

**An Environmental
Assessment
of
Aphthona abdominalis**

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Abstract

Leafy spurge is a long-lived herbaceous perennial weed that was first introduced into the United States circa 1827. Currently there are no satisfactory means of controlling leafy spurge, and it is left to spread unchecked and to displace native flora. Attempts have been made to control leafy spurge with herbicides, but they are only temporarily effective because they fail to destroy the roots. The moist parts of the leafy spurge infestations are particularly sensitive as picloram, the most commonly used herbicide, is highly water soluble and leaches into streams and ponds.

At this time, the most effective biocontrol agents for leafy spurge are those that attack its root system. *Aphthona abdominalis* does this successfully. This insect has a narrow host range and its life cycle is coupled with that of leafy spurge. For these reasons, the release of *Aphthona abdominalis* in the United States is recommended.

Introduction

Leafy spurge is a noxious perennial weed on the Great Plains of the United States and on the prairie provinces of Canada. It is a non-native plant and was not known in the United States until 1827 (Thompson, *et. al.*, 1990). Because it is an introduced plant, leafy spurge has no native natural enemies. The plant is primarily found in non-tilled agricultural land (pasture, rangeland, hayland, and idle cropland) but it is also found along roadsides, river banks, flood plains, ridges, and mountain slopes (Bangsund, *et. al.*, 1991). This noxious weed restricts native plant growth and is not eaten by cattle unless it is given to them in weedy hay or if no other forage is available (Rees and Spencer, 1991). Leafy spurge also produces a toxic latex. The latex causes scours and blisters in cattle, and in large amounts, death. In humans, it causes dermatitis and blisters, and overexposure may lead to blindness. For these reasons, leafy spurge is a serious problem for farmers and ranchers. The area of greatest infestation in North America is defined by a 1,200 mile-diameter circle, centered near Wolf Point, Montana (Spencer, 1990). The circle encompasses parts of 9 states and 5 Canadian provinces and covers nearly 2.5 million acres. The greatest infestations are located in Montana, North Dakota, South Dakota, and Wyoming. The total negative economic impacts in the four states could reach over \$144 million annually by 1995 (Bangsund *et. al.*, 1991). Due to the cost of herbicides and the threat that they pose to the environment, an urgent need has developed to find alternative control methods. Biological control seems to be one answer. The Agriculture Research Service (USDA/ARS) has been researching more effective ways to control leafy spurge for almost twenty years. Nine insects, brought to the US. from the native lands of leafy spurge, have been released in the United States to control leafy spurge, and more are being studied for future release. Several of these biological control agents have already made significant impacts on the spread of leafy spurge. Unlike herbicides, biocontrol agents kill the spurge without causing harm to other plants or animals located in the release areas. In sites where leafy spurge flea beetles were released in 1989, 1990, and 1991, improvements can be readily observed. In the middle of many infestations where flea beetles have been released, large, clear, circular areas have appeared. These areas,

called depressions, show the positive impact of biocontrol agents on leafy spurge infestations. Introduced biocontrol agents are increasing in numbers in the field. Local, state, and federal land owners are excited about this technological method of leafy spurge control. Some of these introduced biocontrol agents are now being released in thirteen states.

Proposed Release

GOALS

The main goal of the proposed release is the establishment of *Aphthona abdominalis* to provide additional control of leafy spurge.

PROCEDURES

A site for release is first chosen based on three main groups of site characteristics. Once a site is chosen, the insects will be released into the area, and then monitored to determine their establishment, effectiveness, and survival rates.

SUMMARY OF SITE CHARACTERISTICS

A site is chosen based on three sets of parameters; physical, biological, and cultural.

- ◆ **Physical:** soil texture, soil moisture, risk of flooding, topography, direction of slope, estimated bare ground at site, annual precipitation
- ◆ **Biological:** weed density, whether the infestation is continuous or interrupted, the amount of ground area shaded by plants, typical mature weed height, trees or shrubs in the release site and surrounding area, amount of shade from shrubs and trees, size of weed infestation
- ◆ **Cultural:** current land use, herbicides applied within the last two years, weed treatments within the last twelve months

Purpose and Need

SIGNIFICANCE OF ACTION

Leafy spurge is a noxious perennial of the Northern Great Plains of the United States. It is hardy, resists control, and forms dense stands that replace grasses and forbs and restrict cattle grazing (Rees and Spencer, 1991). Leafy spurge reproduces by both seeds and vegetative root buds (Spencer, 1991) and therefore has an exceptional ability to thrive and spread. These characteristics have made leafy spurge a serious problem for farmers and ranchers. The most serious infestations are located on the prairies where, because of its deep roots system, it has become the dominant plant on the open sandy soils, displacing native flora and having a corresponding negative impact on native fauna. It also survives, however, on heavy moist soil and in shaded areas. Because of this and climatic reasons, biocontrol agents are the most successful means of controlling leafy spurge.

Leafy spurge produces a toxic latex. This milky substance is poisonous to cattle and to man. In cattle, the latex causes scours and blisters, and in large amounts death (Rees and Spencer, 1991). In humans it causes dermatitis, and blisters, and overexposure may lead to blindness. The continued spread of leafy spurge into grazing and recreational lands is undesirable.

leafy spurge also displaces native plants. The western prairie fringed orchid, *Platanthera praeclara* is one such plant (Gassmann, 1990). It has received threatened status in the United States and remains in danger of leafy spurge invasion. *Platanthera praeclara* is not only forced out of its habitat by leafy spurge, but it is killed by the herbicides used to stop the spread of leafy spurge.

The proposed solution is to use biological control to limit the spread of leafy spurge. *A. abdominalis* would be used in combination with other biocontrol agents to achieve this control.

ALTERNATIVES TO PROPOSED ACTION

Leafy spurge can be controlled through the use of herbicides, but long-term control is very difficult to achieve. Herbicides commonly used in the control of leafy spurge are 2, 4-D, picloram, and dicamba (Lym, 1991a). On non-arable land, picloram is the most persistent and effective herbicide available and re-treatment may not be necessary for 3-5 years (Lym and Whitson, 1991). However, picloram is expensive, extremely persistent, mobile, and kills a broad spectrum of plants. Picloram is highly water soluble, leaches into streams and ponds, and has been known to kill trees. Because of this, the present large scale use of picloram is ecologically undesirable. The best chemical options left are 2, 4-D, and dicamba. However, these herbicides fail to kill the roots of established plants and have to be reapplied every 1-2 years (Lym and Whitson, 1991). Also, large amounts of dicamba harms native forage production. There is an urgent need to develop an alternative to the use of picloram and other herbicides to control the spread of leafy spurge on non-arable land. A much more economical and environmentally acceptable means of controlling this noxious weed would be through biological methods of control.

GOALS OF THE PROGRAM

The goal of this project is to successfully control leafy spurge with the European flea beetle, *Aphthona abdominalis*.

Description of Proposed Release Organism

TAXONOMY

- Order: Coleoptera
- Family: Chrysomelidae
- Subfamily: Alticinae
- Tribe: Aphthonini
- Genus: *Aphthona* Chevrolat
- Species: *Aphthona abdominalis* Duftschmid

The genus *Aphthona* Chevrolat is in the tribe Aphthonini of the Chrysomelid family Alticinae. This genus, comprising about 180 species, is cosmopolitan.

According to Fornasari (unpublished data) *A. abdominalis* is a yellow species that is smaller than *A. cyparissiae*, *A. flava*, *A. nigriscutis*, *A. lacertosa* and *A. czwalinae*.

DISTRIBUTION

The distribution of *A. abdominalis* includes northern and central Italy, Spain, France, southern Poland, Austria, eastern Yugoslavia, the Balkans, Naxos, Hungary, Romania, Bulgaria, southern Soviet Union, Asia Minor, and northwestern Iran (Fornasari, unpublished data).

BIOLOGY

Females lay eggs underground on the plant or in the soil very close to the plant. The larvae move to a root upon emergence and feed on and in the root. Moving from root to root during the larval stage. This species may have 3 or more generations during the summer in North America. Upon hatching from pupae in the soil, the adults feed on the leaves of the plant. The females migrate to the soil to lay eggs.

FIELD HOSTS

Most of the flea beetles in the genus *Aphthona* Chevrolat are confined to species in the genus *Euphorbia* as host plants. *Aphthona abdominalis* has been recorded on *E. cyparissiae*, *E. paralias*, *E. seguieriana*, *E. stricta*, and *Euphorbia* sp. *A. abdominalis* was found on *E. esula* in Italy (Fornasari, unpublished data).

NON - TARGET HOST ORGANISMS

It should be noted that *A. abdominalis* is very host specific and will not attack crops. Grasses, crops, and other plants found in the release areas will not be affected.

Description of Target Organism

TAXONOMY

- Order: Geraniales
- Family: Euphorbiaceae
- Genus: *Euphorbia* L. 1737
- Subgenus: *Esula* Pers.
- Section: *Esula* (Roeper) Koch
- Subsection: *Esulae* Boiss.
- Species: *E. esula* L. (sensu lato) (2n=60); leafy spurge.

Leafy spurge is an introduced species in North America. Native to the Caucasian region, *E. virgata* is a southeastern European-Asiatic species that occurs from eastern Austria and Czechoslovakia to central Asia. The taxonomic status of the introduced North American leafy spurge complex is in a state of confusion. In Europe, there are 105 native *Euphorbia* species in the subgenus *Esula*, the group to which leafy spurge belongs. In North America, there are only 21 native species in the subgenus *Esula* (Muemscher, 1940). Variations in the leafy spurge genotype in North America resulting from new gene combinations and natural selection and adaptation may affect biotic agents introduced from Eurasian areas where these genotypes do not occur. Even more perplexity is added when one considers that this weed may have been introduced from multiple sources throughout Eurasia (Rees and Spencer, 1991).

