored in plastic lettuce crispers or large plastic bags at 2-4° C. until needed. Ragwort will stay crisp for several weeks when stored this way. The diet experiment results indicate that a floral diet will yield larger pupae and more fecund adults, and that shade-grown ragwort should be avoided as a food source.

Large numbers of 1st- or 2nd-instar larvae can be reared in the same container. All larvae within a rearing container should have hatched the same day to avoid developmental stragglers that complicate daily sanitation. A handful of tansy ragwort leaves or flowers is added daily

and the old food removed. The incubation/rearing container should be wide and low as opposed to tall and narrow. This offers more ceiling space which is important because 2nd- through 4th-instar larvae usually crawl up to the horizontal surface to molt. It helps if the lid is easily removable (ie. hard plastic as opposed to opaque rubberized containers) and is transparent. This avoids wasting time securing rubber bands or other make-shift lids.

Small larvae can be maneuvered or moved by catching their silk strand (produced at anterior end) with a thin paint brush or gently flicking them into a new dish. Starting with the 3rd stadium, containers should be changed daily because fecal matter accumulates, holds moisture, contaminates food and aids pathogen growth.

Larvae spend up to one day molting to the next instar. The 2nd through 4th instars move to the lid and secrete silk on the lid and loosely over themselves to serve as substrate and aid in molting. (First-instar larvae usually molt on the ragwort.) Larvae hatching the same day will molt at about the same time within each stadium and container. They usually crowd together in one or two groups as opposed to being regularly or randomly spaced over the container surface. When many larvae are molting on the lid, it can simply be moved to another clean container with fresh food in it.

One method of daily cleaning (4th- and 5th-instar larvae) involves dumping the contents of a rearing container into a sieve. The sieve is shaken back and forth a couple of times; the larvae remaining and the fecal matter and leaf bits dropping out. Any large pieces of ragwort are then removed. The sieve is shaken once to dislodge the larvae and then dumped into a clean container with fresh food. A sieve can be easily made by burning out the bottom of a hard-plastic container about 30 x 25 x 10 cm deep, and burning (or glueing) in a piece of ¼-inch hardware cloth (wire mesh). This mesh size works well for large larvae, but 3rd instars or smaller require a smaller mesh (such as window screening).

The earlier instars do not need containers changed daily as long as moisture does not accumulate.

A clear plastic container of 30 x 25 x 10 cm deep will accomodate about 200-300 5th-instar larvae. It may be necessary to add food twice daily during this stadium when feeding is continuous. A 10 x 10 cm (or larger) hole should be cut in the lid and a fine wire or soft plastic mesh glued in for ventilation. This works well for 3rd through 5th instars. First and 2nd instars usually get through mesh and are best reared in the same size container with no ventilation screen as moisture accumulation is generally not a problem in these early instars.

Careful washing and sterilization of equipment and containers every day may help, but still will not always avoid disease conditions. Disposable rearing containers and petri dishes are easiest to use but are often too expensive for the average research or rearing program. The time and effort spent in sterilization (except between successive rearings in a season) is not as cost effective in rearing healthy larvae as is regular changing of rearing containers and food.

Pupation

Larvae begin rapid motions after they finish feeding in the 5th instar, and pink fecal material appears as the larvae void their guts prior to pupation. Sections of 0.5 cm diameter paper soda straw, 2.5-4.5 cm long, are added to each larval container as pupation nears (at least one straw per larva). Straws can be added singly or in small bundles of 7-20. Larvae readily enter the straws and spin silk to close off the straw ends before pupating.

The straws offer protection and prevent cannibalism that can be a

problem under crowded or scarce food conditions. Paper toweling and tissue have been used as pupation media, but these necessitate daily searches through paper and foliage to pick out pupae. Pupae may remain soft for a couple days after pupation and are readily damaged or broken. The straws can be handled quickly and efficiently with no resultant pupal damage nor time spent being "careful" with the pupae. Thus, straws save time, enhance pupation success, and make daily cleaning easier and faster.

Larvae that fail to pupate with the rest of their cohort or after about 10 days should be discarded. Not only is it an uneconomical use of time and space for a rearing program, but the stragglers are often sick or defective and have poor pupation success.

The straws should be transferred to clean, empty containers when all the larvae within a rearing container have silked up a pupation site. They should be checked occasionally for moisture accumulation and pathogen development. Pupae can be left in the straws for several weeks or until they are needed and time is available to work with them.

Removal from straws involves gently poking the pupa out with a paint brush handle or wooden dowel. This should not be attempted until about seven days after the prepupa silks up, to allow time for pupation and tanning. The extra time required for removal from the straws is negligible compared to the increased pupation success and ease in handling pupae, prepupae and larvae in the same rearing container. Experimentation with leaving pupae in the straws overwinter yielded poor success due to increased moisture accumulation and pathogen growth.

Straws longer than about 4.5 cm should not be used as more than one prepupa may use the straw, complicating pupal removal. One straw-use experiment indicated that mean weight of pupae in double occupancy straws was significantly less than for single occupancy straws, 117.0 ± 9.6 mg ($n = 18$) versus 133.4 ± 2.4 mg ($n = 144$). The smallest of the

pair within each straw was often stunted, cannibalized or a pupation failure. The most obvious reason for this difference in mean pupal size and pupation failure would be some form of pupation interference between two prepupae in close proximity.

Analysis of control and diet experiment pupal data indicated that significantly more pupae were found inside, compared to outside of the straws. Pupae found inside did not differ significantly in size (weight or length) from those found outside of the straws for either sex.

Cinnabar moth literature includes work where high percentages of pupation failure and adult emergence failure (ie. 15-50%) are noted, in the supposed absence of experimental stress (Isaacson 1972, Hawkes 1973, Philogène 1975, Meijden 1976). Meijden (1976) suggests high pupation failure is due to interference amongst larvae and prepupae in the same container during pupation. Use of paper straws yields pupation success of 95% or better. Pupation interference is alleviated and the straws, larvae and debris can still be dumped daily into a sieve to remove feces with no adverse effect on larval feeding and pupating prepupae. Straws are efficient and fulfill the need for a readily available, reliable and easily used pupation medium for laboratory work.

Pupal Overwintering

Overwintering pupae are placed in bark mulch in shallow plastic containers with lids. Water is lightly misted over the mulch weekly or when the mulch appears to be drying. A compound such as petroleum jelly or stop-cock grease may be used to help form a moisture-retaining seal for the lid, but is still no substitute for weekly checking. Pupae prepared this way may be overwintered in an insectary, growth chamber or left out in a room. Watering is especially important in the later two situations which tend to be dry.

Exact relative humidity for optimal cinnabar pupal overwintering is not known but extrapolation from field pupation sites in Oregon would indicate high humidity needs or tolerances. Personal experience indicates that a humidity of 40-50% is probably too low, especially in the spring before pupae metamorphose into pharate adults. Pupae held overwinter in the laboratory with no special care or moisture controls taken will generally still yield adults, but emergence is low (5-50%) compared to the methods described here.

If the pupal container is uncovered in the spring prior to adult emergence, water should still be misted over the mulch weekly or more often. Otherwise, the pupae that are not advanced enough to metamorphose into pharate adults and the pharate adults ready to emerge may desiccate. These emergence failures are characterized by pharate adults unable to break open or fully emerge from the pupal skin. Desiccation is probably the "late factor causing death after the adults have formed" found by Isaacson (1972) in about 20% of his field-collected pupae held for emergence. This type of adult emergence failure is also linked with low pupal weight (due to crowding, insufficient larval food, etc.). Failures due to other causes (diseased prepupae, parasitism, overly humid conditions, etc.) usually involve pupae that are either hollow, filled with mold or fungus, or filled with a putrid liquid.

Dempster (1970) found that pupae could survive a 30% weight loss but died if they were in contact with water and/or sustained a 10% increase in body weight. My field observations indicate that adult emergence is linked with drying out of the area or microhabitat around a pupation site. Schmidl (1972) found adult emergence in Australia increased with sharp increases in temperature. These results contrast with Dempster (1971) who felt that emergence coincided with wet weather for several years in England. It would be of value in emergence prediction to find out in more detail the relationship between humidity and temperature relative to thresholds for overwintering survival, metamorphosing into a

pharate adult, and actual emergence from the pupal skin.

Some work has been performed with the cinnabar moth in attempting to break pupal diapause, especially in conjunction with early attempts at introducing the cinnabar moth to Australia and New Zealand where the seasons are reversed relative to donor sites in Europe. Bornemissza (1961) found better emergence if diapause is extended (to about 450 days) by storage temperature manipulation as opposed to shortened (to about 150 days) compared to the normal pupation period of about 270 days. But, either manipulation severely decreased fecundity. Schmidl (1972) refined techniques somewhat and got emergences as high as 75% with extended diapause. Philogene (1975) showed that the cinnabar pupal diapause does not respond to photoperiod manipulation.

Mating

The Oviposition study for the diet experiment (described in the EGG MASS VARIABILITY AND DIET EXPERIMENTS Section) necessitated having 50 or more females ovipositing or mating at the same time. Space, time and equipment limitations precluded using caged plants for mating and oviposition. The laboratory mating methods used, however, only yielded 60% of the females laying fertile eggs. (Eighty percent of the females laying sterile eggs had not mated).

A small study was set up to compare the growth chamber conditions with field cage conditions to see if the fertilization failure was indeed a mating failure due to experimental conditions. Sixteen virgin females (control experiment and field collected) were each individually caged with a control male in 2 x 2 x 5 dm tall cages over a plant. All females began laying fertile eggs by the third day. Ten virgin females (also controls and field collected) were each paired with a control male under the same conditions as in the Oviposition study of

the EGG MASS VARIABILITY AND DIET EXPERIMENTS. All females laid eggs by the end of 20 days, but only 60% laid fertile eggs. This tends to substantiate the belief that the 40% sterility in the diet oviposition study was due to suboptimal conditions, imposed by the small cups used, that offered no semi-vertical female "perch". (See Description of Field Mating Behavior under MATING BEHAVIOR STUDIES for perch explanation.) I often saw males in the mating cups hovering up to a female that was resting on the lid with her abdomen dangling. Each male literally bumped his head on the lid as he attempted (often unsuccessfully) to grasp the female with his claspers.

These results point to the obvious: mating is predictable and successful under field or caged-plant conditions but haphazard in small cups in the laboratory.

Large-scale methods for laboratory mating of cinnabar females were not worked out. My preliminary work indicates that putting several pairs of moths or several females with one or two males in large cages or containers leads to poorer mating and oviposition success than the 60% found in the diet study. Bornemissza (1966) placed 10-50 adult pairs in field cages (6 x 6 x 9 dm) and only got an average of 10-30 eggs per female (presumably many did not mate and oviposit). These conditions were too crowded to begin with, and adults probably were physically interfering with each other.

Another reason for low fertilization and/or mating success might be some form of pheromonal interference or pheromonal "overkill" from too many virgin females in a small area. I experimented by sending females and males through the mail (10 pairs per gallon ice cream container) to a locality about 80 km from Corvallis, Oregon. In six trials, only one female per container (two females in one case) was mated after the container was opened two days later and allowed to sit for a day. Perhaps females emit a repellant pheromone (or sound?) after they have mated. This might confuse the males and hinder the ability of other

virgins in the same area to be mated.

Individual matings in the laboratory should be successful in pint-sized cups with a thin stick or dowel leaned diagonally inside to serve as a perch for the female. Once a female begins oviposition, she can be moved to a smaller container if space is limited. This method would save enormous space and time over maintaining numerous outdoor cages or potting plants individually for indoor cage studies.

Results from the MATING BEHAVIOR STUDIES section indicate that females only mate once, so males can be removed as soon as females begin to lay eggs. If no mating occurs after three-to-four days of pairing, the male should be replaced with a new male. If females (used in a rearing program) do not lay eggs within a week of initial pairing with males, they are best discarded to maximize space and time utilization. Ragwort is not necessary to initiate mating. Containers with adult moths should have two-to-three water drops added daily for adults to drink.

Oviposition

Mated females readily lay eggs in small confines (ie. petri dishes) in growth chambers. Ragwort is not necessary to initiate oviposition. Drinking water should be added daily to individual oviposition cups as desiccated females oviposit erratically and lay eggs that usually fail to hatch. When allowing females to oviposit on caged plants, water should be lightly misted over the plants daily.

Females placed in small cups with a ragwort leaf are more likely to oviposit on the container than on the leaf. Philogène (1975) noted that eggs laid on cages or plant pot edges generally failed to hatch. I found that eggs laid on cups by fertile females will hatch normally if water drops are added to prevent desiccation. It is actually preferable in a rearing program to encourage females to oviposit directly on cups by omitting leaves which otherwise tend to wilt or decay, decreasing egg hatch and increasing maintenance problems.

Lining oviposition cups with removable wax-paper bottoms is somewhat complicated by the tendency of the females to oviposit on any part of the container. An extract of tansy ragwort painted on the paper might encourage the females to oviposit on the "right" place. These papers are then transferred to a common incubation container.

Ideally, several females could be housed together in a large container and allowed to oviposit for a day and then moved to another container. The eggs could then be incubated together as they will hatch about the same day. There is an indication, however, of some form of interference (mechanical, pheromonal, sonic) in oviposition by females grouped in containers (even though several females can often be seen in the field on the same plant). Females in the laboratory lay more eggs, more regularly when housed separately.

Females lay about 70-75% of their eggs by the fourth oviposition day and 80-90% by the fifth day (at photoperiod 14/10 and thermoperiod 23.9°/10° C.). Eggs laid on or after the sixth day are usually in small batches (15 eggs or less) and have a poorer hatch success than do earlier egg masses. For efficiency in large-scale rearings (where the object is to maximize cinnabar moths reared per cost and labor effort and still maintain some sort of quality) the ovipositing females should be discarded after the fifth day; time being better spent on newer females.

Laboratory Rearing for Colonization

Large-scale rearing for redistribution usually begins with the egg stage or adults. One possibility for cinnabar moth rearing involves field collecting females from high population areas and allowing them to lay eggs for subsequent larval rearing in the laboratory or insectary. This bypasses the heavy early-instar mortality found in the field (Isaacson 1972). Rearing larvae on a floral diet would also yield

larger, more fecund moths. It is estimated that only 8-17 5th-instar larvae at best will result from every 100 eggs laid in Oregon (Nagel and Isaacson 1974). Careful rearing may increase this to 60-90 5th-instar larvae from every 100 eggs brought into the laboratory or laid by females in the laboratory. Thus, 60-plus potential 5th-instar larvae for every 100 eggs from an area might be available for redistribution to form new colonies or to augment previous releases.

All eggs laid after a given time in the summer are doomed to die in areas with consistently large cinnabar populations. Either the leaves the eggs are laid on will be eaten before eggs hatch or the resulting larvae will starve before pupation. This "overload" point in the season occurs when the earlier population builds up sufficiently in numbers and age (ie. 4th- and 5th-instar larvae which account for 90% of the feeding) to cause a seemingly sudden increase in feeding and plant defoliation. Monitoring of high density areas would allow egg harvest before this "outbreak" in feeding. Egg collection effort would be worthwhile as egg masses in such areas could be expected to average better than two per plant. The eggs are then incubated and the larvae reared to the late 4th- or early 5th-instar stage. The larvae could then be released to establish new colonies two-to-three weeks after the eggs were collected. This method bypasses the early-instar field mortality and, at the same time, maximizes rearing effort by avoiding rearing the 5th-instar larvae (the stage that consumes 50-75% of the time, labor, space and plant biomass requirements of a rearing program.)