RELATED ECONOMIC AND NATIVE PLANT

Host specificity tests with the candidate agent are used to determine whether or not it has a restricted host range. If the host range shows a predictable pattern this means that the plants outside of the susceptible group are not at risk (Gassmann, 1990). Plant species will only be attacked if:

1. they occur inside of the climatic region and habitat required of the agent
2. they provide the right structures
3. they occur above a minimum threshold density

The purpose of biocontrol agents, such as the proposed *A. abdominalis*, is to reduce the host to a few scattered plants. Because of this we must be concerned with economic plants acceptable to oligophagous agents as they are often grown in large monocultures. A few scattered plants are generally not at risk unless they occur in the same habitat or close to a large infestation of the target species.

Economically Important Species

The economically most important *Euphorbia* in North America is *E. pulcherrima* Willd. (subgenus *Poinsettia*). It is a perennial which is propagated from cuttings as a Christmas pot plant. This trade has an annual value of \$54 million. Feeding tests on *E. pulcherrima* showed no feeding by *A. abdominalis*.

E. polychroma Kern. (subgenus *Esula*) is a novelty European perennial that in North America is mostly grown from seed as an annual bedding plant. It is not of major economic importance and scattered garden plants are unlikely to be at a high risk from a biocontrol agent.

E. oblongata Griseb. (subgenus *Esula*) is a European annual that has become a waif in California. It is not cultivated and does not require special consideration.

E. antisiphilitica Zuccar. (subgenus *Agaloma*) is a perennial that produces a high quality wax. It is the basis of a small industry in northern Mexico with an annual value of \$1 million. The plant is a tough xerophyte that produces only a few scale like ephemeral leaves. It does not survive in regions with winter frost and so occurs south of the distribution of leafy spurge.

Although there was some adult feeding and survival on *E. antisiphilitica*, no larvae survived on *E. antisiphilitica* in larval survival tests.

Threatened and Endangered Species

Currently the main cause for concern over the introduction of agents for the biocontrol of leafy spurge is the native *Euphorbia* species, especially those in the subgenus *Esula*. The United States Endangered Species Act of 1973 requires that special consideration be given to species designated in the Federal Register as endangered (LE), or threatened (LT) before biocontrol agents can be released into the United States. Category 2 is an entry level and after investigation, the species is moved into Category 3 (not threatened or

endangered) or to Category 1 (species for which there is substantial evidence to support biological susceptibility).

There are only three endangered or threatened native spurges in the United States. These species are *E. deltoides* spp. *deltoides* (endangered), *E. garberi* (threatened), and *E. skottsbergil* var. *kalaeloana* (endangered). The first two species belong to the subgenus *Chamaesyce* and are found in Florida and the last species is native to Hawaii. Eighty percent of the taxa in category 1 (endangered) are Hawaiian and the remainder occupy other habitats.

There are 21 Category 1 spurges in Hawaii which are not at risk to agents released on the mainland. There are also nine Category 1 spurges found on the continent (*E. hooveri*, and four varieties of *E. porterana*). All of these are in the subgenus *Chamaesyce* and are southern USA species. There are nine species in Category three on the North American mainland (one in the subgenus *Esula*, six in *Agaloma*, and two in the subgenus *Chamaesyce*). However, only two of these are sympatric with leafy spurge. Lastly, there are seven species in Category two (subgenus *Esula*, *E. purpurea*, and *E. telephiodes*, and five in *Chamaesyce*). Of these, two are sympatric with leafy spurge: *E. purpurea* (subgenus *Esula*) and *E. fendleri* (subgenus *Chamaesyce*).

The number of American species included in the five subgenera of *Euphorbia* is:

- Agaloma: 26
- Chamaesyce: 58
- Esula: 21
- Poinsettia: 3
- Euphorbium: 0

According to this list, in terms of species numbers, the most important subgenus is *Chamaesyce*. This subgenus is also the most important in terms of rare species. However, all species (except 2) that are under legal review for legal protection are southern US species. Although it is preferable to test all rare plant species or those under review, only those plants which are likely to be at risk need to be tested. The plants that may be at risk are only those plants that occur in the habitats suitable for the beetle's survival.

According to Gassmann, 1990, the most likely native spurges to be attacked by agents introduced to control leafy spurge, based on taxonomy, are in the subgenus *Esula*. *E. roemerana*, which is listed in Category three, occurs in Texas. Two other species, *E. purpurea*, and *E. telephiodes*, are Category two. *E. purpurea* is a widespread but poorly known perennial of swampy woods in the mideastern United States. This region is not usually a leafy spurge habitat and this habitat is not conducive to that required by *A. abdominalis*. The other species, *E. telephiodes*, is an abundant perennial in a restricted pine savanna habitat in southern Florida. This area is also an unlikely area for the establishment of *A. abdominalis* as *A. abdominalis* prefers open sites.

E. skottsbergi var. *kalaeloana* is indigenous to the Hawaiian Islands and are therefore not at risk to the proposed release.

Not much information is available *E. purpurea*. Hence, it is listed as a Category 2. However, *E. purpurea* is found in a wide variety of areas. It can be found from Ohio to Delaware, and south to North Carolina. The wide range of *E. purpurea* should decrease its vulnerability. It is a species of swampy woods and thickets, and these are not the habitats of leafy spurge or *A. abdominalis*.

E. maculata is a common weed of lawns, gardens, and waste ground. It is poisonous to livestock and can lead to photosensitization. It is a problem weed in other parts of the world where it has been introduced. Therefore, a reduction in its numbers would be welcomed. Although *E. maculata* L. proved suitable for *A. abdominalis* under no-choice conditions, this does not necessarily indicate that this plant would be acceptable in natural situations.

There is only one species in Section *Chamaesyce* which is endangered. There is no indication that the proposed release poses a risk to any of the species in this section. Six of the taxa (one endangered, one threatened, and four in Category 1 are restricted to Southern Florida and Alabama. One, in Category 2, is restricted to the southeast coast of the US.. It is unlikely that the beetle would spread into the geographic range of most of these species, and would not invade their habitats. The remaining two *Chamaesyce* taxa, *E. atrococca* and *E. remyi*, (Category 1) are Hawaiian species and thus are not in danger of agent released on the mainland.

There are no species of Sections *Poinsettia* or *Agaloma* which are endangered.

There are no "endangered" or "threatened" species in Section *Tithymalus*. *E. telephioides* is native to Florida and is a Category 2 taxon. *E. austrina*, also of southern Florida, is a 3B taxon. The south Texas *E. roemerana* has been found not to be endangered.

Native Species

E. marginata supported larval development and showed regular adult feeding.

Although *E. lathyris* did support a small percentage of both larval and adult feeding, it is unlikely that this will cause any serious harm to this plant. *E. lathyris*, which was once proposed as a potential use for petroleum production in North America, is not at risk from *A. abdominalis*. *E. lathyris* can only be grown as a garden annual in the North American steppic biom. If it is ever grown commercially in North America, insects introduced for leafy spurge control would pose little threat to *E. lathyris*.

E. abdominalis did show regular feeding on *E. milii*, however, it did not support any larval development. Because of this, there should not be any serious risk to the *E. milii* population.

There was no feeding damage by *A. abdominalis* on the widespread annual *E. spatulata*.

DISTRIBUTION

In continental Europe, leafy spurge is found as far south as central Spain, Italy, and the Balkans, and extends eastward through central Russia into Siberia (Lym, 1991b). In North America, the distribution occurs primarily in the Northern Great Plains. Leafy spurge is practically absent south of 40 degrees north latitude, and almost no 'economic' or 'potentially economic' infestations are found east of the Mississippi River. The most widespread infestation in the US. occurs in Minnesota, but the weed problem is the most severe in North Dakota, followed closely by Montana. It is estimated that about 90% of the leafy spurge in North America may be found within 1000 km of Wolf Point, a small town in northeastern Montana (Spencer, 1990).

ECOLOGY IN NATIVE REGION

Leafy spurge grows on many different types of terrain. It can be found on river banks, sflood plains, grasslands, ridges, and mountain slopes, but it is mainly found in untilled, non-cropland areas such as pastures, rangeland, and roadsides (Lym, 1991b). It also grows in wide variety of environments including dry, subhumid, subtropic, and subarctic (Lym, 1991b). For initial infestation, leafy spurge tends to occupy sites with a high sand content but once introduced into an area, the spurge appears to have no problems adapting and begins its invasion.

BIOLOGICAL CHARACTERISTICS

Rees and Spencer (1991) state that leafy spurge is a herbaceous perennial that spreads by both roots and seeds. It is spread along roadsides by grading and gravelling and the seed itself can be thrown up to 5 meters by the explosive force of the capsule (Rees and Spencer, 1991). Long distance dispersal is by birds and other animals.

The maintenance of a spurge stand is by vegetative reproduction and seed is of little consequence (Gassmann, 1990). The role of seed is the establishment of new stands and the return of old stands after they have been killed by herbicide treatments (Gassmann, 1990). Seed reduction by a biocontrol agent would be beneficial but since spurge is also spread vegetatively on roads and other equipment, spurge is relatively seed independent.

MORTALITY FACTORS

Leafy spurge is sensitive to root damage and is therefore susceptible to *A. abdominalis* as the larvae feed on the roots of spurge. The feeding weakens the spurge's defense mechanisms and makes it more vulnerable to native plant diseases. *A. abdominalis* should be an effective biocontrol agent as it causes considerable damage to the shoots, shoot buds, and roots of leafy spurge.