I feel that rearing methods are sufficiently worked out to enable fairly predictable and competitive sources of adult moths for releasing in the following spring (compared to field collection of larvae which is somewhat unpredictable year to year). No work has been performed with the cinnabar moth, comparing relative success and population build-up of colonies started with adults as opposed to larvae. I feel that 300-500 adults released in the spring would lead to better establishment success and faster population build-up than 1000 larvae released the

previous summer. It remains to be seen if the extra time and money expended in rearing the 5th-instar larvae through to adults the next spring are as cost-effective as rearing larvae to the 5th instar and releasing them the same summer the eggs were laid or collected. (It is suspected that the latter method is cheaper).

In summary, use of paper straws for pupation contributes greatly to high pupation success and eventual adult emergence. The important considerations in larval rearing are to avoid damp and/or crowded conditions, and provide more than adequate amounts of food, preferably floral parts. Eggs, pupae and adults also exhibit "disease" and eventual death due to stress, especially under dry conditions. Disease conditions can be induced in almost any cinnabar population at any time simply by stressing them. Exactly what disease or pathogen is involved, or its source, is secondary to good rearing practices; conditions and attention that will minimize the impact of disease.

MATING BEHAVIOR STUDIES

This short series of field and laboratory studies was performed to gain information and insight into cinnabar moth mating behavior that influences rearing techniques and success. Some of the studies (number of times that females and males mate and field mating behavior) were needed to validate experimental methods used for the ovipositon study in the diet experiment.

Description of Field Mating Behavior

Both males and females can be seen crawling up onto grass and foliage from near the ground around dawn. Males generally become active at lower temperatures and earlier in the day than do females. Males can be seen flapping their wings getting ready for flight, and they sometimes begin flight only to fall to the ground a few times before they are "warmed up". Once they successfully take flight, they tend to fly straight to a virgin female, presumably responding to her sex pheromone. However, the possibility that males also are responding to sonic stimuli from the females has not been explored yet and can not be ruled out.

Females are usually resting head-up, about 2-5 dm above ground, on plant stems and foliage. Males begin to fly up and down vertical outcroppings in search of the female when they are in close proximity to a female. The exact means by which a male recognizes and responds to a nearby female (ie. sonic, chemical, visual, etc.) are not known. The male initiates coupling while he hovers near the female, venter to venter, usually within a minute after he locates her. Mating takes up to about an hour and is probably somewhat temperature dependent.

The cinnabar moth is considered to be diurnal, but behaviorally

the males are crepuscular and the females diurnal. Moths generally do not fly about much during the day, and moths seen flying on clear days are invariably ovipositing females. Observation indicates that the males tend to hide in foliage, shade or near the ground during warm, clear days. Both sexes will take flight when disturbed, but females tend to play dead and fall to the ground in cooler weather or early morning.

Female Attractiveness

Sticky traps baited with female cinnabar moths were used in three field experiments to answer the following questions:

1. Do only virgin females attract males in the field?
2. What time(s) of the day do males respond to females by entering the trap to attempt mating?
3. Is there large variation in relative female attractiveness (as measured by the number of males trapped per female)?

Methods

Each female moth was housed in a small nylon-mesh cylinder suspended in a gallon-sized cardboard ice cream container coated with "stickum" on the inner surface to capture moths entering the trap. Each trap was perforated on the top, bottom and sides. This design was adopted from the traps used by AliNiazee and Stafford (1973) with the grape leaffolder. Pheromone or sound could be released into the area, but the female is hidden from view. (After the first few days, the top hole had to be taped shut to keep out the rain.)

These studies were performed at sites B and C in Linn Co., Oregon. (See APPENDIX 1 for site description.) Traps were set about 10 cm above the ground in an oval-shaped trapline with each trap at least 10 m from the next. Areas with wood debris and slash have very high pupal

populations and more newly emerged cinnabar moths. These trap locations would be expected to have high virgin female concentrations and hence influence males caught in these areas. Therefore, traps were rotated around the trapline each day or checking period to minimize site influence. Table 2 summarizes the pertinent information for these experiments.

Table 2. Design of experiments using sticky traps to investigate female attractiveness and male response for the cinnabar moth, Tyria jacobaeae (L.).

	Experiment 1	Experiment 2	Experiment 3
Number of traps used:	10	10	10
Each baited with:	4 with virgin female 4 with mated female 1 with male 1 empty	virgin female	virgin female
Traps set up:	late June	early July	mid-July
Time:	4 pm	4:45 am	4 pm
Traps were checked:	daily	hourly	every 4 hours
For:	3 days	12 hours	3 days
Location:	site B	site C	site C

Results and Discussion

Virgin female cinnabar moths attract males, presumably with a pheromone, but mated females do not. All moths captured in the sticky traps were found to be males. Mated females either do not emit a sex pheromone or sound (or may emit an anti-attraction pheromone or sound).

The majority of males were attracted to females around sunrise (4 to 6 am) as opposed to early morning (7:30 am or later) as reported in

the literature. Mean number of males caught per female decreased hourly from 2.7 ± 0.8 at 5:45 am, to about 0.4 ± 0.2 per hour until 9:45 am for Experiment 2. No males were ever caught between the hours of 10 am and 10 pm for Experiments 2 nor 3. Some males were caught at night (10 pm to 4 am) for Experiment 3 but it is not known if these catches occured throughtout the night or only just before sunrise, as suspected. The evening matings noted occasionally in the field can not be explained on the basis of these experiments.

Males flew to the traps when they were set up at 3 am, but they were possibly also responding to the flashlight and car lights. Males will fly up from the foliage anytime at night when light is shined on them, but females are not attracted to lights. No females have been collected in several summers of blacklighting by the Oregon Department of Agriculture although hundreds of males have been captured (Isaacson, personal communitcation.)

One unexplained behavior is that after 10 am, many males were sometimes seen resting near a female trap, but not entering. They may have been drawn to the area by traces of pheromone after the female ceased emission. Or perhaps, the female was emitting but lack of some environmental or behavioral cue(s) precluded the males entering the traps to attempt mating. I have also noticed males resting near newly emerged females on cloudy afternoons. There are enough discrepancies in cinnabar moth information, so that the possibility can not be ruled out that virgin females emit pheromone continuously or frequently until mated but males respond differentially due to lack of additional stimuli necessary (such as sound) for mating. Sound production has been demonstrated in the garden tiger moth, Arctia caja L., and other arctiids and may function in both warning and sex calling (Rothschild and Haskell 1966).

There was large variation in relative attractiveness of different females as the number of males caught per female trap per day ranged from 0-40. Daily mean numbers of males caught per female had large standard errors and included: 11.7 ± 3.2 (range 0-28), 4.7 ± 4.4 (range 0-40), 4.2 ± 1.0 (range 0-9), and 3.2 ± 1.6 (range 0-16). Some of this variability may be due to small adult populations with patchy distribution at the

field sites. Large variation in male response to females is a drawback in pheromone work performed with live females, and is the main reason such researches should include means with an estimate of variation instead of catch totals.

Ten percent (3/28) of the traps baited with virgin females failed to catch any males. Fourteen percent (4/28) only captured one male each over testing periods of at least 3 days but some females attracted over 50 males in a comparable period. These data indicate that there probably exists a certain percentage of females in the field populations that fail to attract a mate. Even a delay in mating (of about seven days or more) can be detrimental to fecundity and hatch of eggs (explained in Oviposition section of EGG MASS VARIABILITY AND DIET EXPERIMENTS).

Differences in female attractiveness did not significantly correlate with female size (as measured by wing length). Results are inconclusive concerning effect of age on female attractiveness, but the number of males caught per female decreased while female age increased for the three experiments:

Experiment #:	1	2	3
Female Age:	3.8 ± 0.9	7.7 ± 0.9	10.9 ± 2.4
Number of males: (caught per trap per day)	11.7 ± 3.2	5.4 ± 1.5	4.0 ± 3.0

It would be interesting to use a similar experimental design as reported here to test the hypothesis: Female cinnabar moths reared as larvae on floral parts are more "attractive" to male moths and mate sooner than females reared on leaf diets.

From the second day onward, bait moths began to disappear at a trap site located near a pile of slash in Experiment 1. One female disappeared daily from each trap set at this location for 3 days and the

females were also missing from two nearby traps on the third day. The area for about 0.5 m^2 around the trap would be littered with moth wings. It was obvious that some mammal such as a chipmunk or mouse learned to simply sit by the trap and harvest all the male moths as they flew magically into range (actually being lured in by the virgin female). The bodies were neatly consumed and the wings dropped to the ground in piles. The mammal then learned to enter the trap and scrape the bodies off the sticky container. For the final coup, the mammal would remove the lid from the inner cylinder and consume the lone bait female.

Isaacson (1972) reports predation of cinnabar adults at the Silbernagel site in Linn Co., Oregon during his study, and his trapping yielded a female chipmunk, Eutamias sp., with cinnabar moth heads in the gut. This predator is possibly the same or similar to the mammal responsible for interrupting Experiment 1 and causing Experiments 2 and 3 to be judiciously moved to site C about 0.4 km away. Site C had a smaller cinnabar moth population but also less slash.

Mating Frequency

Since mated females do not attract males, there is no indication that females require more than one fertilization to receive enough sperm to lay a normal complement of eggs. Fertile females in the diet oviposition study laid an average of 359 eggs each (range 68-624). Dissections of 70 of these females yielded only two with two spermatophores; the rest contained one. Both of the second matings occurred the day after the first mating. Presumably the close confinement in the mating cups caused the males to attempt a second mating in the absence of calling from the mated female. It is doubtful that multiple matings occur normally in the field.

Four newly emerged males were paired each with a virgin female (in

1/2-pint waxed cups in a growth chamber with photoperiod 14/10 and thermoperiod 23.9°/10° C.) to determine the number of times a male could mate. Cups were checked daily until the males died, and new females were exchanged for the old females that began laying eggs. The males each mated with, and successfully fertilized, 1-4 females each ($\bar{x}$ = 2.25 $\pm$ 2.25). Mating males lived an average of 8.8 $\pm$ 1.1 days (compared to 65 virgin males in the insectary that lived an average of 17.5 $\pm$ 0.8 days each). One male mated within one day of dying, but the other three mated for the last time 4-5 days before dying.

Hatch success of the eggs laid by each female on the first oviposition day was always 96-100% and was not affected by whether she was the first or fourth female that a male mated with. Total eggs laid by each female on the first oviposition day varied from 45-215 ($\bar{x}$ = 154.8 $\pm$ 24.8), but the experiment was not detailed enough to indicate if the number of eggs a female lays (at least on the first day) is in any way related to the age of the male she mated with or how many females the male had previously mated with.

EGG MASS VARIABILITY (=CONTROL) AND DIET EXPERIMENTS

The egg mass variability (hereafter called control) and diet experiments were run concurrently. Each involved laboratory rearing of larvae from field-collected egg masses. The resulting pupae were overwintered in an outdoor insectary, and adult emergence was monitored late the following spring. Laboratory oviposition studies were conducted by pairing the diet experiment females with control males.

Methods

The source egg masses for the larval rearings were 11 egg masses collected each from both sites A and B in Linn Co., Oregon on July 11, 1975. These sites are near the Silbernagel site (Isaacson 1972). (See APPENDIX 1 for a full description of the field sites.)

Each egg mass on a leaf was kept in a covered glass petri dish in a growth chamber at 23-24° C. and photoperiod 12/12. (See REARING TECHNIQUES for details of rearing methods for each life stage.)

Six egg masses containing 50 or more eggs each (three egg masses from each site) were used in the diet experiment. The remaining 8 of 11 egg masses from both sites were reared as controls. Also, larvae in excess of 50 from each of the diet experiment egg masses were reared as controls. Thus, there were 16 control egg masses plus the excess from the diet egg masses.

Control Experiment

Control larvae from each egg mass were reared as a group, separately

from the other egg masses. Larvae were housed 20 per petri dish during instar I - III and reared under the same conditions as the eggs were incubated. Control larvae were reared on a mixed diet of leaves and flowers, one or the other given randomly each day. (This was equivalent to the M diet described under diet experiment methods in the next section.) Larvae were moved daily to a clean dish with fresh food that was always given in excess. Larvae were reared in groups of about 15 per ½-pint waxed cup for instars IV-V. The cups were covered with plastic screening held in place by a petri dish lid.

Larvae were weighed at the end of each stadium after they ceased feeding and had moved to the container lid prior to molting. First- through 3rd-instar larvae were weighed as a group per replication, and 4th instars were weighed individually. Weights were taken to the nearest 0.1 mg using a Mettler analytical balance. Fifth-instar larvae were not weighed because of time (labor) limitations of this research.

Sections of 0.5 cm diameter paper soda straws, 2.5-4.5 cm long, were added to the larval containers just prior to pupation to be used as pupation sites by the prepupae. Pupae were harvested from the straws and the following data taken for each: sex, length to the nearest 0.1 mm, weight to the nearest 0.1 mg, and whether it pupated inside or outside of a straw.

Pupae were overwintered in an outdoor insectary in ½-pint waxed cups filled 1/3 full with bark mulch and covered with clear plastic household wrap. Plastic petri dishes were substituted as lids in the spring to facilitate daily checking and removal of adults once emergence began. Control adult females were used in the MATING BEHAVIOR STUDIES and control males were used as mates in the oviposition study performed with the diet experiment females.

Diet Experiment

As each egg mass designated for the diet experiment hatched, the larvae were randomly placed in one of five petri dishes, 10 larvae per dish. The five dishes were randomly assigned, one to each of the five following tansy ragwort diets. (The abbreviation for each diet will be used from here on in the text, tables and figures.)

1. L1 Leaves from first-year plants.
2. L2 Leaves from second-year flowering plants.
3. LS Leaves from plants that grow in the shade at least 85% of the daylight hours.
4. F Floral parts (including buds, flowers and pedicels).
5. M Mixture of the above four diets, one diet given randomly each day.

Thus, each diet had six replications of 10 larvae each, and each replication within a diet represented a different egg mass:

Diet	Egg mass 1	2	3	4	5	6
L1	10	10	10	10	10	10
L2	10	10	10	10	10	10
LS	10	10	10	10	10	10
F	10	10	10	10	10	10
M	10	10	10	10	10	10

= 30 replications of 10 larvae each

Replications were based on egg mass because nothing was known beforehand about the potential variability between egg masses. This experimental design would help to avoid potential bias if it was later found that significant differences existed between cinnabar larvae and/or adults attributable to different egg masses.

Diet experiment larvae were reared at lower densities per rearing container than were control experiment larvae. All other aspects of rearing were the same for the diet experiment as described for the

control experiment. The adult males emerging the following spring from the diet reared pupae were left in cups in the insectary to determine longevity in the absence of mating.

Diet experiment females were mated with control experiment males (for standardization) in an oviposition study to determine fecundity and percent egg hatch. This study was performed in a growth chamber with photoperiod 14/10 and thermoperiod 23.9°/10° C. to simulate field conditions more closely than a single temperature.

Each adult pair (diet female and control male) was housed with a ragwort leaf in a ½-pint waxed cup with a plastic petri dish as a cover. Adults were always paired on the first day the female emerged, and the male was a newly emerged virgin whenever possible. Water drops were added daily for the adults to drink. The male was replaced by a new male if mating failed to occur after about 3-4 days. The replacement process continued until the female died or laid eggs, at which time the male was removed.

Each ovipositing female was moved to a new cup every second day. Cups with eggs were labeled by female and incubated to determine percent hatch. Newly dead females were dissected to look for a spermatophore, a round ball-shaped object with a small hard coil, located in the bursa copulatrix (?). The right wing was measured to the nearest 0.1 mm using a dissecting microscope and following the method of Green (1974).