There are no known native predators or parasites of leafy spurge because it is not a plant species native to North America. The latex that spurge produces is a natural barrier that keeps most grazing animals away (Lym, 1991b). Cattle will usually not eat leafy spurge unless it is given in weedy hay or better forage is not available. Although sheep and goats will eat leafy spurge, they fail to completely kill leafy spurge because they do not destroy the roots. Only the upper seed producing area is eaten, and the spurge is still able to

spread and grow again. The grasshopper is the only insect known to consume spurge but it only happens in times of drought (Gassmann, 1990). The only known organisms able to kill leafy spurge are those that have been introduced to do so.

Research in Support of Release¹

COUNTRY OF ORIGIN FIELD INVESTIGATIONS

Life History

Observations were made on all of the stages of the flea beetle and on their development, focusing on the key aspects of development and influence of abiotic factors.

Eggs

The pre-eclosion period and the degree of fertility were recorded for eggs laid by adults reared on *E. esula* in the laboratory garden, where the eggs were kept in the dark at ambient temperatures and humidity. Groups of twenty newly laid eggs (less than five hours old) were collected with a camel hair brush and placed in 35 ml plastic cups which were provided with a layer of moistened plaster on the bottom and covered with a nylon net to protect them from predators. Each cup represented a replicate. A total of 2658 eggs were distributed in 133 cups, and were tested during the oviposition period. Checks were made twice daily and the number of hatched or collapsed eggs was recorded. Testing began during early March, when the first adults appeared, and finished at the beginning of December, 1988, when the adults began to diapause. The size of the eggs was also measured.

Preliminary observations conducted during 1987 on 150 eggs kept under different humidity conditions showed the need for high humidity to hatch, similar to that of their natural habitats in the soil near the plants. The eggs hatched from May to October. Eggs laid during April and after October 20 did not hatch. The incubation period, under the experimental conditions, lasted from a minimum of three days during June, July, and August, to a maximum of 16 days during May. These differences were due to the different conditions of temperature and humidity in the micro-environment. The lowest mortality was recorded during May, June, and July. The optimum temperature for egg development was during July, when a mean of 25.2 +/- 3.6 °C, range 16-34°C, was recorded. During this period, the eggs hatched, on an average, within 4.7 +/- 0.7 days (r= 3-6 days) and the mortality of eggs was only 1.9 %.

Larvae

Preliminary observations were conducted during 1986 on the behavior of larvae on *E. esula*. Since a colony of *A. abdominalis* was established on leafy spurge plants in the Rome laboratory garden, the aerial and terrestrial parts of ten potted plants were examined on May 9 and July 22, 1986. In addition, two laboratory trials were also made during

¹ Information in this section obtained from unpublished data from Fornasari & Pecora, USDA/ARS/EBCL Montpellier, France.

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¹ All information in this section was obtained from Luca Fornasari of ARS/BCW Lab. -

September by placing neonate larvae on root shoots, root buds, and pieces of roots on leafy spurge. Thirty neonate larvae (one larva per piece of plant part) were tested for each plant part and equally distributed in glass vials, gelatine capsules, and Petri dishes. Each dish was provided with moist blotting paper at the bottom, and kept at a constant temperature ($25 \pm 0.5^\circ\text{C}$). Plant parts were dissected at four to six day intervals and living larvae were transferred onto fresh plants.

Development of the larvae

To investigate development of larvae and determine the number of instars, neonate larvae were placed on selected healthy potted leafy spurge plants (pots 14 cm diameter). Ten larvae were placed on the collar of each plant, 1-3 cm below the soil level. A total of two hundred and twenty plants and 2,200 larvae were used. Each day five plants that had been infested for three days were dissected and the soil that they were growing in was examined. The study was conducted from June 5 to August 24, 1988, in a laboratory greenhouse where temperatures and relative humidity were $22.1 \pm 5.0^\circ\text{C}$ and $60.8 \pm 31.5\%$.

In the laboratory, the preliminary feeding tests with first instar larvae on different parts of the plants suggest that they develop on root buds, roots, shoot buds, and underground shoots. Five days after the beginning of the experiment, living larvae were found in the inner part of young adventitious shoots. Soon after eggs hatch, neonate larvae move rapidly downwards in the soil, attempting to penetrate into the plant through clefts and openings of the root bark, or through feeding on shoots or on the tender parts at the axils of root buds. In the laboratory, the larvae did not remain inside the same plant part, but exited and penetrated repeatedly into new parts. The same kind of damage on shoots, adventitious subterranean stem buds, and roots was found on the leafy spurge plants heavily infested by *A. abdominalis* in the garden. On ten potted plants dissected, about 50 shoots were attacked.

The larvae were found feeding mainly on subterranean shoots and young roots. Most of the first instar larvae and second and third instar larvae penetrated and fed inside shoots, root apices, and root buds. There were three larval instars. Larval development required 18 to 21 days. One larva was found and observed during the molt to the third instar. At 8:00 a.m. its cephalic cuticle was separated in two parts along the suture, and the larva pushed on the soil and stones, moving backward and lifting up its head, trying to remove the old head capsule. After a few minutes it managed to remove the old head capsule, exposing the new, completely white head capsule. The new head width was 247 micrometers. Larvae that hatched from eggs at the end of October or beginning of November did not survive.

Pupae

To study the development of pupae to adults, pupae less than 12 hours old were searched for in the soil of 200 pots containing plants previously infested with ten larvae each. The pupae were kept individually until adults emerged. Development from pupa to adult required on an average 10 to 11 days at a temperature of 20 to 21°C.

Adults

Studies were conducted on over 2,000 adults. Data on pre-oviposition and oviposition periods, egg production, daily activity, and number of days of survival were obtained on 100 adults that were collected on leafy spurge plants in the Rome laboratory garden on April 4, 1986. These adults were equally distributed in 50 cylindrical Plexiglas cages (10 cm high, 7 cm diameter) whose tops were tightly covered with a fine mesh nylon net. Vials with bouquets of leafy spurge were introduced through holes at the bottom of these cages. Inspections were made every three to four days, bouquets were replaced with fresh ones, eggs were counted and collected, and the number of living and dead individuals was recorded. Observations were conducted on these adults throughout their life span until they died, during the autumn. This trial, which started on April 4 and ended on September 9, was conducted in an outdoor insectary where temperature and humidity were recorded.

From the end of March to the beginning of December, 1986, *E. esula* was inspected at regular intervals in a field at San Rossore (Pisa), and in the laboratory garden (plants coming from San Rossore). Six hundred and forty-two adults were collected in the garden at the beginning of October, 1986 to conduct observations on their ability to overwinter. They were placed in 32 plastic containers (11 x 11 x 16 cm), with a 5 cm layer of soil, plant debris, and wood chips as shelter. The containers were provided with aeration holes and kept in an outdoor insectary.

The oviposition behavior was investigated on potted plants of leafy spurge. On July 29, 1986, ten pots (10 cm diameter) with *E. esula* from San Rossore were caged with ten ovipositing adults each (15 to 20 days old). The plants were inspected on August 7, 1986 and the number of eggs was recorded. Observations on the feeding behavior were carried out on four groups of ten insects each placed in transparent plastic tubes (60 cm high 20 cm diameter) on larger potted plants (22 cm diameter).

To observe adult oviposition and feeding behavior in more detail, 12 plants of *E. esula* were placed in special cages constructed for this purpose. Roots with soil and the lower part of the stem were sandwiched between two glass panes (16 x 31 cm with a Plexiglas frame inside to separate the glass and provide space for the soil and roots. The frame was made of Plexiglas in order to provide transparency and perfect closure, since the insect is very small. The plant stem passed through a hole at the top of the frame so that the aerial portion was outside the cage. Ten ovipositing *A. abdominalis* adults (15 to 20 days old) were placed in each cage and observed at regular intervals throughout each day. Ten days later, the cages were opened to search for oviposition sites and the number of eggs laid was recorded.

Feeding of Adults

A specific study was conducted on the feeding behavior and the amount of feeding throughout the life of adults. For this purpose, 102 newly emerged adults (emerged between June 9 and November 1, 1988) were caged in groups of the same age in 13 transparent plastic cages with the top covered with a nylon screen to provide aeration.

Also, 42 newly emerged adults were caged individually, and their feeding throughout their life was recorded. Another eighteen adults were collected in the field on March 10 to 15 to observe and compare their feeding behaviors with adults newly emerged from potted plants in the laboratory. Bouquets of leafy spurge in vials filled with water were put in each cage. The plants were examined and replaced every three days, and evaluated for feeding. The observations were conducted throughout the life of adults whose longevity was recorded. These studies started on June 9, 1988 and ended on August 14, 1989.

The adults fed on the leaves of leafy spurge, having a preference for the youngest leaves, at the tip of the stems and on the shoots. The stems of shoots were also destroyed. Young leaves were eaten completely. On well-developed leaves, feeding usually began on the lower side of the leaf, and sometimes the thin epidermis of the upper side of the leaf was left. The daily amount of feeding throughout the life of the adults studied, beginning with emergence, was on an average, $11.04 \pm 1.8 \text{ mm}^2$ ($n=35$) per day and the amount of feeding by the field collected adults during the same period was $8.6 \pm 3.2 \text{ mm}^2$ ($n=18$) per day. It appears that because of different emergence periods, feeding remains constant in spite of differences in longevity. Adults that emerged in July and August had a higher metabolism with higher feeding, but they had shorter life cycles.

Fertility and Longevity of Adults

Observations were conducted during 1988 on the fertility and longevity of 886 adults collected in the laboratory garden as they started their activity during the spring, and on newly emerged adults obtained from larval survival tests and rearings on *Euphorbia esula*. One pair (one female and one male) of newly emerged adults was placed in multiple transparent plastic cages (11 x 11 x 16 cm) with leafy spurge bouquets, replaced three times per week. These cages were kept in an outdoor insectary. Data were recorded on pre-oviposition period, oviposition period, fertility, and longevity. Observations were also conducted on groups of about 20 adults (newly) that emerged from June to October, using the methods described above.

Additional fertility and survivorship studies were conducted during 1989 on 124 overwintering adults (61 males and 63 females that emerged during 1988). They were kept in transparent plastic cages (11 x 11 x 16 cm) containing a layer of 2 to 3 cm of soil, dry stems, and leafy spurge. During these observations the cages containing the insects were kept outdoors, partly buried in the soil, to reproduce natural conditions and protect them from freezing. Other studies were also conducted on adults of the different generations to verify which ones overwinter. At the end of the winter, when adults became active again after diapause, leafy spurge bouquets were provided as food. The bouquets were replaced as necessary, at least twice per week. For these adults, data was recorded on duration of diapause, pre-oviposition period, oviposition period, fertility, and longevity. Temperature and humidity conditions during these studies were also recorded.

On December 10, 1988, 188 of the adults belonging to the group of 410 adults under study, which had emerged during 1988, were still alive. At that time, only the adults that had

emerged from the beginning of August to the beginning November were alive. Ninety-five percent of the adults that entered diapause survived. The later the adults emerged during the year, the higher was their percentage of survival after diapause. The individuals of the first generation that emerged during June and July had a shorter life cycle and these adults died earlier but laid many eggs. The maximum number of eggs laid by a female (emerged during July) was 104. The life span of the adults was quite variable. The average life span of the adults that emerged during June and July was 54.0 ± 26.7 (n=20) and 41.6 ± 8.4 (n=20) days, respectively. This difference is mainly due to the higher temperatures and longer photoperiods during July, which accelerate the life cycle, thus shortening the life span of adults.

When the observations required single pairs or an equal number of males and females, mating pairs were selected. Nevertheless, the observations conducted from 1986 through 1990 showed that the sex ratio of the various generations of this species is nearly 1:1. The pre-oviposition period was 8.9 ± 2.8 days for 232 adults observed. During this period, temperatures and humidity conditions were, respectively, $24.2 \pm 1.7^\circ\text{C}$, and $63.6 \pm 5.6\%$. The oviposition period was 48.2 ± 11.3 (n=232) days (temperature $23.0 \pm 1.6^\circ\text{C}$, relative humidity $67.8 \pm 7.6\%$). These adults stopped laying eggs on November 5, 1988, but all of the eggs laid after October 20 either did not hatch or collapsed, even when kept at optimal conditions of temperature and humidity.

Diapause of the group of adults (n=124) that emerged during 1988 and overwintered until 1989, lasted 96.49 ± 2.56 days on an average, ranging from 92 to 101 days. The average temperature during the diapause period was $4.2 \pm 4.6^\circ\text{C}$ (r = -6 to 19°C), and the average relative humidity was $71.6 \pm 17.5\%$ (r = 25-92%). Adults belonging to the second, third, and fourth generations, that emerged from the beginning of August, overwintered. Adults became active again on March 10, 1989, and started feeding again on March 15. The mean pre-oviposition period was 69.01 ± 12.28 days. During pre-oviposition, the average temperature was $11.88 \pm 3.46^\circ\text{C}$ (r=0- 24°C), and the average relative humidity was $67.19 \pm 7.19\%$ (r=23-96%). The oviposition period of overwintering adults, after diapause, lasted a mean of 56.56 ± 26.05 days, ranging from 29 to 101 days, from mid May to the end of July. During this period, the average temperature was $22.69 \pm 4.22^\circ\text{C}$ (r=12 to 34°C), and the average relative humidity was $68.13 \pm 16.25\%$ (r=25 to 96%). All overwintering adults died by August 19. Higher temperatures shortened the life cycle of adults. At higher temperatures the females laid more eggs, there was more feeding, and the life span was shorter. When temperatures were lower, fewer eggs were laid. The total number of eggs laid per female was higher for females that emerged during June and July, though their life span was shorter. Females that emerged during August, September, and October laid eggs before and after diapause. Females that emerged at the end of October and during November did not lay eggs until June of the following year, after diapause, while females that emerged earlier started oviposition again much earlier. In a few cases, females that emerged at the end of October and during November laid some unfertile eggs before diapause.

Number of generations per year

Preliminary observations, conducted during 1986 and 1987, showed that *A. abdominalis* overwinters as an adult, becomes active during March, and starts oviposition again at the end of April to the beginning of May. In 1988, 34 adults were collected from March 8 to 15, on potted plants in the laboratory garden, as they began to be active. On April 26, they were released on 20 uninfested plants (which had three to four stems each) of *E. esula* in two 55 cm diameter pots under nylon screen cages. Seventeen days later, on May 13, the adults were collected, the cages were removed, and they were caged with a leafy spurge bouquet to determine if they were still ovipositing. When newly emerged adults were found on the plants in the 55 cm pots, the nylon screen cages were replaced to prevent their dispersal, they were left to feed and oviposit for 10 to 20 days (since the pre-oviposition period is shorter at higher temperatures), and then collected. New 55 cm pots containing the same number of uninfested plants of *E. esula* were used for each generation. This procedure was repeated until new adults were found. All of the adults collected were placed in transparent plastic cages in a laboratory with natural lighting and with bouquets of leafy spurge as food. They were observed until they died in order to record their longevity and to conduct observations on their biology.

To verify these observations, another study was conducted in the laboratory at ambient temperatures and natural lighting, following the development of each generation. A colony of overwintering adults was kept in outdoor cages. As soon as new eggs were laid by overwintering adults during the spring, these eggs were collected and the neonate larvae obtained were transferred onto uninfested plants of *E. esula* in pots. When adults emerged they were transferred into cages containing leafy spurge bouquets. The neonate larvae obtained were transferred onto uninfested potted plants of leafy spurge, and this procedure was repeated until new adults emerged. The adults that were re-collected on May 13, 1988, on the plants in the outdoor 55 cm diameter pots, were found to be still ovipositing. About one month later, on June 20, three teneral adults were found and the pots were caged once again. They were allowed to feed and oviposit for 14 days. On July 4 they were collected (13 were found) and put in a transparent plastic cage (11 x 11 x 16 cm) with a leafy spurge bouquet for observations on their feeding, fertility, and longevity. The nylon screens were removed. No adults were found during the following days. Three teneral adults were found on July 31 and the pots were caged again. They were allowed to feed and oviposit for 14 days, until August 14, when 14 adults were collected using a D-VAC suction sampler. No new adults were found until September 7 when three adults were seen, and the pots were caged again. On September 9, six adults were collected with a D-VAC, and the nylon screens were removed. On October 22, two teneral adults were found and the pots were caged. Adults were left on the plants for 20 days, and on November 11, 1988, five adults were collected, using a D-VAC. Afterwards, no adults were found. Four generations were observed.

The studies on the number of generations of *A. abdominalis* conducted in the laboratory confirmed the finding of four generations. In the laboratory, overwintering adults laid their first fertile eggs on May 4, 1988, 60 days after they started their activity again. The incubation period of eggs (n=53) laid on May 6 was about 13 days, the development of larvae to pupae took about 28 days, and the development from the eggs to the adults of

the first generation took about 52 days. The adults of the first generation emerged beginning June 28. Under higher temperature conditions the life cycle was shorter, and the adults belonging to the second, third, and fourth generations emerged beginning on August 4, September 10, and October 29, respectively.

The investigation on oviposition and feeding behavior, conducted on potted plants in the laboratory, on plants in glass cages, and in the garden, led to the following results:

1. During the night and until early morning (4:00 to 7:00 a.m.) the adults were motionless on the soil or on the plants. During the day they crawled around and fed on leaves, especially on the upper part of the plants. Movement by this insect was very limited when it had its host plant available.
2. Females laid eggs underground on the plant or in the soil very close to the plant, singly or in groups of two to six eggs. The eggs were laid at one five cm below the soil level and were found both along the primary stem and the young adventitious shoots and stem buds on the roots.
3. On October 31, 1986, the adults kept outdoors stopped laying eggs and reduced their feeding and general activity although they were under favorable temperature conditions (25°C), with a photoperiod L:D = 11:13. Forty of these adults, transferred into a temperature cabinet at 25°C and with long daylight conditions (L:D = 15.9), started to feed and lay eggs again normally. This behavior continued until mid December when they died. The same experiment was repeated under the same results were obtained. This shows the role of photoperiod in inducing diapause.
4. Six hundred and forty-two adults were collected in the garden at the beginning of October, 1986 and kept in cages in the garden. These adults stopped feeding on December 9, 1986 (temperature: min. = 5°C; max. = 17°C) and started to diapause. At the end of December they were still alive and overwintered, hiding among plant debris and under stones on the soil. Of these, 238 adults survived and started to become active again during March, 1987.

Host Specificity

To establish the host specificity of *A. abdominalis*, 56 plant species in 21 families, in addition to the control, were exposed to adults and larvae of this flea beetle. Included in this plant list were species in the order Euphorbiales and other orders of the super-order Rosidae, closely related to *Euphorbia*, economic or ornamental plants in other super-orders, plus plant species attacked by other *Aphthona* spp.

No-choice feeding tests

The adults used in the no-choice feeding tests were collected during June and July 1984 and 1986 at San Rossore, Italy, on plants of *E. esula*. Paper cups (200 cc) with perforated plastic lids (to allow aeration) were used as cages. Five adults were placed in each cup and the test plants were added as bouquets and replaced twice weekly. The feeding damage on the leaves of the test plants was estimated using a transparent grid divided into one millimeter squares. Since there were no apparent external morphological characters to separate living males and females, unsexed insects were used in the experiments and their sex was determined after death by dissecting them under a stereomicroscope. These tests were conducted in a greenhouse laboratory during 1984 and

1986. One replication per test plant species was made in the preliminary experiment conducted during 1984 and ten replications in the experiment conducted during 1986.