Statistics

One- and two-way analyses of variance for the diet experiment data were performed using the *ANOVA12 statistical program (version 3.5) on the OS-3 computing system at the Oregon State University Computer Center. When significant differences for diet or for egg mass means were found,

the Neuman and Keuls sequential variant of the Q (Studentized Range) method (Snedecor and Cochran 1967) was used to pick out the means that differed significantly from others. This method is more conservative than the LSD (Least Significant Difference) method and sometimes indicated no significant differences between means when the analysis of variance indicated significant diet and/or egg mass differences. These cases are indicated in the appropriate data table.

One-way analyses of variance with unequal sample sizes for the control experiment data were computed using the unequal sample size method described in Snedecor and Cochran (1967; page 277).

Results and Interpretation: Control Experiment

Field Collected Egg Masses

The 22 egg masses collected from sites A and B formed the basis or source of all subsequent diet and egg mass variability studies performed for this thesis. Most statistical differences between site A and B data were found to be non-significant. Data for the two sites were therefore usually pooled.

It is recognized here that the 11 egg masses collected from each site constitute too small a sample size for adequate site comparison. It can not be ascertained if any "significant" site differences are real or due to "founder effect" induced by the small number of egg masses collected. Likewise, any "non-significant" site differences may represent real site equalities or simply, statistical equalities due to large variance and small sample size. No conclusions will be made in this thesis concerning any supposed biological differences between the two sites used relative to the cinnabar moth.

Mean egg batch size for eggs collected at sites A and B was 46.5 ± 7.0 (range 5-143) and average egg hatch was 85.7% (Table 3). Mean batch size and percent hatch did not differ significantly for the two sites, and variance was large, especially for site B.

Table 3. Egg batch size, sex ratio and percent hatch for field-collected egg masses of the cinnabar moth, Tyria jacobaeae (L.), from Linn Co., Oregon.

Site	Egg masses, n	Egg batch size $\bar{x}$ [a] ± S.E.	s^2	Sex ratio[b] $\bar{x}$ [a] ± S.E.	% Egg hatch $\bar{x}$ [a] ± S.E.
A	11	43.1 a ± 7.3	585.7	0.51 a ± 0.03	95.9 a ± 2.4
B	11	50.0 a ± 12.3	1666.6	0.52 a ± 0.02	75.5 a ± 14.9
A + B	11	46.5 ± 7.0	1085.0	0.51 ± 0.02	85.7 ± 7.7

a/ Means followed by the same letter within a column do not differ significantly at the .05 level.
b/ Number of females divided by total

Larval Development

Highly significant differences were found between mean 4th-instar weights for different egg masses (Anova, APPENDIX 2a)[4] Thus, there is significant variation in larval size attributable to the egg mass that a larva came from. These differences could be due to the quantity or quality of the food that the parent obtained as a larva.

Size of larvae does not appear to be related to the batch size of

4/ The notation "Anova, APPENDIX 2a" indicates that the significance of differences in means was determined by performing an analysis of variance test as described in "Statistics" and that the "Anova" table for this test can be found in APPENDIX 2a.

the original egg mass as no significant correlations were found between larval weight and number of eggs in the source egg mass. Also, there was no significant correlation between larval weight and sex ratio. Green (1974) found that 1st instar survival generally increased with increasing egg batch size up to about 50 eggs but tended to decrease for batches larger than 50 eggs. A possible reason for this could be that larger egg masses have larger (more fit) larvae than smaller egg masses, but lack of correlation tends to discount this possibility.

Survival per instar was always 97-100%, and total mean larval survival was 95%. Larval deaths were usually associated with improper or incomplete molting that rendered the mouthparts inoperative. Most larval deaths in this study were probably due to mechanical disturbance during molting.

Once larvae select pupation sites, they secrete silk strands to seal off the ends of the pupation straw, or they may cover themselves loosely with silk and bring plant or other material around them. This period from silking to formation of a pupa takes 1-4 days. The pupa itself may require 1-3 days to harden and tan to a dark red-brown color. For purposes of this study, larval developmental time is defined as the period between egg hatch and the day a prepupa silks up a chosen pupation site. This avoided potential problems of disturbing prepupae (they will sometimes vacate the pupation site and search for another) or damaging the fragile new pupae if daily checks were made to see if each prepupa within a straw had actually pupated. These developmental periods will therefore be shorter than others reported in the literature by about 1-4 days.

Larval development required 18-24 days ($\bar{x} = 19.1$) at 23.9° C. and photoperiod 12/12. The first four stadia averaged 3 days each (3.3, 2.9, 3.0 and 3.2), and the 5th stadium lasted 6.7 days. Developmental time period did not differ between the two sites or between different egg masses. In the field, Cameron (1935) found developmental periods of 5-6 days each for

the first four stadia and 10-11 days for the 5th stadium. Isaacson (1972) found significantly differing developmental periods for larvae reared at different temperatures but no significant differences in developmental periods for the five stadia. He questions the lack of significant differences between 5th-stadium and earlier-stadia developmental periods, however. Stimac (1977) assumes equal developmental periods of one week each for all stadia in his cinnabar moth-tansy ragwort model that has a weekly resolution. A more realistic model would probably have the duration of the 5th stadium twice as long as that for each of the earlier stadia.

Pupation

Highly significant differences between mean female pupal weights for different egg masses were found (Anova, APPENDIX 2b). The same highly significant differences were found for male pupal weight, and for female and male pupal length (Anovas, APPENDIX 2c-2e). This difference in pupal size potential amongst different egg masses should be taken into consideration when designing experiments involving size of cinnabar with pupae.

Mean pupal length for males (11.70 ± 0.06 mm) was significantly greater than for females (11.47 ± 0.08 mm). Female pupae outweighed males in 12 of 18 egg masses, but overall female pupal weight (140.8 ± 2.5 mg) was not significantly heavier than that of males (137.6 ± 2.2 mg). Females generally had more variable pupal weights and lengths than males.

Figure 2 depicts simple linear regressions of mean pupal weight on mean pupal length by egg mass for both sexes. The R^2 value is lower for males, indicating that weight and length do not correlate as closely for males as for females. Pupal weight and length ratios (as expressed by regression slopes) are about the same for females and males. Female pupae generally weigh about 10 mg more than males at a given length, and

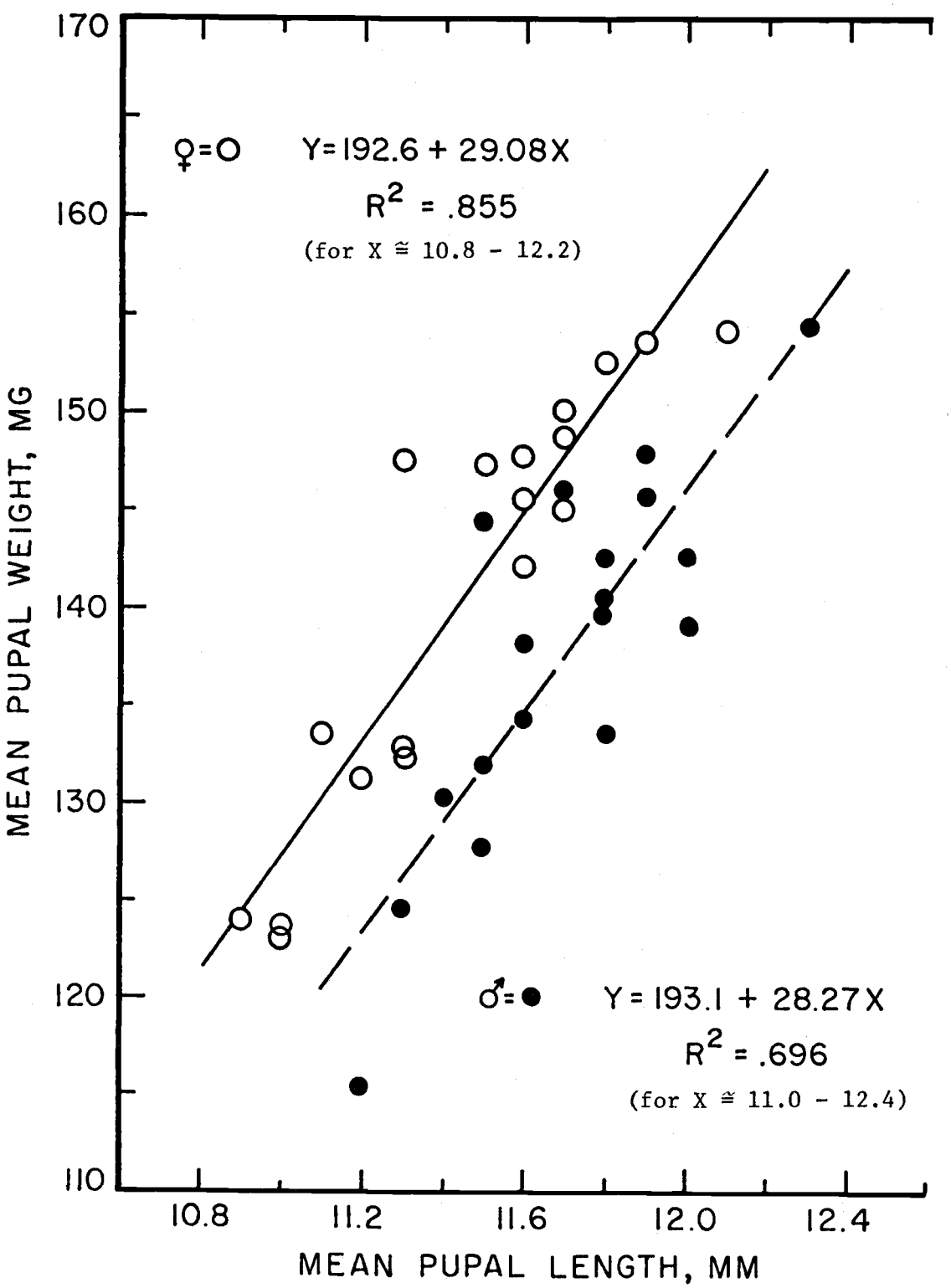

Figure 2. Pupal weight regressed on pupal length for female and male cinnabar moths, Tyria jacobaeae (L.). Data represent means for 18 egg masses.

male pupae are usually about 0.4 mm longer than female pupae at a given weight. Highly significant correlations were found between female and male pupal weights and pupal lengths within each egg mass (Table 4). This indicates a similar growth potential for females and males within each egg mass and doubtless influences the parallel regressions in Figure 2.

Table 4. Correlation coefficients betwen egg mass size, sex ratio, and pupal size for the cinnabar moth, Tyria jacobaeae (L.). Data represent means for progeny from 18 field-collected egg masses from Linn Co., Oregon

	Egg batch size	Sex ratio (% ♀♀)	Pupal weight ♀	Pupal weight ♂	Pupal length ♀
Sex ratio (% ♀♀)	-.125	---	---	---	---
Pupal weight: ♀	.150	.672 **	---	---	---
Pupal weight: ♂	.304	.432	.902 **	---	---
Pupal length: ♀	.174	.629 **	.938 **	.874 **	---
Pupal length: ♂	.092	.496 *	.794 **	.846 **	.890 **

* Correlation coefficient is significant at .05 level; $|r| > .468$
** Correlation coefficient is significant at .01 level; $|r| > .590$

Egg batch size was not significantly correlated with pupal size (Table 4). Interestingly, sex ratio per egg mass highly correlated with female pupal weight and length, correlated with male length, but did not significantly correlate with male weight. The question that arises is why do egg masses with relatively more females than males yield larger female pupae and possibly larger male pupae. Inspection of Table 4 shows that female weight and length are most highly correlated with each other. Male pupal weight and length, however, are each most highly correlated with female weight and length and not each other. This would seem to indicate that female and male size potential is very similar within each

egg mass, and this potential is somehow correlated with the sex ratio of an egg mass. This may be a "nonsense" correlation, but it would be interesting to explore it by testing the hypothesis: Large female moths lay egg masses yielding large progeny, and these include a higher proportion of females than do egg masses laid by smaller females. (Note that this has nothing to do with egg batch size.)

Adult Emergence

Control experiment adults began to emerge May 6 (about 280 days after pupation). Emergence continued for a 50-day period ending June 24. Females emerged over a 49-day period versus 39 days for males. The 70-100% cumulative emergence period was longer for females than for males, 33 versus 27 days (Figure 3). Although females tended to emerge later, mean days to emergence after pupation did not differ significantly for females and males (292.7 ± 1.3 versus 289.0 ± 1.0). Cumulative total emergence (females plus males) after emergence began was: 10% by day 4, 25% by day 6, 50% by day 9, 75% by day 15, 90% by day 23, and 100% by day 52.

Mean days to emergence differed highly significantly for adult females from different egg masses (Anova, APPENDIX 2f). Differences in male emergence for different egg masses were also highly significant (Anova, APPENDIX 2g). Pupal size, egg batch size and sex ratio were not signifcantly related to emergence timing as evidenced by non-significant correlations (Table 5). Green (1974) also found no correlation between size (adult) and emergence date for females or for males.

Egg mass variability is the most important influence on timing of emergence for adults reared as larvae under the same conditions. This is presumably a parental influence but it is not known if it is genetic or perhaps, environmentally induced by some aspect of adult nutrition. It must be kept in mind that all adults for this experiment

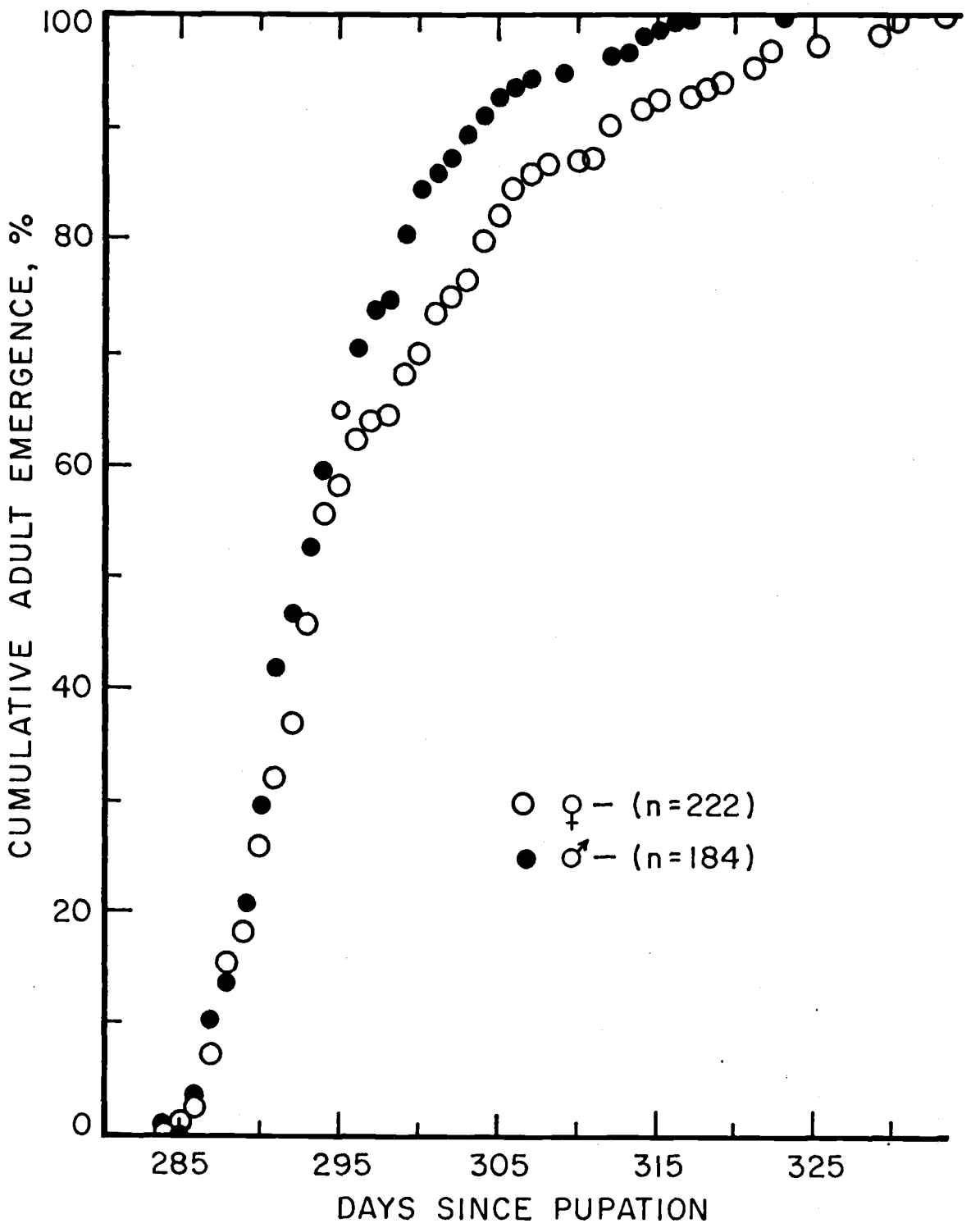

Figure 3. Cumulative emergence for female and male cinnabar moths, Tyria jacobaeae (L.).

came from eggs collected on a single day in the summer season, and all were probably laid within a week of each other. Possible seasonal effects on emergence timing can not be discerned from this research.