Of the 54 plant species or varieties in twenty-one families tested in 1984 and 1986, 17 species in the genus *Euphorbia*, (including the American populations of leafy spurge) were readily accepted and fed upon by adults of *A. abdominalis*. Whereas nibbling was recorded on *Ricinus communis* and *Codiaeum variegatum* (Euphorbiaceae), *Lythrum salicaria* L. (Lithraceae), *Pelargonium zonale* Ait. (Geraniaceae), *Ficus elastica* Roxb. (Moraceae), *Ipomoea alba* L. (Convolvulaceae) and *Helianthemum apenninum* L. (Cistaceae), no feeding occurred on *Linum usitatissimum* and *L. flavum*.

Larval survival test

The objective of this trial was to determine the ability of neonate larvae to develop on the test plants, on which adult feeding activity was observed in the no-choice feeding tests. Five neonate larvae were put on the root collar of the plants at soil level and two weeks later transparent plastic tube cages were placed over the plants to check for the emergence of new adults. Fifteen replications were made on each test plant and the number of newly emerged adults was recorded. This test was conducted in a greenhouse laboratory from May 15 to July 15, 1990 (mean temperature 23.81 +/- 4.18°C, r=14-34°C, mean relative humidity 67.43 +/- 16.54%, r=25-96%). Compared to the control plant, the number of larvae of *A. abdominalis* which completed development was significantly different on *E. maculata*, *E. corrolata*, *E. lathyris*, and *E. lucida*. That number was also significantly different when comparing the American populations of leafy spurge with the other species of *Euphorbia* used in the test. No larval development occurred on *Ricinus communis* and *Acoiaeum variegatum*, two plant species of economic importance on which adult nibbling occurred in the adult no-choice feeding test.

Oogenesis

A no-choice test was conducted to assess the ability of overwintering adults of *A. abdominalis*, kept in outdoor cages, to produce and lay fertile eggs on the test plant species. The adults used in this test were collected in Rome on September 9, 1986, and kept in outdoor cages with soil and wood pieces as shelter. In addition to *Euphorbia esula* as control, *E. lathyris*, *E. marginata*, and *Linum usitatissimum* were tested. The experiments started on April 2, 1987, when the adults began their activity. The test plants were placed in bouquets into cardboard cups (200 cc) covered with a fine mesh nylon screen to allow aeration. Eight adults were put in each cup and four replications were made for each test plant. Twice a week the bouquets were checked and replaced with fresh ones. As the adults died, they were dissected to check for development of retained eggs. The experiment was conducted in a laboratory room with natural light conditions, an average temperature of 19.36 +/- 5.64°C, r= 6-32°C, and an average relative humidity of 64.90 +/- 21.59% (r=27 and 93%). It ended when the last adult on the test plants died on June 19, 1987.

Oviposition occurred on the control (248 eggs) and on *E. maculata* (12 eggs). While the eggs laid by adults reared on *E. esula* hatched, the eggs laid by adults reared on *E.*

maculata did not hatch. No egg development was found at dissection of the adults which had fed on the other test plant species.

Host suitability test

The objective of this test was to verify the ability of *A. abdominalis* to complete development on the following test plants: *E. maculata*, *E. supina*, *E. tirucalli*, *E. marginata*, and *E. corollata*. *Euphorbia esula* was used as the control. Ten ovipositing adults were placed on each potted plant, placed under transparent plastic tubes (the same kind used in the larval survival test). Ten replications were made for each plant species. Fifteen days after these adults were re-collected and the tubes removed. A week later the tubes were placed on the plant again and were checked twice a day for 60 days, to observe the emergence of adults of the new generation. This test was conducted during 1987 and 1988 in a greenhouse laboratory. During 1987 (from August 6 to October 9) the mean temperature was 21.73 +/- 3.98°C, range 13-31°C, and the mean relative humidity was 69.36 +/- 32.90%, range 32-97%, during 1988 (from September 6 to October 25) the mean temperature was 19.82 +/- 3.60°C, range 11-29°C, and the mean relative humidity was 71.95 +/- 15.31%, range 33-96%.

Results.....Thirty-three adults emerged from the control plants, two from *E. corollata*, and one from *E. marginata*.

Free-choice field test

The objective of this test was to verify if under semi-natural conditions, feral adults of *A. abdominalis* would be able to oviposit, feed, and complete their life cycle on some North American populations of leafy spurge, on species closely related to *E. esula*, and plants of economic importance. The trials took place during July and August 1986 and 1988 at Castel Porziano (Rome), a natural preserve where *A. abdominalis* did not occur. No other species of *Euphorbia* were present in the experimental area.

Experiment A..... This experiment was conducted during July and August 1986 on (1) plant species on which positive results were obtained in the laboratory experiments, (except American leafy spurge), on *E. spathulata*, an American species, and on *Ricinus communis* and *Linum usitatissimum*, two species of economic importance. *Euphorbia esula* from Italy was used as the control. The size of the plot was 9.6 by 8.0 m. During 1988, an additional experimental plot was set up to test *E. corollata*, *E. supina*, *E. marginata*, *E. maculata*, and *E. serpyllifolia*. *Euphorbia esula* from Italy was once again used as the control. The size of the plot was 4.8 by 8.0 meters. In the field, five to fifteen adults were seen on each control plant, when checks were made throughout the experiment. Damage was mainly to the leaves of the new shoots of control plants. No adults nor damage were found on the other test plants. All the plants were in very good conditions until the end of the experiment. When the test plants were subsequently observed at the laboratory, 96 adults emerged from the control plants, and no adults from the other test plant species.

Experiment B.....This experiment was conducted during 1986 to measure the preference of *A. abdominalis* on American populations of leafy spurge versus *E. esula* of Italian origin.

The following test plants were exposed to adults: leafy spurge from Montana, Nebraska, and Wisconsin, and from Italy as the control. The size of the plot was 3.2 by 8.0 meters. In both experiments the test plants, in 22 cm terra-cotta pots, were buried with the tops of the pots at ground level. The experimental design was a randomized complete block and each plot had ten blocks. The distance between plants was 80 cm and the plants were put in the ground one week before starting the tests. The insects used in these tests were collected in the laboratory garden on plants of *E. esula*. Each potted plant received ten ovipositing adults (total 2,000 adults in experiment A and 400 in experiment B). Twelve days after the insects were released, those remaining on the plants were dug up and brought back to the laboratory. At the laboratory, the adults recovered were checked to see if they were still ovipositing. The potted plants were then caged in transparent plastic cylinder cages and kept in a greenhouse laboratory until new adults emerged, about a month later. All American populations of leafy spurge tested were suitable hosts for *A. abdominalis*. Two hundred and six adults emerged from the control, 150 from the plants of leafy spurge from Nebraska, 66 from Wisconsin and 35 from Montana. The statistical analysis of this data showed a separation between two groups: leafy spurge from Nebraska and the control on one side, and leafy spurge from Wisconsin and Montana on the other. The adults of *A. abdominalis* recovered were found still ovipositing.

The results of the studies conducted show that *A. abdominalis* has a narrow host range. In the laboratory, under no choice conditions, feeding of adults and development of larvae occurred on *E. maculata*, but in the oogenesis test the eggs laid by adults that fed only on *E. maculata* did not hatch. In the host suitability test in the laboratory, under no-choice conditions, one individual completed development to adult on *E. marginata* and two adults on *E. corollata*. Nevertheless, under field conditions, these species were not suitable hosts for *A. abdominalis* and were not even fed upon. When the plant species that showed to be susceptible to attack by *A. abdominalis* in the laboratory were exposed to adults of *A. abdominalis* under field conditions, only the plants of leafy spurge were attacked and infested. *E. pulcherrima* and *Ricinus communis*, important economic plants closely related to leafy spurge, proved not to be susceptible to *A. abdominalis*. American populations of leafy spurge from Montana, Nebraska, Wisconsin, and Wyoming were readily accepted, infested, and *A. abdominalis* completed development also under field conditions.

Environmental Consequences of Proposed Release

SITE DESCRIPTION

The potential site is located in Billings County, North Dakota, section 21, township 141 N, range 102 W. The soil is sandy loam and consists of 78.7% sand, 11.3% silt, and 10.0% clay. The site is in a hilly, well-drained area with no flood risk. There is no shade in the area, and the infestation is continuous. *A. abdominalis* will not interfere with any other biocontrol agents as there are none at this site. *Aphthona nigriscutis* were released at this site before, but did not establish. There is no grazing, mowing or spraying in the area. *Aphthona abdominalis* will not interfere with any of the insects, plants, or animals native to the proposed release site.

PHYSICAL ENVIRONMENTAL RISKS

- ◆ Air The establishment of *Apthona abdominalis* will pose no threat to air quality.
- ◆ Water *Apthona abdominalis* will not have any negative effect on water quality. In fact, it may indirectly help to improve the water quality by reducing the amount of herbicides used to control leafy spurge.
- ◆ Land *Apthona abdominalis* will not have any adverse effects on land quality. The value of spurge infested land should increase as biocontrol takes effect.

HUMAN HEALTH RISKS

The establishment of *A. abdominalis* will have no detrimental effects on humans. However, leafy spurge does have negative effects on human health. The latex produced by spurge causes dermatitis and may even cause blindness. Therefore, any reduction in the spread of leafy spurge will be beneficial to humans.

ECOLOGICAL IMPACTS

Wildlife...*Apthona abdominalis* will not have a negative effect on wildlife. By controlling leafy spurge, more diverse vegetation will result which will be beneficial to wildlife.

Invertebrates...Native insects will not be threatened (by interference or exploitation) by *Apthona abdominalis*. Leafy spurge is not a native plant species and therefore it is free of specialized native herbivores (Gassmann, 1990). It is seldom attacked by invertebrate phytophages except for grasshoppers in times of drought (Gassmann, *op cit*).

Domestic Animals...*A. abdominalis* will not cause any adverse effects on domestic animals and livestock. On the contrary, the latex in leafy spurge gives cattle scours, mouth blisters and in large quantities can cause death (Rees & Spencer, 1991). This causes the cattle to avoid grazing in areas with moderate to high spurge densities. The reduction of spurge will in fact cause a resurgence in vegetation for all animals.

Pollinators...Even though spurge does produce abundant amounts of honey in open nectaries, it is not regarded by beekeepers as an important honey producing plant. The replacement of vegetation may, in fact, supply a more continuous flow of honey (Gassmann, 1990). Gassmann (1990) stated that the honey from some South Africa *Euphorbia* species is toxic and it is not known if this applies to leafy spurge honey.

Other Biocontrol Agents...The establishment of *A. abdominalis* should not cause any problems for any other previously established biological control agents.

Threatened and Endangered Species...The establishment of *A. abdominalis* should not have a negative effect on endangered or threatened plant species. In fact, at least one species will benefit. In the United States, the western prairie fringed orchid, *Platanthera praeclara*, was declared a threatened species partly because of its susceptibility to the herbicides used to control leafy spurge (Gassmann, 1990). The legally protected species *Euphorbia* are not at risk because of the limited climatic range of *Apthona abdominalis*.

POTENTIAL FOR DISPERSAL FROM THE RELEASE AREA

The potential dispersal from the release area is not known at this time. However, *A. abdominalis* is unlikely to travel large distances from the original release area. Although many insects tend to immigrate rapidly from one area to another, *Aphthona* tend to aggregate. Releases of *Aphthona* in Montana have shown that within a year of the release, the colonies can be found within a meter or two of the original release site.

Cumulative Impacts

The establishment of *A. abdominalis* will complement the effect of the other biological agents released for the control of leafy spurge. Thus, a reduction of the populations of the weed is expected. This will allow many indirect beneficial economic and ecological impacts: Improved environments for native plants due to competition from leafy spurge, increased sustainable productivity on rangelands and pastures, reduction in the application of herbicides, and enhancement of recreational lands.

Aphthona abdominalis will help to increase plant diversity on a rather narrow range of sites currently dominated by leafy spurge. The main effect of *Aphthona abdominalis* on wildlife, both vertebrate and invertebrate, will be to increase their diversity. Their increased diversity will be due to the larger diversification of plant life.

Effective spurge biocontrol will reduce the amount of herbicides used to control spurge and their contamination of ground water. Pressure to cultivate on light soils to control leafy spurge will also be reduced with the achievement of biocontrol (Gassmann, 1990). This reduction of cultivation will help to decrease erosion and maintain a prairie habitat.

Mitigative Measures

If for some reason it should become necessary to decrease the number of *A. abdominalis*, the method of control currently used by APHIS (Animal and Plant Health Inspection Service) against grasshoppers, could be used effectively. In general, the most satisfactory and consistent results are obtained by the use of ultra-low-volume (ULV) sprays. One treatment would not eradicate the insect. Instead, three separate treatments at a minimum should be used. There are many different insecticides that could be used, but the three that would probably work best are Malathion ULV, Carbaryl/Sevin-4-Oil, and Carbaryl/ULV. The same treatment methods and dosage that are currently used to control grasshoppers could also be used to control *A. abdominalis*.

Dosage

Insecticide	Per hectare	Per acre
Malathion ULV 91.0-95.0%AI	428 ml ULV (0.65 Kg AI/hectare)	8.0 fluid oz. ULV (0.58 lb. AI/acre)
Carbaryl Sevin-4-Oil	1.46 liters total material (1.17 liters of formulation plus 292.23 ml diesel) (0.42 Kg AI/hectare)	20 fluid oz. Total material (16 oz. formulation plus 4.0 oz. diesel) (.5 lb. AI/acre)
Carbaryl/ULV	2.34 liters total material (876.90 ml of formulation plus 219. 22 ml of diesel) (0.42 Kg AI/hectare)	15.0 fluid oz. total material (15.0 fluid oz. total material (12.0 oz. of formulation plus 3.0 oz. diesel) (0.375 lb. AI/acre)

Conclusion

Studies on the life history showed that this flea beetle appears to be a very good candidate for biological control of leafy spurge since it has four generations, or more, per year, depending on the climatic conditions. The larvae, the stage which causes the major damage, are very mobile. They displayed a different behavior from other species of *Aphthona* already studied and released, and they occupy a unique ecological niche. In fact, they feed on shoots and shoot buds underground, as well as on roots as do the other species. This kind of damage severely stresses leafy spurge and prevents the growth of new stems, thus the spread of the plant and the production of seeds. Therefore, the damage by *A. abdominalis* does not conflict with, and could very effectively complement the other biocontrol agents already released. The release of *Aphthona abdominalis* is recommended.

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Table 1. Period of emergence and survival of adults of *A. abdominalis*, 1988-1989.

Date of Emergence	No. of Adults Emerged (%)	Entered Diapause	Living After Diapause (%)
June 1-15	31	0	0
June 15-30	44	0	0
July 1-15	46	0	0
July 15-31	48	0	0
August 1-15	36	22.2	19.4
August 15-31	56	55.3	53.5
September 1-15	39	69.2	59
September 15-30	66	60.6	54.5
October 1-15	37	97.3	97.3
October 25-30	29	93.1	93.1
November 1-15	22	86.4	86.4

Table 2. Synopsis of oviposition and longevity of *A. abdominalis* from emergence to death, 1988-189. (females collected in the field after diapause).

Month of emergence	No. of females	Mean Longevity (days+/-SD)	Dead Before Diapause (%)	Longevity of Overwintering females	Dead after Diapause (%)	Mean No. of Eggs Laid per Female Before Diapause	Mean No. of Eggs Laid per Females After Diapause	Mean No of Eggs Laid per Female (Total/Range)
June	60	54.05 +/-26.70	100	---	---	56.45 +/-10.90	---	56.45 +/-10.90 29-73
July	60	41.65 +/-8.40	100	---	---	61.47 +/-10.60	---	61.47 +/-10.60 35-82
August	60	54.33 +/-18.87	66.7	270.67 +/-61.88	33.3	33.66 +/-19.60	15.63 +/-17.31	49.29 +/-14.61 19-69
September	60	59.90 +/-23.89	50	252.25 +/-56.49	50	18.87 +/-20.41	30.80 +/-17.87	49.19 +/-11.63 19-104
October	60	28.33 +/-1.15	3	231.28 +/-87.26	97	17.83 +/-19.71	30.36 +/-15.60	49.35 +/-9.8 19-69
November	60	23.91 +/-5.26	12	238.72 +/-83.23	88	---	49.35 +/-9.83	49.35 +/-9.83 21-65
March/ Garden	18	---	---	132.44 +/-22.63	---	---	38.20 +/-5.29	38/20 +/-5.29 23-46

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Table 3. Synopsis of study on number of generations of *A. abdominalis*, 1988, in outdoor pots.

First Adults Released on	Released Adults Re-collected on	No. Adults Released	New Adults Found on	No. New Adults Found	New Adults Left Until	Adults allowed to feed for (days)
April 26	May 13	34				17
			June 20	13	July 4	14
			July 31	14	August 14	14
			September 7	6	September 9	14
			October 22	5	November 11	20

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Table 4. Development & number of generations of *A. abdominalis* in the laboratory.

	Pre-oviposition period	Incubation Period	From Larvae to Pupa	From Pupa to Adult	From Egg to Adult Days
	Days+/-SD(n) Temperature (°C) R.H. (%)	Days+/-SD(n) Temperature (°C) R.H. (%)	Days+/-SD(n) Temperature (°C) R.H. (%)	Days+/-SD(n) Temperature (°C) R.H. (%)	
Overwintering Adults	61.40+/-10.81(20)	13.00+/-0.75(43)	28.11+/-1.87(40)	11.21+/-0.78(20)	52.32
Began activity again at the beginning of March	10.8+/-4.9 68.4+/-15.7	18.2+/-3.9 70.7+/-12.78	20.9+/-3.5 87.7+/-16.4	21.5+/-1.3 88.9+/-17.8	
Adults of First generation	8.11+/-0.68(40)	4.87+/-0.68(52)	18.00+/-0.83(40)	10.00+/-70(40)	32.67
Emerged since End of June	25.1+/-2.5 69.1+/-12.4	23.3+/-5.7 84.7+/-20.5	23.8+/-5.8 57.7+/-20.2	21.7+/-8.0 60.4+/-18.8	
Adults of second generation	8.29+/-0.85(40)	3.88+/-1.20(48)	18.94+/-1.69(38)	10.13+/-1.21(40)	33.05
Emerged since beginning of June	24.7+/-4.2 57.7+/-18.7	23.9+/-4.1 87.2+/-22.7	23.6+/-3.7 67.8+/-14.2	21.2+/-3.5 75.2+/-15.9	
Adults of third generation	10.28+/-1.81(40)	7.15+/-1.28(40)	22.08+/-2.29(40)	11.98+/-0.78(28)	40.8
Emerged since First Decade of September	22.0+/-3.4 81.3+/-14.7	21.4+/-3.1 80.1+/-15.3	20.22+/-2.9 78.8+/-13.5	18.8+/-2.2 78.5+/-12.2	
Adults of Fourth generation	12/25+/-1.59(23)				
Emerged since end of October	18.2+/-2.8 87.4+/-14.8				

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Table 5. Incubation period of eggs of *A. abdominalis* throughout the year under semi-natural conditions, Rome, 1988.