Only 5% (24/445) of the pupae failed to yield adults. These emergence failures showed no apparent pattern relative to site, sex or egg mass. Pupae failing to emerge were dissected in August. They were moldy, dried up, or filled with a putrid brown liquid and presumably diseased. None had metamorphosed to a pharate adult within the pupal case. Many were pupae that weighed less than 100 mg the previous summer and were at the time considered to be possible pupation failures.

Table 5. Correlation coefficients for mean days to emergence and egg mass size, sex ratio and pupal size for the cinnabar moth, Tyria jacobaeae (L.). Data represent means for progeny from 18 field-collected egg masses from Linn Co., Oregon.

Mean days to emergence after pupation	Egg batch size	Sex ratio (% ♀♀)	Pupal weight ♀	Pupal weight ♂	Pupal length ♀	Pupal length ♂
♀	-.199[a/]	.344	.075	-.158	-.088	-.247
♂	-.401	.353	.093	-.026	-.044	-.134
♀ + ♂	-.319	.384	.092	-.110	-.076	-.218

a/ Absolute value of .468 or greater needed for significant correlation at the .05 level.

Results and Interpretation: Diet Experiment

Larval Development

In general, diet did not significantly affect 1st- through 4th-instar larval weights. Specifically, non-significant diet effects were found for instars I, II and IV (Anovas, APPENDIX 2h-2j) and significant diet effects were found for instar III (Anova, APPENDIX 2k). Larval weights are listed in Table 6. (Note that the Anova for instar III indicated significant diet effects but the multiple-mean differences test employed indicated no significant differences in diet means.)

The analyses of variance indicated highly significant weight differences attributable to differing egg masses for instars I-III but not for instar IV. This later finding would seem to contradict the control data analysis that indicated significant egg mass effects on 4th instar weights. It is likely that the smaller sample size of egg masses for the diet compared to the control experiment (6 versus 18) and higher variance influenced this lack of statistical significance, in addition to diet influences on between-egg mass differences.

Total larval development periods ranged from 18-20 days at 23.9° C. and photoperiod 12/12 (Table 7). The number of days per instar was only recorded with an accuracy of ± 0.5 day, so statistical analysis was not performed. Meijden (1976) found developmental periods for cinnabar larvae on leaf and leaf-plus-flower diets to be 40.96 and 39.36 days at 20° C. (He states the difference between these two means is "highly significant".) His leaf-plus-flower diet is equivalent to my F, and perhaps M, diets. Looking at Table 7, there is no reason to suspect that leaf diets alone require longer developmental periods than the F or M diets. Meijden's overall developmental periods are substantially longer than those I found, even when differences in rearing temperatures are taken into consideration. Differing humidities might explain this developmental difference as might real differences in developmental rates for the Oregon and The Netherlands cinnabar larvae (given equal temperatures). But it is difficult to explain the differences in our

Table 6. Comparison of larval weights for the cinnabar moth, Tyria jacobaeae (L.), reared on one of five diets and emerging from one of six egg masses.

Source	Replications, n	INSTAR: I weight, mg $\bar{x}$ a/ ± S.E.	II weight, mg $\bar{x}$ a/ ± S.E.	III weight, mg $\bar{x}$ a/ ± S.E.	IV weight, mg $\bar{x}$ a/ ± S.E.
Diet					
L1	6	1.01 a ± .02	5.43 a ± .20	25.30 a b/ ± .90	87.17 a ± 3.18
L2	6	1.01 a ± .06	5.04 a ± .36	22.63 a ± 1.58	87.70 a ± 1.48
LS	6	.99 a ± .05	5.32 a ± .31	25.51 a ± .95	93.92 a ± 2.34
F	6	.95 a ± .06	5.15 a ± .29	23.83 a ± 1.23	90.60 a ± 1.24
M	6	1.03 a ± .06	5.03 a ± .29	23.91 a ± .87	90.18 a ± 2.46
Egg mass					
1	5	.91 ab ± .02	4.53 a ± .14	19.76 a ± 1.38	87.83 a ± 2.33
2	5	1.06 c ± .04	5.63 c ± .11	25.53 bc ± .39	93.58 a ± 2.11
3	5	.99 bc ± .02	5.01 ab ± .07	24.01 b ± .42	91.70 a ± 1.95
4	5	.86 a ± .03	4.61 ab ± .25	24.04 b ± 1.02	84.18 a ± 2.93
5	5	1.23 d ± .02	6.24 d ± .17	26.88 c ± .60	89.89 a ± 1.71
6	5	.94 ab ± .03	5.16 bc ± .18	25.20 bc ± .56	92.29 a ± 2.69
Control	18	.97 ± .03	4.88 ± .16	22.88 ± .47	82.37 ± 1.29

a/ Diet means followed by the same letter within a column are not significantly different at the the .05 level. The same applies to Egg mass means.

b/ Analysis of variance indicated significant differences in 3rd instar weight means attributable to diet but the multiple-mean difference test employed does not show this.

Table 7. Comparison of larval developmental periods and survival for the cinnabar moth, Tyria jacobaeae (L.), reared on one of five diets and emerging from one of six egg masses. Developmental periods carry an error of ± 0.5 days in data recording.

		INSTAR: I		II		III		IV		V		TOTAL	
Source	Replications, n	Days $\bar{x}$ a/ (range)	% Survival $\bar{x}$	Days $\bar{x}$ a/ (range)	% Survival $\bar{x}$	Days $\bar{x}$ a/ (range)	% Survival $\bar{x}$	Days $\bar{x}$ a/ (range)	% Survival $\bar{x}$	Days $\bar{x}$ a/ (range)	% Survival $\bar{x}$	Days $\bar{x}$ a/ (range)	% Survival $\bar{x}$ b/ (± S.E.)
Diet													
L1	6	3.0 (3)	100	2.7 (2-3)	100	3.0 (3)	98	3.3 (3-4)	100	6.3 (6-7)	100	18.3 (18-19)	98 a (± 2)
L2	6	3.0 (3)	100	2.8 (2-3)	98	2.8 (2-3)	97	3.3 (2.5-4)	98	6.6 (6-7)	100	18.4 (18-19)	93 a (± 5)
LS	6	3.0 (3)	100	2.8 (2-3)	98	2.8 (2-3)	98	3.0 (3)	100	5.9 (5.5-6)	100	17.6 (17-18)	97 a (± 2)
F	6	3.0 (3)	100	3.0 (3)	98	2.6 (2-3)	100	2.8 (2-3)	100	6.2 (5.5-7)	100	17.6 (17-18.5)	98 a (± 2)
M	6	3.0 (3)	100	2.8 (2-3)	100	2.8 (2-3)	100	3.2 (3-4)	100	6.5 (5-8)	100	18.3 (18-20)	100 a (± 0)
Control	20	3.3 (3-4)	97	2.9 (2.5-3.5)	100	3.0 (2.5-3)	100	3.2 (3-5)	98	6.7 (6-9)	99	19.1 (18-24)	95 (± 2)

a/ Developmental periods carry an error of ± 0.5 days in data recording.
b/ Means followed by the same letter do not differ significantly at the .05 level.

conclusions about diet effect on duration of the larval period.

Diet did not significantly affect instar survival (range 97-100%), nor total larval survival that ranged from 93-100% (Table 7 and Anova, APPENDIX 2m).

Weight variation is thus related more to egg mass variability (ie. parental influences) than to diet in the first four instars. But, niether diet nor between-egg mass variability significantly affected duration of larval development and survival.

Pupation

Significant differences were found in female pupal weights attributable to diet, but not to egg mass (Anova, APPENDIX 2n). The same results were found for male pupal weight, and for female and male pupal lengths (Anovas, APPENDIX 2p-2r). Female pupae reared on the F and M diets were significantly larger than pupae reared on the LS diet (Table 8). Diets ranked by decreasing female pupal size are: F, M, L2, L1, LS. Pupal weights for males reared on the F diet were significantly heavier than on the LS diet, and male pupal lengths on the F, M and L2 diets were significantly longer than on the LS diet (Table 8). Diets ranked by decreasing male pupal size are: F, L2, M, L1, LS. Feeding on a diet that includes floral parts (F and M diets), usually imparts a greater pupal size potential to larvae than diets containing only leaf parts. It would also be selectively advantageous for larvae to avoid feeding on shade-grown plants and for adult females to avoid ovipositing on these plants as they impart a lower growth potential to larvae and a female's progeny.

These results about floral-diet effects on pupal size are similar to the significant size differences Meijden (1976) found in female pupal widths on two diets. He found female pupae reared on a leaf-plus-flower diet to be significantly wider than those reared on a leaf diet. His male pupal widths followed the same trend but were not significantly

Table 8. Comparison of pupal size for the cinnabar moth, Tyria jacobaeae (L.), reared on one of five diets and emerging from one of six egg masses.

Source	Replications, n	Pupal weight, mg: Females $\bar{x}$ a/ ± S.E.	Pupal weight, mg: Males $\bar{x}$ a/ ± S.E.	Pupal length, mm: Females $\bar{x}$ a/ ± S.E.	Pupal length, mm: Males $\bar{x}$ a/ ± S.E.
Diet					
L1	6	154.65 ab ± 5.09	144.82 ab ± 3.90	11.79 ab ± .07	11.93 ab ± .04
L2	6	159.28 ab ± .81	157.58 ab ± 3.18	11.90 ab ± .05	12.18 b ± .10
LS	6	143.70 a ± 6.95	142.17 a ± 7.03	11.56 a ± .14	11.71 a ± .18
F	6	167.45 b ± 5.34	159.83 b ± 3.07	12.15 b ± .19	12.30 b ± .10
M	6	161.95 b ± 2.43	156.98 ab ± 2.46	12.03 b ± .10	12.12 b ± .09
Egg mass					
1	5	149.06 a ± 5.67	147.78 a ± 5.44	11.61 a ± .14	11.87 a ± .13
2	5	160.52 a ± 7.85	155.26 a ± 4.37	12.06 a ± .19	12.10 a ± .10
3	5	164.53 a ± 3.51	154.82 a ± 1.55	11.90 a ± .11	12.01 a ± .03
4	5	158.31 a ± 1.47	154.96 a ± 3.34	11.88 a ± .08	12.11 a ± .07
5	5	154.04 a ± 8.45	147.71 a ± 9.05	11.82 a ± .19	11.96 a ± .28
6	5	157.95 a ± 6.82	157.92 a ± 5.85	12.02 a ± .18	12.24 a ± .15
Control	18	140.8 ± 2.5	137.6 ± 2.2	11.47 ± .08	11.70 ± .06

a/ Diet means followed by the same letter within a column are not significantly different at .05 level. The same applies to Egg mass means.

different. My pupae reared on the F and M diets were generally larger than those on any of the three leaf-only diets. The differences in weight and length were not always significant, except that the F diet pupae were always significantly larger than the LS diet pupae for both sexes.

Since pupal size is significantly correlated with fecundity, Meijden (1976) assumed that the larger pupae reared on the leaf-plus-flower diet were more fecund than those on the leaf diet. My research confirms this assumption but discussion is postponed until the "Oviposition" section.

Findings concerning the presence of egg mass variability in pupal size for the control and diet experiments contrast each other; the same situation was found for 4th-instar weights. Highly significant pupal size differences between egg masses were found for the controls, but non-significant egg mass differences were found in the diet experiment (APPENDIX 2b-2e compared to APPENDIX 2n-2r). This non-significance for the diet experiment may be due to smaller sample size and larger variance. Given equal rearing conditions, differences in pupal size are probably attributable mainly to parental influence. But pupal size potential imparted to progeny by different females can be changed (increased or decreased) by larval diet. There is no reason to believe that a given diet will affect all larvae the same way. Increased size potential imparted by a floral diet may have a synergistic effect on pupal size attained by progeny of one egg mass compared to another. Pupal size can also be influenced by rearing density (Bornemissza 1966, Dempster 1971, Schmidl 1972, Meijden 1976) and food deprivation at any stage of larval development (Meijden 1976).

The pupal weights and lengths for the diet experiment did not correlate with sex ratio as did the pupal weights and lengths in the control group. This indicates that diet can also override the size potential for female pupae in a given egg mass that is for some reason

highly correlated with sex ratio.

The only two diet experiment larvae that failed to pupate were reared on the LS diet. Meijden (1976) also found no significant differences in pupation success between larvae reared on a leaf and a leaf-plus-flower diet (89% versus 92%).

Adult Emergence

Adults from the diet experiment emerged from May 8 to June 28. The 3% (8/264) failing to emerge did not show any apparent pattern relative to site, diet, sex or egg mass. Meijden (1976) had 73% pupal mortality (= emergence failure) with the leaf diet compared to 44% with the leaf-plus-flower diet. Though the level of mortality in Meijden's experiment is similar to some other literature values, it is so high that the results are probably influenced by disease or desiccation of the pupae.

Females reared on the F diet emerged significantly later than those reared on the other four diets (Table 9 and Anova, APPENDIX 2s). The analysis of variance performed on the mean days to emergence for females also indicated highly significant egg mass variability in emergence timing; the same was found for control female emergence. Even though both diet and egg mass had highly significant effects on mean days to emergence, diet appears to also affect the overall emergence pattern compared to egg mass (Figures 4 and 5). Diet appears to have a synchronizing affect on emergence in that uniform diet shortens the duration of the 1-80% cumulative emergence period by reducing the heterogeneity or spread of emergence found amongst different egg masses. The result is that emergence appears to be "late" for the F diet (or "early" for the other four diets), with elimination of a "middle" emergence as found when comparing different egg masses.

Male emergence data listed in Table 9 include the total mean

Table 9. Comparison of mean days to emergence after pupation for cinnabar moths, Tyria jacobaeae (L.), reared on one of five diets and emerging from one of six egg masses.

Source	Replications, n	Days to emergence after pupation: Female moths $\bar{x}$ a/ ± S.E.	Male moths $\bar{x}$ b/ ± S.E.
Diet			
L1	6	297.5 a ± 3.6	22 290.3 ± 1.7
L2	6	297.5 a ± 4.5	24 290.9 ± 1.8
LS	6	297.7 a ± 2.9	25 293.4 ± 1.9
F	6	308.2 b ± 2.5	31 299.6 ± 1.9
M	6	295.0 a ± 2.1	31 292.1 ± 1.5
Egg mass			
1	5	300.7 ab ± 3.2	24 291.9 ± 1.0
2	5	294.5 a ± 2.4	24 288.1 ± .8
3	5	293.7 a ± 3.4	20 287.1 ± .8
4	5	309.1 c ± 3.3	25 301.8 ± 2.8
5	5	293.4 a ± 2.6	19 294.2 ± 1.8
6	5	303.6 bc ± 3.4	21 297.4 ± 1.9
Control	18	292.7 ± 1.3	18 289.9 ± 1.0

a/ Diet means followed by the same letter within a column are not significantly different at the .05 level. The same applies to Egg mass means.

b/ Data are not available for individual repetition means for diets and for egg masses. Therefore, the means listed are weighted means for all males within a diet and for all males within an egg mass.

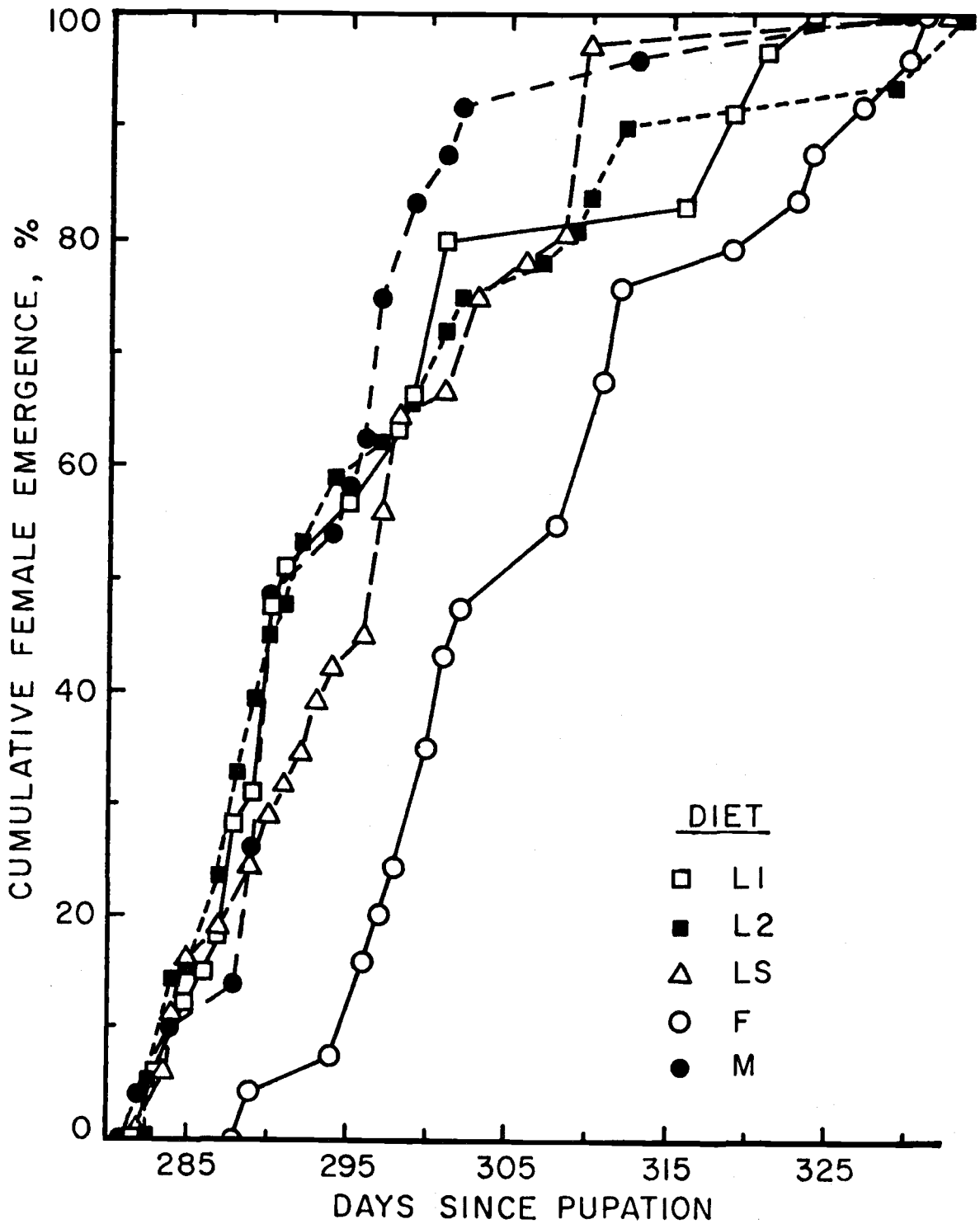

Figure 4. Cumulative emergence of female cinnabar moths, Tyria jacobaeae (L.), reared on one of five diets.

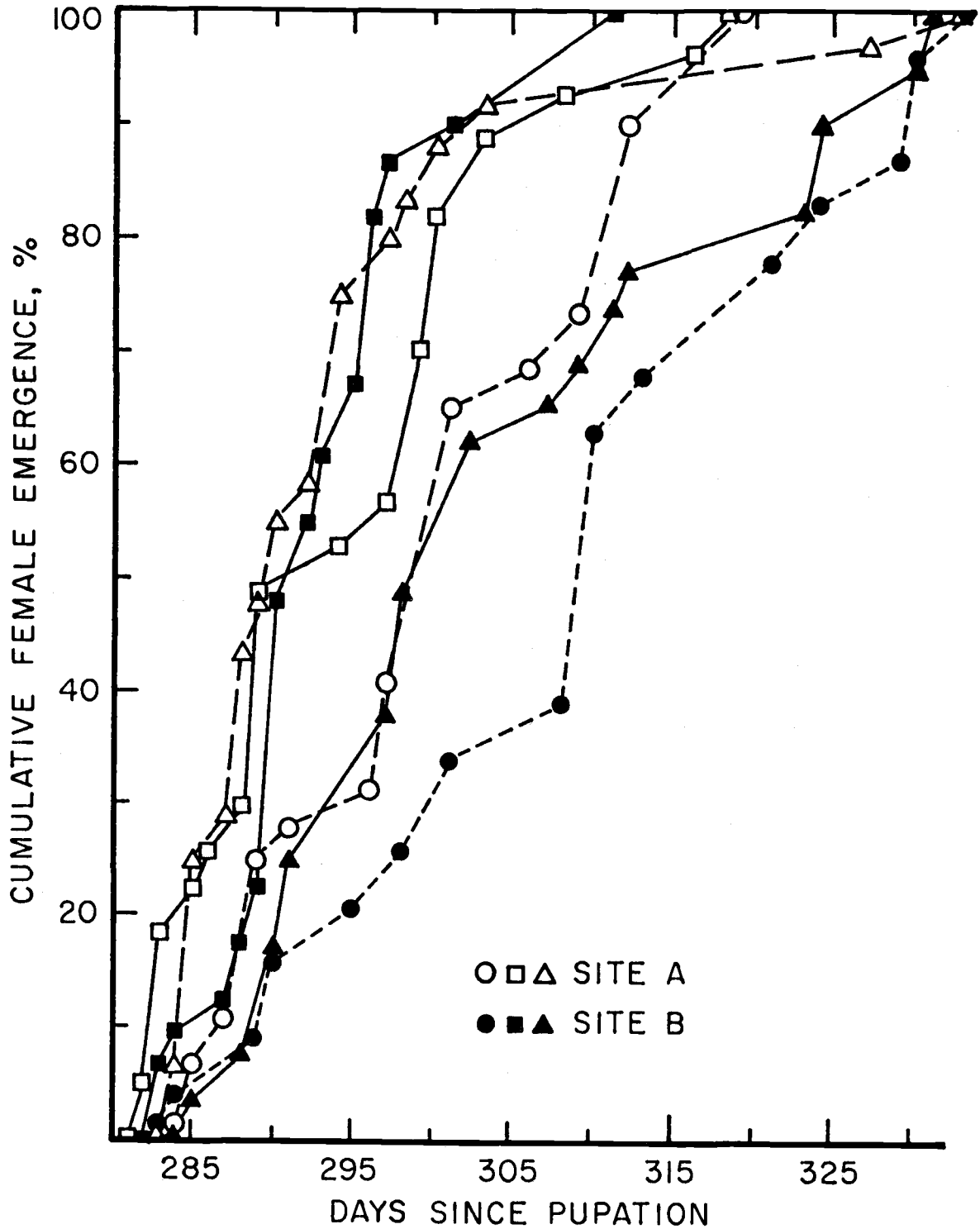

Figure 5. Cumulative emergence of female cinnabar moths, Tyria jacobaeae (L.), reared from one of six egg masses.

emergence dates per egg mass and total mean emergence dates per diet. The specific replication means were lost, so analysis of variance can not be used with this data. Inspection of means and S.E. for diet and for egg mass however, seems to indicate similar diet effects on male emergence as for females. Males reared on the F diet emerged later, and cumulative diet emergence patterns are similar for both sexes (Figure 4 compared to Figure 6).

The mean days to emergence after pupation were lower for the controls than for the M diet (Table 9). These differed only in rearing densities, so this is a possible indication that increased larval rearing density might be associated with earlier emergence.

Diet experiment pupae were weighed in the spring after 244-250 days in the insectary. Mean pupal weights in April, expressed as a percent of the previous August weights were 94.0 $\pm$ 0.5% (n = 30) for females and 94.6 $\pm$ 0.5% (n = 30) for males. Actual weight retention for the different replications ranged from 85-97%. Diet and egg mass did not significantly affect overwintering weight retention (Anova, APPENDIX 2t). Only 2% (6/264) of the pupae weighed at this time were considered dead as indicated by pupal weights of less than 75 mg.

Recalling the lack of correlation of pupal size with emergence date for the control experiment, it is unlikely that late emergence of females on the F diet is due to a need for an increased pupation period with increased pupal weight. The late emergence on the F diet is probably a qualitative effect of floral parts as a diet. It could be that some nutritive or chemical influence in eating floral parts triggers a genetic mechanism that causes late emergence.

One could hypothesize that larvae will always attempt to feed on floral parts, but a high population density will prevent this from occuring (ie. not enough floral parts available for all larvae). This may then trigger a genetic mechanism that is associated with early

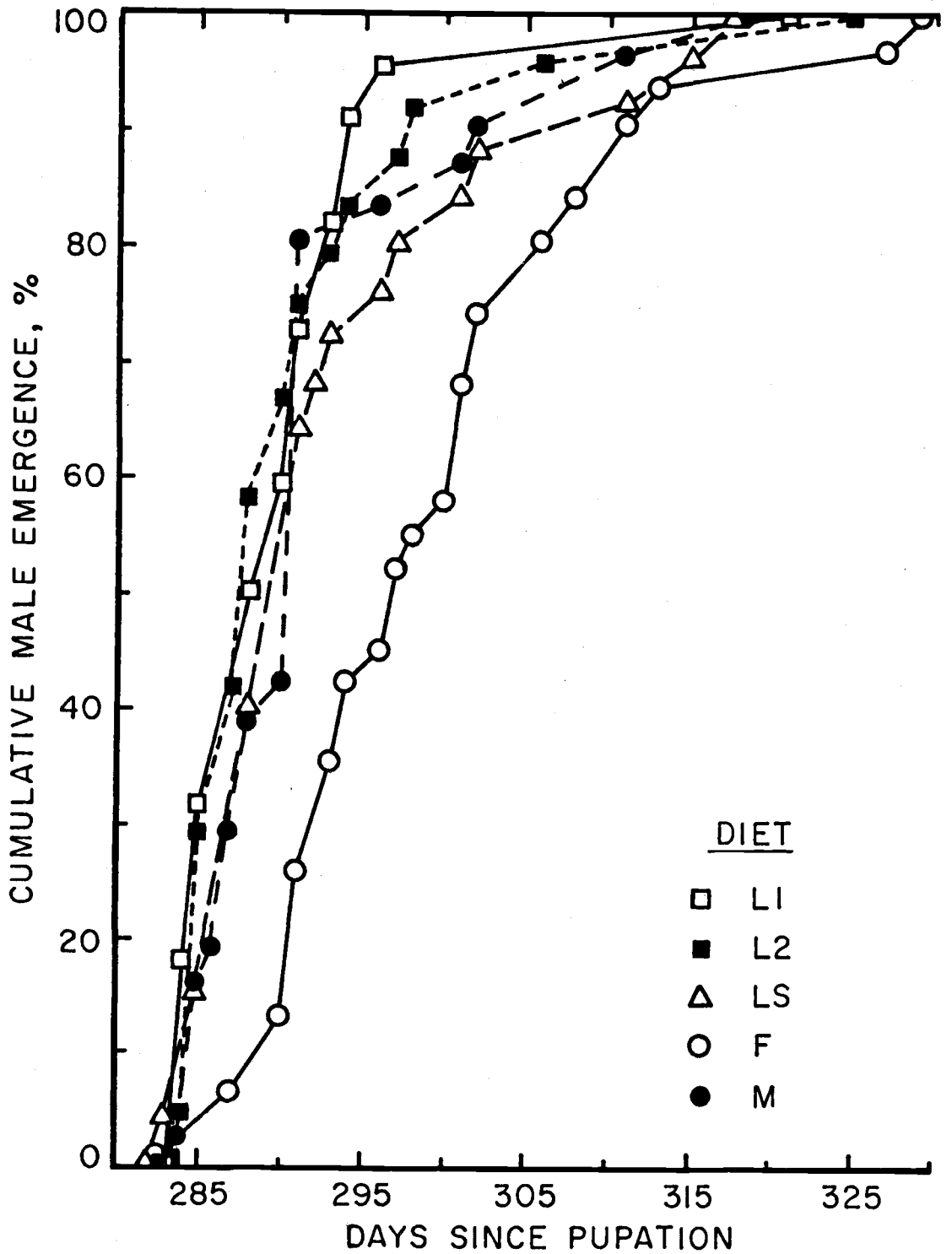

Figure 6. Cumulative emergence of male cinnabar moths, Tyria jacobaeae (L.), reared on one of five diets.

emergence to avoid starvation, because high proportions of larvae being forced to feed on leaves one year may be indicative of a potential increase in starvation the next year due to increased larvae per food unit. Or perhaps, risk of later emergence is simply a disadvantage imparted by a floral diet that would otherwise be advantageous due to presumably increased fecundity. The possibility of floral-reared adults being more able or likely to emigrate in search of oviposition sites or less crowded mating conditions would influence this "disadvantage".

The control and diet experiment data demonstrated that progeny emerging from different egg masses have different potentials for emergence timing in spite of uniform diet. This would suggest a maternal and/or paternal influence on emergence timing. It remains to be investigated if this influence is environmental (perhaps imposed by the mother's diet) or truly genetic. If the influence is dietary, then a female's diet can influence not only her progeny's size, but the emergence of her progeny as adults a year later. Diet is thus potentially important in timing and coordination of cinnabar population emergence. In addition, density may influence emergence timing in that adults reared as larvae at densities of 15-20 per container tended to emerge sooner than those reared at 10 per container.