Period when Eggs were laid	No. of Eggs	Incubation period (Days) Mean \pm -SD (Range)	Mortality (%)	Temperature ($^{\circ}$ C) Mean \pm - SD (Range)	Relative Humidity (%) Mean \pm -SD (Range)
March	0	—	100	9.35 \pm -1.4 (0.24)	68.86 \pm -9.1 (23-90)
April	14	—	100	16.06 \pm -4.0 (10-24)	62.46 \pm -14.03 (30-90)
May	451	12.40 \pm -1.70 (8-16)	13.6	19.39 \pm -2.0 (11-26)	67.30 \pm -7.5 (36=95)
June	415	7.10 \pm -2.29 (3-9)	6.7	21.76 \pm -3.6 (13-31)	68.45 \pm -16.58 (31 \pm -95)
July	452	4.66 \pm -0.68 (3-6)	1.9	25.24 \pm -3.06 (16-34)	6.84 \pm -16.55 (31-95)
August	392	6.30 \pm -1.70 (3-10)	19.3	23.25 \pm -5.06 (16-33)	61.10 \pm -15.16 (30-870)
September	370	7.70 \pm -1.70 (4-13)	19.6	23.67 \pm -1.86 (20-27)	66.31 \pm 6.46 (44-80)
Oct. 1-19	512	7.60 \pm -0.70 (4-15)	44.7	22.94 \pm -1.07 (19-24)	73.68 \pm -5.49 (54-82)
Oct. 20-31	69	—	100	20.38 \pm -3.54 (16-27)	81.33 \pm -14.75 (46-97)
November	13	—	100	9.89 \pm -6.58 (1-20)	69.10 \pm -23.38 (25-91)
December	0	—	—	3.78 \pm -3.95 (-6-14)	71.54 \pm - 18.06 (32-93)
Total	2688				

Table 6. Head capsule width of larvae of *A. abdominalis*.

Larval Stage	Head Capsule Width
L1	125.2 +/- 9.5 / 22
L2	187.8 +/- 22.1 / 29
L3	248 +/- 16.8 / 59

Table 7. Results of the no-choice adult feeding test with *A. abdominalis*, Rome, 1984 and 1986.

PLANT SPECIES	Mean Daily Feeding per adult (mm ² +/- SD)	Feeding Range (mm/day/adult)	Percentage feeding ^a	Mean Survival (days +/- SD)
1984 ^b :				
<i>E. esula</i> Italy	13.3+/-3.84	8.1+/-10.4	100	38.8+/-26.75
<i>E. lucida</i>	18.5+/-5.11	12.0+/-26.2	124.1	28.0+/-2.24
<i>E. prob. triangularis</i>	15.5+/-14.42	10.8+/-41.0	118.5	31.8+/-4.60
<i>E. maculata</i>	14.2+/-10.83	0.0+/-32.7	106.8	58.2+/-28.43
<i>E. trigona</i>	8.3+/-5.34	4.5+/-11.8	89.9	30.1+/-18.91
<i>E. peplus</i>	7.9+/-1.82	5.9+/-10.3	58.4	17.8+/-7.80
<i>E. mū</i>	7.2+/-5.01	0.33+/-18.0	54.4	58.2+/-8.28
<i>E. tirucalli</i>	8.0+/-4.68	0.0+/-14.5	54.1	24.0+/-23.75
<i>E. marginata</i>	5.9+/-8.90	0.0+/-21.7	44.4	48.8+/-2.19
<i>E. esula</i> from Montana	4.8+/-1.50	2.8+/-8.0	34.8	101+/-3.15
<i>E. esula-virgata</i> Nebraska	3.8+/-2.15	0.0 - 8.9	27.1	40.8+/-41.78
<i>E. prostrata</i>	3.5+/-2.28	2.5 - 8.8	26.3	18.4+/-11.33
<i>E. esula-virgata</i> Wyoming	3.2+/-2.12	0.1 +/- 8.0	24.1	78.5+/-28.88
<i>Ricinus communis</i>	3.13+/-3.30	0 - 8.70	23.3	42.6+/-18.39
<i>E. lathyris</i>	1.2+/-2.18	0 - 3.75	8	8.0+/-3.00
<i>E. antisiphilitica</i>	1.0+/-1.41	0 - 2.0	7.5	7.0+/-0.00
<i>Codiaeum variegatum</i>	0.3+/-0.68	0 - 1.67	2.2	10.0+/-8.63
<i>E. characias</i>	0.1+/-0.15	0 - 0.33	0.7	8.8+/-5.89
<i>Pelargonium zonale</i>	0.2 +/-0.38	0 - .67	1.5	5.4+/-3.13
<i>Lythrum salicaria</i>	1.9+/-2.06	0 - 4.78	14.3	20.4+/-10.78
<i>Ficus elastica</i>	0.8+/-0.94	0 - 2.00	4.5	14.0+/-3.08
<i>Ipomoea alba</i>	0.1+/-0.18	0 - 0.33	0.7	8.8+/-2.18

(Euphorbiaceae:)				
<i>Euphorbia pulcherrima</i>	0	0	0	
<i>E. heterophylla</i>	0	0	0	
<i>Mercurialis</i> sp.	0	0	0	
<i>Manihot palmata</i>	0	0	0	

(Geraniaceae:)				
Geranium rotundifolium	0	0	0	
(Linaceae:)				
Linum flavum	0	0	0	
(Rosaceae:)				
Rosa sp.	0	0	0	
Prunus avium	0	0	0	
(Labiatae:)				
Salvia splendens	0	0	0	
Thymus serpyllum	0	0	0	
(Apocinaceae:)				
Nerium oleander	0	0	0	
Vinca sp.	0	0	0	
(Ebenaceae:)				
Diospyros kaki	0	0	0	
(Juglandaceae:)				
Juglans regia	0	0	0	
(Leguminosae:)				
Medicago sativa	0	0	0	
(Rutaceae:)				
Ruta graveolens	0	0	0	
(Vitaceae:)				
Vitis vinifera	0	0	0	
(Gramineae:)				
Zea mays	0	0	0	
(Asclepiadaceae:)				
Asclepias curassavica	0	0	0	

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(Chenopodiaceae:)				
<i>Beta vulgaris</i>	0	0	0	
(Compositae:)				
<i>Lactuca sativa</i>	0	0	0	
<i>Cynara scolymus</i>	0	0	0	
<i>Carthamus tinctorius</i>	0	0	0	
<i>Tanacetum</i> sp.	0	0	0	
<i>Cichorium intibus</i>	0	0	0	
<i>Sonchus</i> sp.	0	0	0	
(Umbelliferae:)				
<i>Daucus carota</i>	0	0	0	
(Araliaceae:)				
<i>Hedera helix</i>	0	0	0	

1986^d:

<i>E. esula</i> from Italy	11.3+/-2.01	7.5+/-13.3	100	28.8+/-0.45 ^{d/}
<i>Helianthemum apenn.</i>	0.1+/-0.00	—	0.9	6.4+/-3.51
<i>E. supina</i>	8.8+/-1.67	6.0+/-11.3	77.9	24.4+/-5.13
<i>E. serpyllifolia</i>	4.1+/-2.65	2.25+/-6.0	36.3	5.8+/-3.83
<i>Linum usitatissimum</i>	0	—	—	—

^a % feeding = $\frac{\text{mm}^2 \text{ eaten per insect per day on test plant}}{\text{mm}^2 \text{ eaten per insect per day on control}} \times 100$

b/ Five adults were used per test plant (one replicate) in the preliminary test conducted during 1984

^c Ten replicates (5 adults per replicate) were used for each test plant in the test conducted in 1986.

^d Four adults were still living

Table 8. Results of the larval survival test with *A. abdominalis* ^{a/}.

Plant species	No. of adults emerged ($\bar{x} \pm \text{SD}$, n=15)	Homogeneous groups ^{b/}	% survival
<i>E. esula</i> Italy	3.60 \pm 1.18	A	72
<i>E. esula-virgata</i> Nebraska	2.73 \pm 1.16	B	55
<i>E. esula-virgata</i> Montana	2.33 \pm 1.05	BC	47
<i>E. esula-virgata</i> Wyoming	1.80 \pm 1.01	CD	36
<i>E. lucida</i>	1.33 \pm 1.18	DE	27
<i>E. lathyris</i>	0.80 \pm 0.86	E	16
<i>E. corollata</i>	0.67 \pm 0.82	E	13
<i>E. maculata</i>	0.47 \pm 0.64	E	9
<i>E. trigona</i>	0	—	0
<i>E. milii</i>	0	—	0
<i>E. antisiphilitica</i>	0	—	0
<i>E. tirucalli</i>	0	—	0
<i>E. characias</i>	0	—	0
<i>E. supina</i>	0	—	0
<i>E. serpyllifolia</i>	0	—	0
<i>E. peplus</i>	0	—	0
<i>E. marginata</i>	0	—	0
<i>Ricinus communis</i>	0	—	0
<i>Codiaeum variegatum</i>	0	—	0
<i>Linum usitatissimum</i>	0	—	0
<i>Helianthemum apenninum</i>	0	—	0
<i>Lythrum salicaria</i>	0	—	0
<i>Ipomoea grandiflora</i>	0	—	0
<i>Ficus elastica</i>	0	—	0
ANOVA	F = 18.30 $\chi^2 = 8.06$	P = 0.000 P = 0.3271	

^{a/} Fifteen replications (plants were used per plant species and five larvae were used per replication).

^{b/} Means followed by the same letter are not significantly different ($P \leq 0.05$, Newman-Keuls test).

Table 9. Results of the free choice field test with *A. abdominalis*.