Oviposition

Fertility and Sterility

Eggs laid per female ranged from 0-624, and only 4.2% (5/119) of the diet experiment females failed to lay eggs. By comparison, Stimac (1977) found fecundities in Oregon varying from 18-433 eggs per female. He states that unmated females deposited small egg batches of 10 or fewer eggs, but I found some unmated females laying egg batches of 50 or more. Stimac (1977) also states that 7.4% (2/27) of his females laid a small egg mass and then "behaved unusually". I found females nearly always laid several egg masses whether or not they had mated and whether or not their eggs were fertile. It has been my experience that female moths that emerge and begin oviposition without free water available for drinking may oviposit for a day or so and then either cease or lay more eggs that fail to hatch due to maternal desiccation. This might happen if females were held in containers without water for a day or so after emergence before field caging as described in Stimac's (1977) methods.

Females in this oviposition study fell into one of three categories:

1. Fertile — Most eggs laid (70% or more) appeared fertile and hatched (or at least showed signs of development).
2. Fertile? — A few eggs laid (up to 20%) developed or hatched. These eggs were usually the first or last laid eggs.
3. Sterile — None of the eggs developed of hatched, no matter how many eggs were laid.

The general egg laying pattern of females laying sterile eggs was similar to that of females laying fertile eggs. If the eggs had not been kept to determine hatching success, one might have assumed these were mated females laying a usual complement of fertile eggs. Table 10 compares data for the three categories of females. The "Fertile?" class only involved 6 females that appear to have mated, and then some unknown factor caused the eggs not to hatch (perhaps desiccation). The following discussion ignores this minority of females.

Table 10. Comparison of female cinnabar moths, Tyria jacobaeae (L.), classified by percent hatch of the eggs they laid.

Female classification (% egg hatch)	n (% of total)	% with spermatophore	Pupal weight, mg $\bar{x}$ a/ (± S.E., n)	Days since pupation $\bar{x}$ a/ (± S.E., n)	Life span, days $\bar{x}$ a/ (± S.E., n)	Day # the first eggs were laid $\bar{x}$ a/ (± S.E., n)	Oviposition period, days $\bar{x}$ a/ (± S.E., n)	Total eggs laid $\bar{x}$ a/ (± S.E., n) range
Fertile (70% +)	74 (61)	92	150.5 a (± 2.0, 72)	297.6 a (± 1.6, 74)	14.5 a (± 0.5, 70)	5.1 a (± 0.4, 72)	7.2 a (± 0.3, 71)	359.1 a (±13.2, 70) 68-624
Fertile? (1-20%)	6 (5)	75	151.6 a (± 5.3, 6)	300.2 a (± 7.1, 6)	26.0 b (± 1.3, 6)	12.2 ab (± 3.6, 6)	5.5 ab (± 0.8, 6)	226.7 ab (±50.8, 6) 26-357
Sterile (0%)	41 (34)	21	150.5 a (± 2.6, 41)	300.6 a (± 1.8, 38)	22.1 b (± 0.8, 40)	17.3 b (± 1.2, 36)	2.9 b (± 0.4, 40)	98.9 b (±14.4, 41) 0-321

a/ Means followed by the same letter within a column are not significantly different at the .05 level.

More fertile egg-laying females had spermatophores than did sterile egg layers, 92% compared to 21% (Table 10). The 8% fertile females without a spermatophore indicates that the spermatophore may not always be distinct or visible, or perhaps had already begun to be reabsorbed. It appears that 21% of the females in the sterile class had mated but laid sterile eggs due to some fertilization failure. All females, whether mated or not, were full of eggs, so failure of ovarial maturation must be ruled out as a cause of infertility. It seems that the majority of females laying sterile eggs simply failed to mate, in spite of having had several males available to them. I feel this is due to the small size of the mating container and lack of a semi-vertical perch for the female to rest on while the male hovers up to and a little above her in the courtship behavior (see MATING BEHAVIOR STUDIES and Mating in REARING TECHNIQUES for further elaboration.)

Pupal weight was not related to sterility (Table 10). There was a slight trend for later emerging females to lay sterile eggs, but this was not significant. Presumably these later females may have been somewhat stressed relative to lack of moisture or higher temperatures experienced in the insectary as the season progressed. There is also a possibility of a slight but gradual "disease" build-up in the oviposition growth chamber through time.

Females laying fertile eggs laid significantly more eggs than sterile females, 359.1 versus 98.9 (Table 10). Fertile females lived shorter lives than sterile females (14.5 versus 22.1 days) but began oviposition earlier (5.1 versus 17.3 days) and laid eggs for a longer period. Sterile females generally laid eggs only during the last 2-3 days of their lives, perhaps a sort of "last ditch" effort to oviposit before they die. Occasionally females laid 1-5 eggs before they mated.

The comparison of fertile and sterile females in Table 10 suggests that even if a female finally does mate late in her life, retention of eggs for an extended period reduces their viability or their ability to

be fertilized. Thus, the longer a female goes without mating, the fewer fertile eggs (if any) she will lay. It can not be ascertained if mating delay causes the sterility or if the sterility and mating delay are merely highly correlated and are together caused by another factor, such as inadequate pheromone production, "disease" manifestations, etc.

Nothing is reported in the cinnabar moth literature about the percent of females in the field failing to mate or, once mated, failing to lay viable eggs. The MATING BEHAVIOR STUDIES in this thesis give some indication that some females do indeed fail to attract a mate in the field. But it is not known if they fail to emit a sex pheromone or sound or if they emit a poor quality or quantity of either. Estimates of the percentage of eggs in the field that fail to hatch due to infertility and hatch failure include: 0.8-4.4% and 4.3-16.8% (Dempster 1971), 7-8% (Hawkes 1973), and 3.1% (Isaacson 1973).

Fecundity

Though it became apparent that the small oviposition cups were probably responsible for some mating failure, the experimental design was not altered for fear of biasing data for later emerging females. Table 11 presents a breakdown of the female fertility classes by diet and by egg mass. A greater mean percentage of the F diet females laid fertile eggs than did females on the other four diets. Neither diet nor egg mass, however, had a significant effect on fertility or sterility (Anovas, APPENDIX 2u-2v). There remains the suspicion that females reared on the F diet are somehow more fit or robust relative to mating and fertility than are females reared on the other four diets. Fecundity analysis was restricted to the 61% (74/121) females laying fertile eggs (ie. at least 70% of their total eggs hatched).

Females reared on the F, M and L2 diets laid significantly more eggs than those on the LS diet (Table 12 and Anova, APPENDIX 2w). Those reared on the L1 diet did not lay a significantly different number

Table 11. Comparison of relative fecundity of female cinnabar moths, Tyria jacobaeae (L.), reared on one of five diets and emerging from one of six egg masses.

Source	Replications, n	Percent of females laying eggs that were: Fertile[a] $\bar{x}$ [d] ± S.E.	Fertile??[b] $\bar{x}$ ± S.E.	Sterile[c] $\bar{x}$ [d] ± S.E.
Diet				
L1	6	51 a ± 12	0 ± 0	49 a ± 12
L2	6	51 a ± 10	16 ± 7	33 a ± 9
LS	6	62 a ± 14	0 ± 0	38 a ± 14
F	6	78 a ± 14	0 ± 0	22 a ± 14
M	6	51 a ± 8	10 ± 6	39 a ± 8
Egg mass				
1	5	37 a ± 12	13 ± 8	50 a ± 13
2	5	72 a ± 11	7 ± 7	21 a ± 12
3	5	64 a ± 12	0 ± 0	36 a ± 12
4	5	48 a ± 16	4 ± 4	48 a ± 16
5	5	72 a ± 14	0 ± 0	28 a ± 14
6	5	59 a ± 12	7 ± 7	34 a ± 11

a/ 70% or more of eggs hatched
b/ 1-20% of eggs hatched
c/ No eggs hatched
d/ Diet means followed by same letter within a column are not significantly different at the .05 level. The same follows for Egg mass means.

Table 12. Fecundity and percent egg hatch for female cinnabar moths, Tyria jacobaeae (L.), reared on one of five diets and emerging from one of six egg masses.

Source	Replications, n	Total eggs laid (fertile females only) $\bar{x}$ [a] ± S.E.	100(S.E.)/$\bar{x}$	Eggs laid per mg pupal weight	Total eggs laid (all females) $\bar{x}$ [a] ± S.E.	Percent hatch of all eggs $\bar{x}$ [a] ± S.E.	Mean percent hatch of egg batches $\bar{x}$ [a] ± S.E.
Diet							
L1	6	358.1 ab ± 18.3	5.1	2.32	235.5 a ± 38.8	92.0 a ± 3.8	91.4 a ± 2.7
L2	6	417.0 b ± 38.8	9.3	2.62	282.3 a ± 39.8	95.4 a ± 1.4	93.2 a ± 1.6
LS	6	274.0 a ± 21.7	7.9	1.91	209.4 a ± 22.7	91.1 a ± 2.7	86.2 a ± 4.2
F	6	437.6 b ± 24.6	5.6	2.61	347.2 a ± 58.5	97.0 a ± .9	96.4 a ± .9
M	6	391.4 b ± 32.1	8.2	2.42	243.6 a ± 37.8	89.7 a ± 4.5	90.2 a ± 3.4
Egg mass							
1	5	313.2 a ± 82.9	26.5	2.10	228.2 a ± 28.6	86.1 a ± 4.1	84.4 b ± 4.0
2	5	364.8 a ± 41.4	11.3	2.27	303.2 a ± 42.2	93.2 a ± 2.4	90.8 ab ± 3.9
3	5	382.0 a ± 46.0	12.0	2.32	237.4 a ± 38.7	96.9 a ± 1.2	96.3 a ± 1.8
4	5	288.8 a ± 74.3	25.7	1.82	201.8 a ± 61.8	96.6 a ± .7	96.4 a ± .8
5	5	365.6 a ± 34.6	9.5	2.37	278.4 a ± 38.4	94.8 a ± .6	90.6 ab ± 1.9
6	5	407.2 a ± 60.2	14.8	2.58	332.8 a ± 60.4	90.6 a ± 5.6	90.4 ab ± 3.5

[a] Diet means followed by same letter within a column are not significantly different at .05 level. The same applies to Egg mass means within a column.

of eggs than those on the other four diets. There were no significant differences in eggs laid per female attributable to diet if all eggs laid (fertile as well as sterile) are considered, but the same trends exist in diet effect on relative numbers of eggs laid (Table 12 and Anova, APPENDIX 2x). Females reared on the F diet laid the most eggs, followed by diets L2, M, L1, and LS.

It should be noted that the number of eggs laid per mg pupal weight (Table 12) is not fixed but is highest for the F and L2 diets, followed by the M and L1 diets and lowest for the LS diet. It is not known if F and L2 diet females laid larger eggs than did LS females. It may simply be that F and L2 diet females had a greater propotion of pupal weight available for egg production than did LS females.

The fecundities reported here are generally higher than any others reported for the cinnabar moth. Mean fecundities based on laboratory and field data fall in the high 100's to mid 200's (Cameron 1935, Dempster 1971, Stimac 1977, etc.). This may be due to real differences between populaitons at different localities through time, and laboratory versus field conditions. Philogene (1975) found that differing rearing conditions (photoperiod and temperature regimes) did not significantly affect fecundity. Generally, laboratory studies are expected to yield ideal or maximum fecundities due to lack of predation and adverse environmental effects. But one wonders how much laboratory work has been performed on the cinnabar moth in which experimental laboratory or insectary conditions or manipulations actually lowered fecundity and fertility due to adult desiccation, interference with mating due to crowding and pheromonal effects, etc. This would generally pass unnoticed by the researcher unless eggs were held for hatching to look for potential maternal stress.

Analysis of the diet experiment data indicated non-significant egg mass variability, but I suspect significant differences do exist in mean fecundities between progeny of different egg masses. This belief is justified by remembering that the pupal weight and length analyses

indicated non-significant egg mass effects for the diet experiment but significant egg mass effects for the control experiment. Again, small sample size may have influenced the lack of significance for the diet data compared to the control data. Significant pupal weight differences would be expected to yield significant fecundity differences. In view of the inconclusive data on possible significance of between-egg mass variabilty in fecundity, future research on the cinnabar moth should probably include careful design of experiments to avoid introducing bias.

Eggs laid by females reared on the F diet had a higher percent hatch than did eggs laid by females from the other diets as measured both by total eggs hatching and by mean percent hatch of the egg batches (Table 12). But these diet effects on hatch were not significant (Anovas, APPENDIX 2y-2z). Eggs laid by females from different egg masses had significantly differing hatches as measured by mean percent hatch of egg batches but not as measured by total eggs hatching (Table 12). Diet and egg mass variability did not significantly affect female longevity or duration of oviposition period (Anovas, APPENDIX 2aa-2bb).

Females laying the most eggs can be characterized as having emerged from large pupae and laying eggs over a longer period of time as indicated by correlation coefficients between various oviposition, size and pupation parameters (Table 13). Longevity is most correlated with the day number that oviposition begins after adult emergence. Regardless of pupal size, if the onset of oviposition is delayed (ie. if mating is delayed) a female will lay fewer viable eggs over a shorter oviposition period. This contrasts with Dempster (1971) who found that larger females live longer than smaller females ($r = .622$ for adult weight and life span). No significant correlation was found between life span and pupal size (which is highly correlated with adult size) in the present study (Table 13).

Table 13. Correlation coefficients for various oviposition, pupation and size parameters for female cinnabar moths, Tyria jacobaeae (L.), laying fertile eggs. Data represent 70 females reared each on one of five diets and emerging from one of six egg masses.

	Pupal weight	Days since pupation	Wing length	Total eggs laid	Number of egg batches	Day # 1st eggs were laid	Oviposition period length	Life span	Total % egg hatch
Days since pupation	.182	---	---	---	---	---	---	---	---
Wing length	.807 **	.197	---	---	---	---	---	---	---
Total eggs laid	.658 **	.172	.538 **	---	---	---	---	---	---
Number of egg batches	.380 **	.136	.364 **	.497 **	---	---	---	---	---
Day # 1st eggs were laid	-.040	-.109	-.044	-.281 *	-.123	---	---	---	---
Oviposition period length	.309 **	-.009	.227	.597 **	.668 **	-.279 *	---	---	---
Life span	.136	-.050	.101	-.084	.161	.767 **	.168	---	---
Total % egg hatch	.179	.035	.249 *	.505 **	.400 **	-.592 **	.379 **	-.434 **	---
$\bar{x}$ % egg hatch for batches	.304 *	.096	.328 **	.561 **	.455 **	-.546 **	.487 **	-.271	.871 **

* Correlation coefficient is significant at .05 level
** Correlation coefficient is significant at .01 level

The results of this oviposition study indicate that there probably exist selective advantages for a female to oviposit on certain types of tansy ragwort compared to others. These advantages relate to the quality of food that progeny will obtain and pupal size potential imparted by differing diets that will unltimately affect her progeny's fecundity. For example, larvae fed on floral parts yield larger pupae than those fed on leaves only, and larvae fed on second-year leaves will be larger than those feeding on first-year or shade-grown leaves. It is therefore adaptive for a female to oviposit on second-year plants. A diet of shade-grown ragwort imparts the lowest growth potential, so it is also advantageous for a female to select sun-grown plants over shade-grown plants.

Green (1974) showed that larvae dispersing from overloaded ragwort plants gained if they were able to successfully locate a non-crowded plant. Meijden (1976) felt that it is advantageous for a larva to risk dispersal-related mortalities if dispersal allows the larva to feed on floral parts and gain in weight and fecundity. My work confirms Meijden's hypothesis about increased fecundity imparted to the cinnabar moth by a floral diet.

The potential size and qualitative advantages in avoidance of shade-grown ragwort as a diet can also be weighed against risk in dispersal. This risk might have significant influence in areas where ragwort has been "forced" into the shade habitat (not ragwort's optimal habitat) due to effects of cinnabar populations, cultivation, grazing, etc. As a larger proportion of a cinnabar population feeds on suboptimal plant parts and suboptimal plants (ie. shade), the effect should be smaller, less fecund individuals that are possibly also less fit or robust. A greater proportion of such a larval population would probably suffer from dispersal mortality. It would be interesting to test the hypothesis: Feeding on suboptimal plants and plant-parts leads to overall reduction of cinnabar population size, fecundity and vigor. This may

lead to localized cinnabar population extinction and allow tansy ragwort to recover or prosper under reduced feeding pressure.

Stimac's (1977) model included a percentage for dispersal mortality of cinnabar larvae. For one of the years he modeled, he had to increase this dispersal mortality by a factor of about 13 to account for the low pupal population resulting from the plant biomass that year. He noted that ragwort regeneration (after defoliation) had also been poor that year. Application of my results would suggest that poor quality of ragwort may indeed have forced a higher proportion of larvae to disperse (perhaps in the earlier, more vulnerable stages) in search of better food compared to other years. This indicates a need for a food "quality" factor in Stimac's model as relates to percentage of larvae dispersing. Also, the quantity and quality of food available per successful pupator could be used to predict pupal weight and fecundity instead of simply assuming the same average fecundity every year.

Egg Laying Pattern

The basic pattern of egg deposition for each female cinnabar moth is illustrated in Figure 7; mean batch size and percent hatch decrease with time throughout the oviposition period. Cumulative eggs laid increases rapidly due to the majority of a female's eggs being laid in a few large batches early in her oviposition period (Figure 8). Increased hatch failure is therefore associated with small egg masses that accounted for about 1/3 of the batches laid (Figure 9).

Variability in clutch size is widely reported in the cinnabar moth literature (Dempster 1971, Green 1974, Meijden 1976, Myers and Campbell 1976b). Green (1974) found no differences in mean clutch size between eggs laid on rosettes and on second-year ragwort. He felt clutch size was adapted to the average amount of potential food available per average-sized plant. However, Meijden (1976) found clutch size unrelated to size

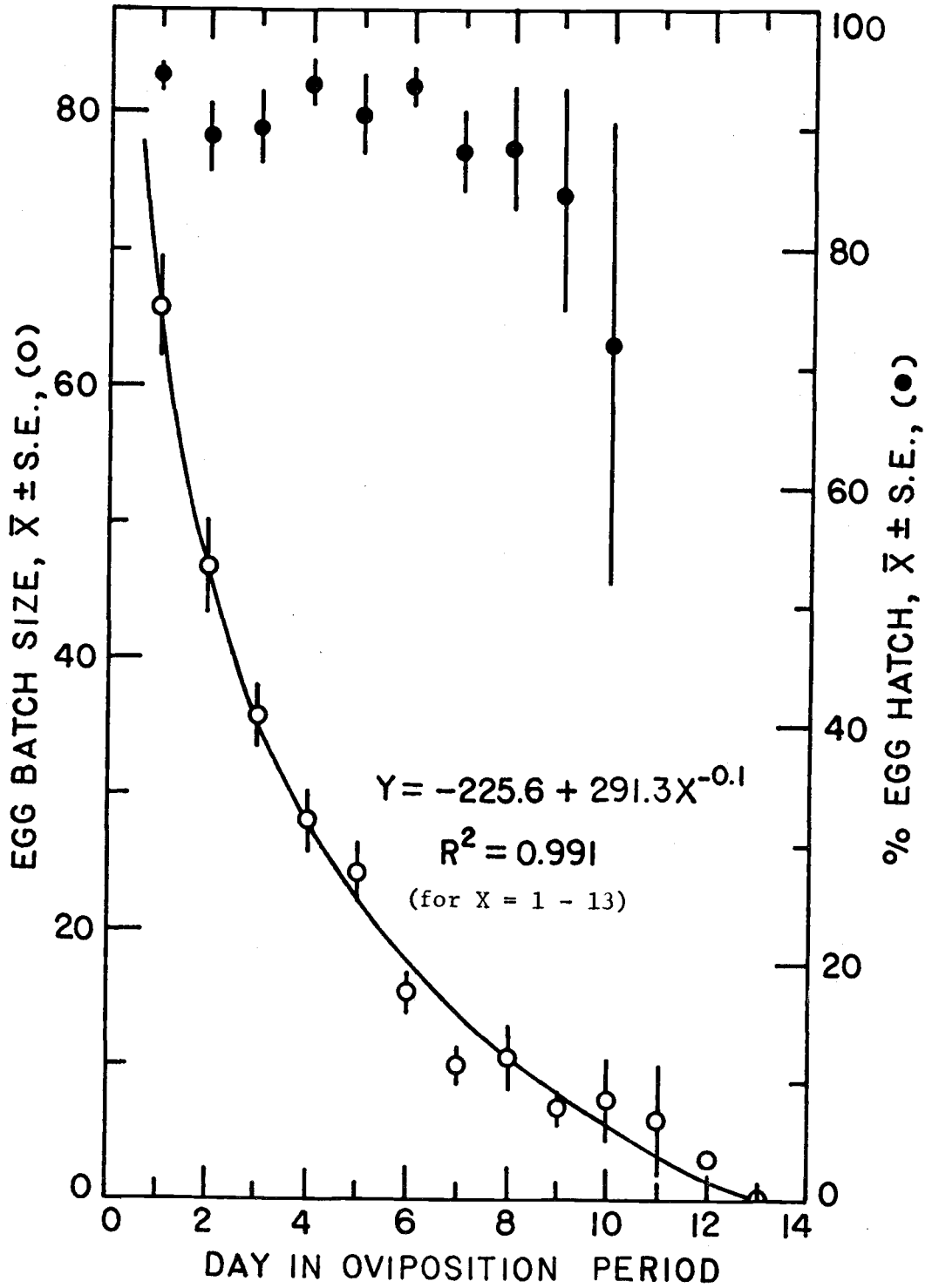

Figure 7. Batch size and hatch for eggs laid by 70 female cinnabar moths, Tyria jacobaeae (L.), and day number in oviposition period.

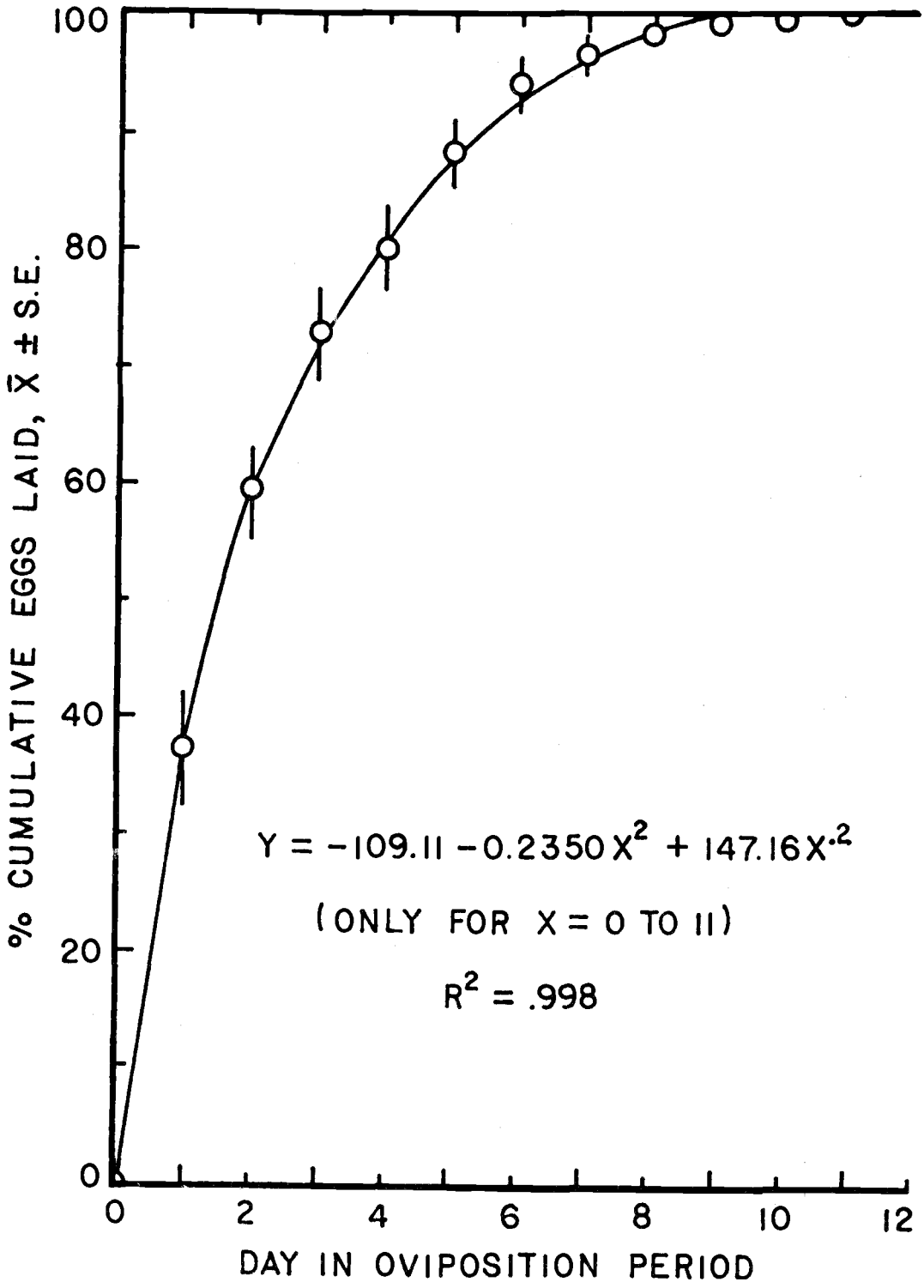

Figure 8. Cumulative eggs laid by 70 female cinnabar moths, Tyria jacobaeae (L.), and day number in oviposition period.

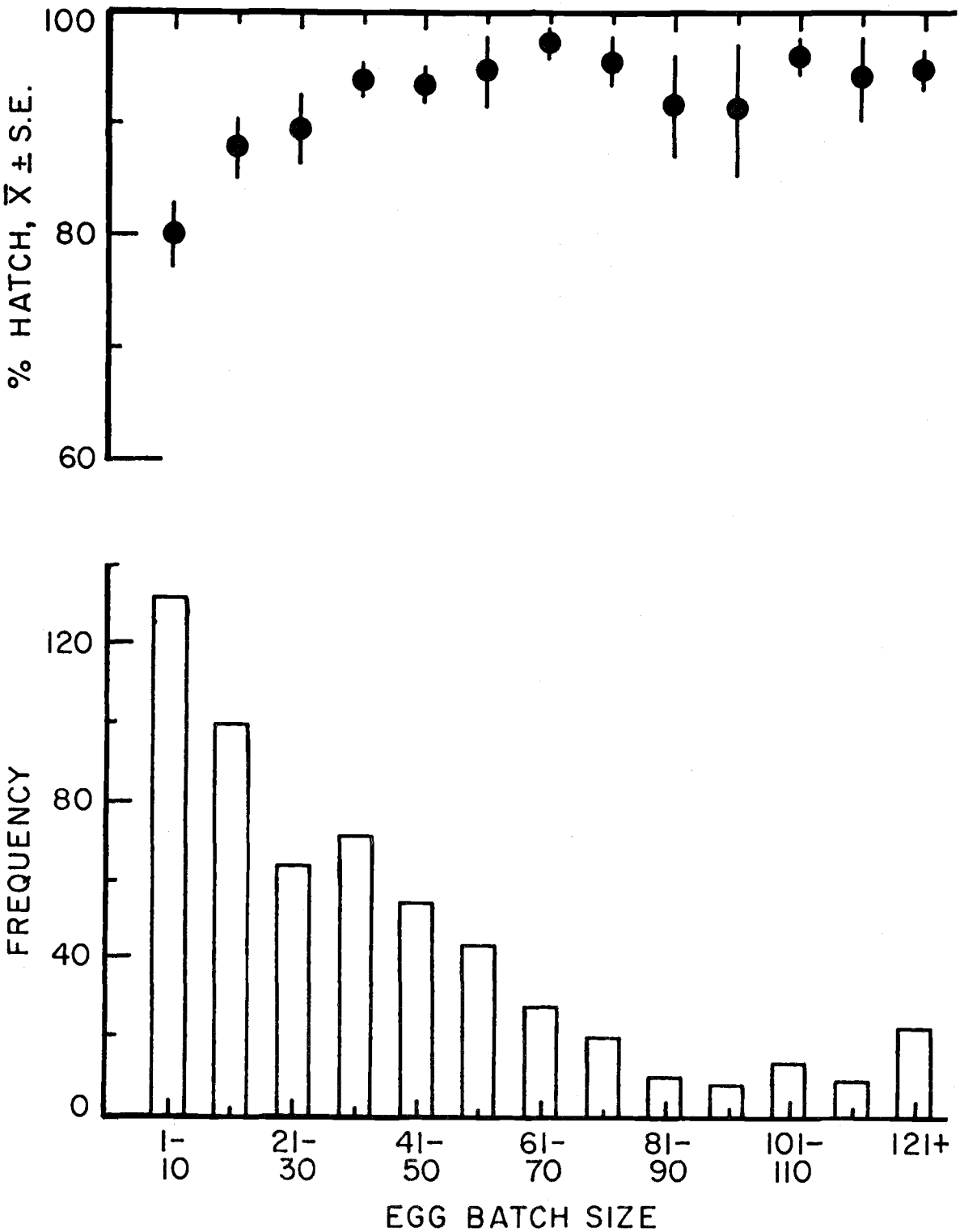

Figure 9. Frequency and hatch of different sized egg batches laid by 70 female cinnabar moths, Tyria jacobaeae (L.).

and other characteristics of the ragwort plant. He felt that the adult behavior of laying eggs in larger egg masses and the early gregarious larval habits lead to overall increased survival.

Variability in clutch size in this research would appear to be most related to the basic ovipositional behavior of the female in laying larger egg masses at the beginning of ovipositon with egg batch size steadily decreasing throughout the oviposition period to very small egg masses at the end (assuming no predation, etc.). Thus, mean batch size for each female will have a rather larger variance due to each female having laid large- and small- in addition to medium-sized egg masses.

High egg mortality (from all causes) is linked to small egg batch size (Dempster 1971, Green 1974, Meijden 1976). Survival of 1st-instar larvae is also lower for smaller egg masses than for larger ones (Green 1974, Meijden 1976). Green (1974) caged females in the field for one day, classified each to one of four relative age-classes (on the basis of wing condition), and compared the mean batch size for the four age-classes. He thus demonstrated that older females probably laid eggs in small batches and these were possibly less viable than the larger egg batches laid by younger females. Meijden (1976) also suggests that the poorer hatch-to-early-instar survival of small egg masses is related to quality. These views are substantiated by my research that shows that smaller egg masses have an inherently lower hatch than do larger egg masses, and this is related to physiology in the absence of field mortality. This decreased hatch is also related to increased delay in mating and resultant delay in oviposition. Therefore, low hatch of smaller egg masses is indeed a quality effect and influenced by the extended time the eggs are held by the female. It remains for future research to study if, or how, this quality influence is manifested in subsequent larval survival, pupal weight, and adult emergence and fecundity.

Weather influences ovipositional pattern through time for cinnabar

moths. Green (1974) suggested that eggs are not laid on poor (cold or rainy) days, and egg batches laid the day after a poor day will tend to be larger than average because the female is laying a two-day complement of eggs. Females that I had ovipositing in field cages also showed a tendency not to oviposit or move about on inclement days, but after ceasing oviposition for a day of "poor weather" they would generally oviposit the next day regardless of weather. This tendency to not postpone oviposition was especially noticeable in the first few days of a female's oviposition period. Older females that only lay eggs in small (20 eggs or less) batches are more likely to halt oviposition for two or three days, often in spite of good weather. There was also a tendency for older females to remain and oviposit on the same plant for several days, especially in poor weather. This would tend to influence egg distribution and contagion.

Green (1974) dismissed the probability of females laying more than one egg batch per day and also stated that egg laying was always in the afternoon hours. Stimac's (1977) model assumes that each female will lay five egg batches (averaging 40 eggs each) for only one week. I found oviposition beginning well before noon on warm days. In the laboratory (photoperiod 14/10 and thermoperiod 23.9°/10°) females frequently laid two or more egg batches in one day. Of 70 females, 68 deposited two egg masses on at least one day, 30 on at least two days, and 10 on at least three days. Field data for 13 females yielded 11 laying two egg masses on one day and 5 laying two egg masses on two days. The tendency to lay more than one egg mass per day occurred most often at the beginning of a female's oviposition period. Also, towards the end of a female's life, she is likely to lay more than one small (15 eggs or less) egg mass per day. Green (1974) felt that the reserve of eggs remaining after each batch was laid was insufficient for further oviposition on a given day. Higher latitude and lower overall temperatures could explain some discrepancies between Green's (1974) work and mine relative to number of batches laid per day. He did not monitor female oviposition throughtout female's lives as I did.

CONCLUSIONS

Diet can significantly affect certain aspects of the cinnabar life cycle. Specifically, pupal weight and resultant fecundity, and timing of adult emergence respond to diet manipulation. Larvae that feed on floral parts generally yield larger, more fecund adults. Diet may be viewed as a strategy to improve a growth potential that would otherwise relate mainly to maternal influences (assuming other variables such as crowding, etc. are uniform). Viewed as a sort of continuum, it is more advantageous for a larva to feed on floral parts than leaves, but in the absence of flowers, second-year leaves are more nutritious than first-year leaves. Shade-grown leaves are the least desirable food as they impart the lowest growth potential.

The increased growth potential of feeding on floral parts may be a qualitative as well as a quantitative gain. Floral fed females lay egg masses that have a higher percent hatch than egg masses from females fed on leaf diets. An application of this work is to laboratory rearings in which floral diets could be used to increase egg production or yield more robust, fecund adults for field liberation.

The behavior of a female moth in choosing second-year versus first-year ragwort for oviposition is therefore adaptive in that it tends to insure good quality, as well as quantity, of food for her progeny. Likewise, the avoidance of shade-grown ragwort as oviposition sites by females and the avoidance of feeding on shade plants by dispersing larvae is adaptive.

Diet has potential as a source of between-years feedback for a cinnabar population. Populations in the growth or colonization stage would be expected to expand and increase rapidly for a few years because there should be enough floral biomass so that the majority of the larvae can utilize this optimal food source, leading to large, fecund moths. Eventually, the population would become dense enough that more and more

larvae would be forced to suboptimal (ie. rosettes and shade-grown plants) food sources, and increased dispersal, competition and mortality would occur. Population levels fluctuating between starvation and abundant food-for-all are noted for the cinnabar moth and would appear to be influenced by food quality as well as food quantity.

It remains for future researchers to look more closely at possible diet effects on dispersal by larvae and adults. Such behavior might relate to the significantly late adult emergence found for adults reared on a floral diet compared to other diets (or early emergence on a leaf diet compared to a floral diet). Feeding on flowers may trigger some genetic mechanism that causes late emergence. This would appear to be disadvantageous because late emergers run the risk of no food for their progeny. This interesting aspect of diet should be further explored along with the indication from my research that increased larval rearing density may tend to decrease pupation periods, also leading to early emergence.

Significant variation between progeny of different egg masses was demonstrated in this research. Aspects of the life cycle found to differ between egg masses include larval and pupal weights, adult emergence timing, and possibly, fecundity. These differences were not related to size of the egg mass, but rather to some aspect of maternal (or perhaps, paternal or both) size, quality or genotype. It is not known if the increased or decreased size potential (relative to a population mean) passed to an egg mass is environmental (such as parental diet or rearing density) or a true genetic potential. If the influence was found to be substantially dietary, then diet would seem to have an important influence in the field on cinnabar population growth and dynamics.

Egg mass variability should be treated as an independent variable when setting up experiments with the cinnabar moth (assuming it is not

the variable being investigated). Design of experiments should endevor to improve reliability by properly controlling or randomizing between-egg mass variability.

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APPENDIX 1

Site Description

Sites used for egg mass collection and field work in this research are located in Linn County, Oregon (Section 33, R.1E., T.10S.) about 6-8 km S. of Jordan. (These sites are about 7-8 km S.S.W. of the cinnabar moth-tansy ragwort research site known as Silbernagel (Isaacson 1972) that is presently maintained by the Entomology Department at Oregon State University. The sites are along or just off the main Burmeister Creek road that runs due S. of Jordan. Individual site descriptions follow:

Site A: Roadside ragwort pockets along the main gravel/dirt road. Site distrubed by road traffic. Cinnabar moth has been present at this site at least since 1973. Elevation about 380 m.

Site B: Ragwort in a clearcut on a hilltop sloping S. and surrounded by low forest on E., N., and W. Clearcut is at the end of BLM road (# 10-1E-33.2) put in just prior to logging the area in 1972. Site is about 0.4 km N.E. of site A. Clearcut was reforested in spring of 1974. Cinnabar population was large enough to defoliate all ragwort in this area the summer of 1974. Large adult emergence in spring of 1975 but almost no ragwort present (apparently no larvae reached pupation that year at this site). Elevation about 450 m.

Site C: Pockets of roadside ragwort along BLM road (# 10-1E-33.1, put in same year as site B road). Area not distrubed by much traffic. Site about 0.4 km N.W. of site B. Elevation about 400 m.

APPENDIX 2

Analysis of Variance Tables

Analysis of variance tables for cinnabar moth data are listed in this appendix. The F values listed in each table can be compared with a statistical table of F values to determine (lack of) significance for differences between means. Values of F followed by * are significant at the .05 level, and ** are highly significant at the .01 level. Values of F followed by N.S. are not significant at the .05 level. Values of S.S. (Sum of Squares) and M.S. (Mean Squares) are rounded off from the numbers listed on the original computer print out. Therefore, Total S.S. and arithmetic divisions may appear to vary numerically (in thousanths) due to rounding.

2a. Effects of egg mass on 4th-instar larval weights for the control experiment.

Source	D.F.	S.S.	M.S.	F
Between Egg masses	18	1.563×10^{-2}	8.684×10^{-4}	16.386 **
Within Egg masses	470	2.491×10^{-2}	5.300×10^{-5}	
Total	488	4.054×10^{-2}		

2b. Effect of egg mass on female pupal weight for control experiment.

Source	D.F.	S.S.	M.S.	F
Between Egg masses	16	2.900×10^{-2}	1.813×10^{-3}	9.345 **
Within Egg masses	251	4.870×10^{-2}	1.940×10^{-4}	
Total	267	7.770×10^{-2}		

2c. Effect of egg mass on male pupal weight for control experiment.

Source	D.F.	S.S.	M.S.	F
Between Egg masses	16	2.090×10^{-2}	1.306×10^{-3}	10.993 **
Within Egg masses	223	2.650×10^{-2}	1.188×10^{-4}	
Total	239	4.740×10^{-2}		

2d. Effect of egg mass on female pupal length for control experiment.

Source	D.F.	S.S.	M.S.	F
Between Egg masses	16	3.407×10^{-1}	2.129×10^{-2}	12.673 **
Within Egg masses	259	4.363×10^{-1}	1.685×10^{-3}	
Total	275	7.770×10^{-1}		

2e. Effect of egg mass on male pupal length for control experiment.

Source	D.F.	S.S.	M.S.	F
Between Egg masses	16	1.994×10^{-1}	1.246×10^{-2}	10.479 **
Within Egg masses	229	2.723×10^{-1}	1.189×10^{-3}	
Total	245	4.717×10^{-1}		

2f. Effect of egg mass on mean days to emergence after pupation for adult females in control experiment.

Source	D.F.	S.S.	M.S.	F
Between Egg masses	16	5.836×10^{3}	3.647×10^{2}	4.505 *
Within Egg masses	204	1.652×10^{4}	8.096×10^{1}	
Total	220	2.235×10^{4}		

2g. Effect of egg mass on mean days to emergence after pupation for adult males in control ecperiment.

Source	D.F.	S.S.	M.S.	F
Between Egg masses	16	3.476×10^{3}	2.172×10^{2}	6.448 **
Within Egg masses	166	5.592×10^{3}	3.369×10^{1}	
Total	182	9.068×10^{3}		

2h. Effects of diet and egg mass on 1st-instar larval weights for the diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	2.459×10^{-8}	6.147×10^{-9}	2.319 N.S.
Egg mass	5	4.289×10^{-7}	8.577×10^{-8}	32.359 **
Diet x Egg mass	20	5.301×10^{-8}	2.651×10^{-9}	
Total	29	5.065×10^{-7}		

2i. Effects of diet and egg mass on 2nd-instar larval weights for the diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	7.357×10^{-7}	1.839×10^{-7}	1.497 N.S.
Egg mass	5	1.050×10^{-5}	2.101×10^{-6}	17.098 **
Diet x Egg mass	20	2.457×10^{-6}	1.229×10^{-7}	
Total	29	1.370×10^{-5}		

2j. Effects of diet and egg mass on 4th-instar larval weight for the diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	1.746×10^{-4}	4.365×10^{-5}	1.874 N.S.
Egg mass	5	2.970×10^{-4}	5.940×10^{-5}	2.550 N.S.
Diet x Egg mass	20	4.658×10^{-4}	2.330×10^{-5}	
Total	29	9.374×10^{-4}		

2k. Effects of diet and egg mass on 3rd-instar larval weight for the diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	3.362×10^{-5}	8.405×10^{-6}	3.675 *
Egg mass	5	1.485×10^{-4}	2.969×10^{-5}	12.982 **
Diet x Egg mass	20	4.575×10^{-5}	2.287×10^{-6}	
Total	29	2.278×10^{-4}		

2m. Effects of diet and egg mass on total larval survival (egg hatch to end of the 5th instar) for the diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	1.515×10^{-2}	3.787×10^{-3}	1.027 N.S.
Egg mass	5	3.315×10^{-2}	6.270×10^{-3}	1.701 N.S.
Diet x Egg mass	20	7.373×10^{-2}	3.687×10^{-3}	
Total	29	1.202×10^{-1}		

2n. Effects of diet and egg mass on female pupal weight for diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	1.924×10^{-3}	4.810×10^{-4}	3.702 *
Egg mass	5	7.117×10^{-4}	1.423×10^{-4}	1.095 N.S.
Diet x Egg mass	20	2.599×10^{-3}	1.229×10^{-4}	
Total	29	5.235×10^{-3}		

2p. Effects of diet and egg mass on male pupal weight for the diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	1.310×10^{-3}	3.274×10^{-4}	2.909 *
Egg mass	5	4.571×10^{-4}	9.140×10^{-5}	0.812 N.S.
Diet x Egg mass	20	2.251×10^{-3}	1.126×10^{-4}	
Total	29	4.018×10^{-3}		

2q. Effects of diet and egg mass on female pupal length for the diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	1.220×10^{-2}	3.051×10^{-3}	3.850 *
Egg mass	5	6.487×10^{-3}	1.297×10^{-3}	1.637 N.S.
Diet x Egg mass	20	1.585×10^{-2}	7.924×10^{-4}	
Total	29	3.454×10^{-2}		

2r. Effects of diet and egg mass on male pupal length for the diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	1.275×10^{-2}	3.189×10^{-3}	4.474 **
Egg mass	5	4.252×10^{-3}	8.504×10^{-4}	1.194 N.S.
Diet x Egg mass	20	1.425×10^{-2}	7.125×10^{-4}	
Total	29	3.125×10^{-2}		

2s. Effects of diet and egg mass on mean days to emergence after pupation for adult females in the diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	6.420×10^{2}	1.605×10^{2}	6.274 **
Egg mass	5	1.026×10^{3}	2.053×10^{2}	8.025 **
Diet x Egg mass	20	5.116×10^{2}	2.558×10^{1}	
Total	29	2.180×10^{3}		

2t. Effects of diet and egg mass on pupal weight retention after 244-250 days pupation period for the diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	4.836×10^{-3}	1.209×10^{-3}	1.939 N.S.
Egg mass	5	1.200×10^{-3}	2.400×10^{-4}	0.385 N.S.
Diet x Egg mass	20	1.247×10^{-2}	6.236×10^{-4}	
Total	29	1.851×10^{-2}		

2u. Effects of diet and egg mass on percentage of females laying fertile eggs (ie. 70% or more hatched) for the diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	3.425×10^{5}	8.562×10^{4}	1.029 N.S.
Egg mass	5	4.720×10^{5}	9.441×10^{4}	1.135 N.S.
Diet x Egg mass	20	1.664×10^{6}	8.318×10^{4}	
Total	29	2.478×10^{6}		

2v. Effects of diet and egg mass on percentage of females laying sterile eggs for diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	3.266×10^{5}	8.166×10^{4}	0.887 N.S.
Egg mass	5	3.140×10^{5}	6.281×10^{4}	0.682 N.S.
Diet x Egg mass	20	1.841×10^{6}	9.205×10^{4}	
Total	29	2.482×10^{6}		

2w. Effects of diet and egg mass on fecundity of fertile females for diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	9.856×10^{4}	2.464×10^{4}	4.405 *
Egg mass	5	1.424×10^{4}	2.849×10^{3}	0.509 N.S.
Diet x Egg mass	20	1.119×10^{5}	5.594×10^{3}	
Total	29	2.247×10^{5}		

2x. Effects of diet and egg mass on fecundity of all females (fertile as well as sterile) for the diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	6.877×10^4	1.719×10^4	1.789 N.S.
Egg mass	5	6.154×10^4	1.231×10^4	1.281 N.S.
Diet x Egg mass	20	1.922×10^5	9.611×10^3	
Total	29	3.225×10^5		

2y. Effects of diet and egg mass on percent hatch of all eggs laid by each fertile female for the diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	2.285×10^4	5.714×10^3	1.267 N.S.
Egg mass	5	4.188×10^4	8.375×10^3	1.858 N.S.
Diet x Egg mass	20	9.016×10^4	4.508×10^3	
Total	29	1.549×10^5		

2z. Effects of diet and egg mass on mean percent hatch of egg batches laid by each fertile female for the diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	3.395×10^4	8.488×10^3	2.486 N.S.
Egg mass	5	4.943×10^4	9.887×10^3	2.895 *
Diet x Egg mass	20	6.830×10^4	3.415×10^3	
Total	29	1.517×10^5		

2aa. Effects of diet and egg mass on longevity of fertile females for the diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	1.120×10^1	2.800×10^0	0.293 N.S.
Egg mass	5	5.327×10^1	1.065×10^1	1.116 N.S.
Diet x Egg mass	20	1.909×10^2	9.543×10^0	
Total	29	2.553×10^2		

2bb. Effects of diet and egg mass on duration of oviposition period for fertile females in the diet experiment.

Source	D.F.	S.S.	M.S.	F
Diet	4	2.051×10^{1}	5.127	1.815 N.S.
Egg mass	5	2.438×10^{1}	4.876	1.726 N.S
Diet x Egg mass	20	5.651×10^{1}	2.825	
Total	29	1.014×10^{2}		