Plant species ^{a/}	No. of Adults Emerged	Homogeneous Groups ^{b/}
<i>E. esula</i> Italy	18.20+/-10.92	B
<i>E. esula</i> Nebraska	15.10+/-10.33	B
<i>E. esula-virgata</i> Wisconsin	5.80+/-10.13	A
<i>E. esula-virgata</i> Montana	3/50+/-2.55	A
ANOVA	F = 6.02 X ² = 15.13	P = 0.0020 P = 0.0019

^{a/} Each plant species was replicated ten times

^{b/} Means followed by the same letter are no significantly different ($P \leq 0.05$, Newman-Keuls test).

Table 10. Plant species used in the host specificity testing with *A. abdominalis* (Plants attacked by other species of *Aphthona*).

Test Plants	Subgenus	No. Plants Tested	No. Plants Dissected Oct. 2, 1985	No. Plants Infested	No. Larvae Found	No. Adults Emerged June 1986	No. Adults Emerged July 1986
<i>Euphorbia virgata</i> "group" (control)	(<i>Esula</i>) Romania	22	10	3	3	2	2
<i>E. virgata</i> "group" Nebraska	(<i>Esula</i>)	22	10	3	3	2	1
<i>E. virgata</i> "group" Montana	(<i>Esula</i>)	35	20	6	6	3	—
<i>E. virgata</i> "group" Wyoming	(<i>Esula</i>)	6	6	2	2	—	—
<i>E. virgata</i> "group" Oregon	(<i>Esula</i>)	6	6	2	2	—	—

Table 11. Plant species used in the host specificity testing with *A. abdominalis*. Plants related to leafy spurge (Euphorbiales, Euphorbiaceae)

SUBGENUS	SPECIES	TYPE OF TEST*
Esula	<i>Euphorbia esula</i> from Italy	ABCDE
	<i>Euphorbia esula</i> from Nebraska	ABE
	<i>Euphorbia esula</i> from Montana	ABE
	<i>Euphorbia esula</i> from Wyoming	AB
	<i>Euphorbia esula</i> from Wisconsin	BE
	<i>Euphorbia lathyris</i> L.	ABC
	<i>Euphorbia characias</i> L.	AB
	<i>Euphorbia lucida</i> W. et. K.	AB
	<i>Euphorbia peplus</i> L.	AB
	<i>Euphorbia spathulata</i> Lam.	E
Agaloma	<i>Euphorbia marginata</i> Pursh.	ABCDE
	<i>Euphorbia antisiphilitica</i> Zuccar.	ABE
	<i>Euphorbia corollata</i> L.	BDE
Poinsettia	<i>Euphorbia pulcherrima</i> Willd.	A
	<i>Euphorbia heterophilla</i> L.	A
Euphorbium	<i>Euphorbia millii</i> Ch. des Moulins	ABE
	<i>Euphorbia tirucalli</i> L.	ABCDE
	<i>Euphorbia trigona</i> Haw.	ABE
	<i>Euphorbia</i> prob. <i>triangularis</i> Desf.	A
Chamaesyche	<i>Euphorbia maculata</i> L.	ABCDE
	<i>Euphorbia prostrata</i> Aiton	A
	<i>Euphorbia supina</i> Rafin.	ABCDE
	<i>Euphorbia serpyllifolia</i> Pers.	ABE
	<i>Codiaeum variegatum</i> Blume	AB
	<i>Mercurialis</i> sp.	A
	<i>Ricinus communis</i> L.	ABE
	<i>Manihot palmata</i> Mull. Arg.	A

Table 12. Plants attacked by other species in the genus *Aphthona*.

ORDER	FAMILY	SPECIES	TYPE OF TEST*
Geraniales	Geraniaceae	<i>Geranium rotundifolium</i>	A
Geraniales	Geraniaceae	<i>Pelargonium zonale</i> Ait.	A
Geraniales	Linaceae	<i>Linum flavum</i> L.	A
Geraniales	Linaceae	<i>Linum usitatissimum</i>	ABCE
Myrtales	Lythraceae	<i>Lythrum salicaria</i>	AB
Rosales	Rosaceae	<i>Rosa</i> sp.	A

Table 13. Economic or ornamental plants

ORDER	FAMILY	SPECIES	TYPE OF TEST*
Rosales	Rosaceae	<i>Prunus avium</i> L.	A
Lamiales	Labiatae	<i>Salvia splendens</i> Ker. Gavl.	A
		<i>Thymus serpyllum</i> L.	A
Gentianales	Apocynaceae	<i>Nerium oleander</i> L.	A
		<i>Vinca</i> sp.	A
Ebenales	Ebenaceae	<i>Diospyros kaki</i> L.	A
Juglandales	Juglandaceae	<i>Juglans regia</i> L.	A
Fabales	Leguminosae	<i>Medicago sativa</i> L.	A
Sapindales	Rutaceae	<i>Ruta graveolens</i> L.	A
Rhamnales	Vitaceae	<i>Vitis vinifera</i> L.	A
Poales	Gramineae	<i>Zea mays</i> L.	A
Gentianales	Asclepiadaceae	<i>Asclepias curassavica</i> L.	A
Urticales	Moraceae	<i>Ficus elastica</i> Roxb.	AB
Violales	Cistaceae	<i>Hleianthemum apenninum</i> L.	AB
Cariophyllales	Chenopodiaceae	<i>Beta vulgaris</i> L.	A
Asterales	Compositae	<i>Lactuca sativa</i> L.	A
		<i>Cynara scolymus</i> L.	A
		<i>Tanacetum</i> sp.	A
		<i>Cichorium intibus</i> L.	A
		<i>Sonchus</i> sp.	A
Umbellales	Umbelliferae	<i>Daucus carota</i> L.	A
	Araliaceae	<i>Hedera helix</i>	A
Polemoniales	Convolvulaceae	<i>Ipomoea alba</i> L.	AB

* A = No-choice adult feeding test

B = Larval survival test

C = Oogenesis test

D = Host suitability test

E = Free-choice field test

Figure Captions

1. Adult of *A. abdominalis*.
2. Feeding and activity pattern of *A. abdominalis*, 1988-1989.
3. Longevity and oviposition of *A. abdominalis* throughout its life, 1988-1989. Temperature and humidity conditions during this study are also reported.
4. Longevity and oviposition of adults of *A. abdominalis* collected in the field after diapause. Temperature and humidity conditions during this study are also reported.
5. Life cycle of *A. abdominalis* on *E. esula* in Rome, Italy, under semi-natural conditions.
6. Eggs of *A. abdominalis*
7. First instar of larva of *A. abdominalis*
8. Damage of larvae of *A. abdominalis*.
9. Third instar larva of *A. abdominalis*.
10. Larva of *A. abdominalis* penetrating into a shoot of *E. esula*.

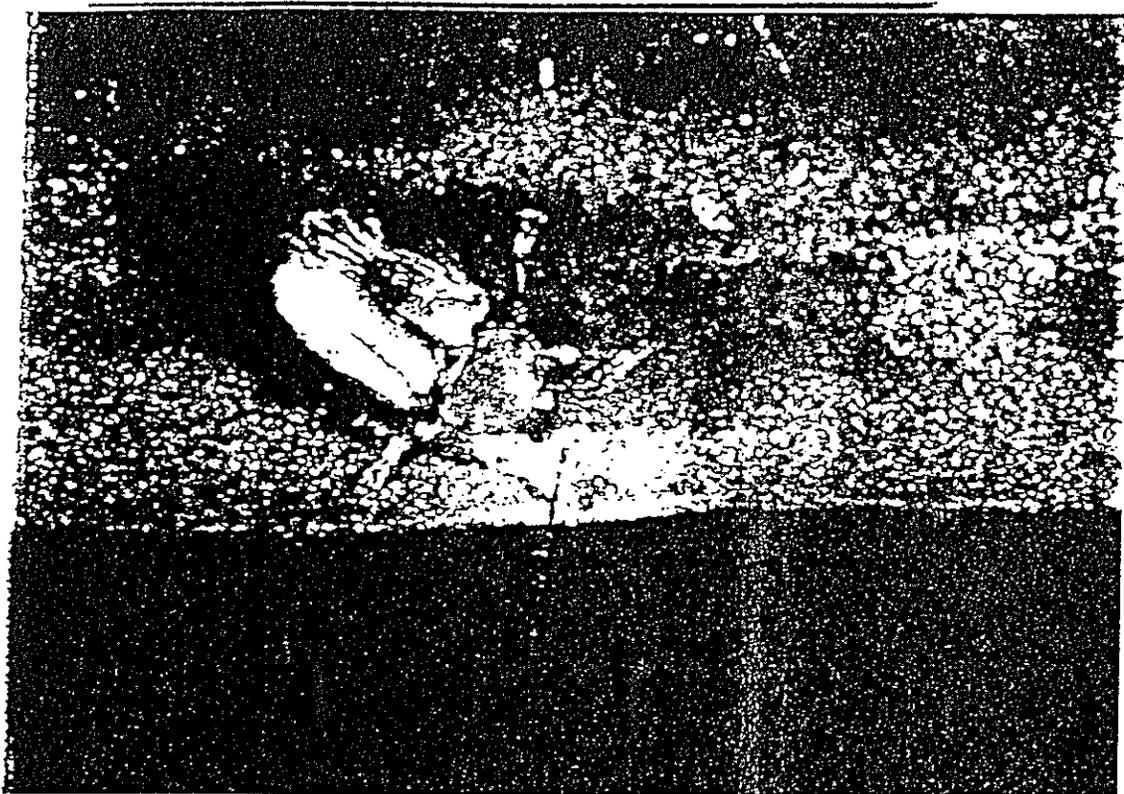


Figure #1

Figure #2

Fig. 3

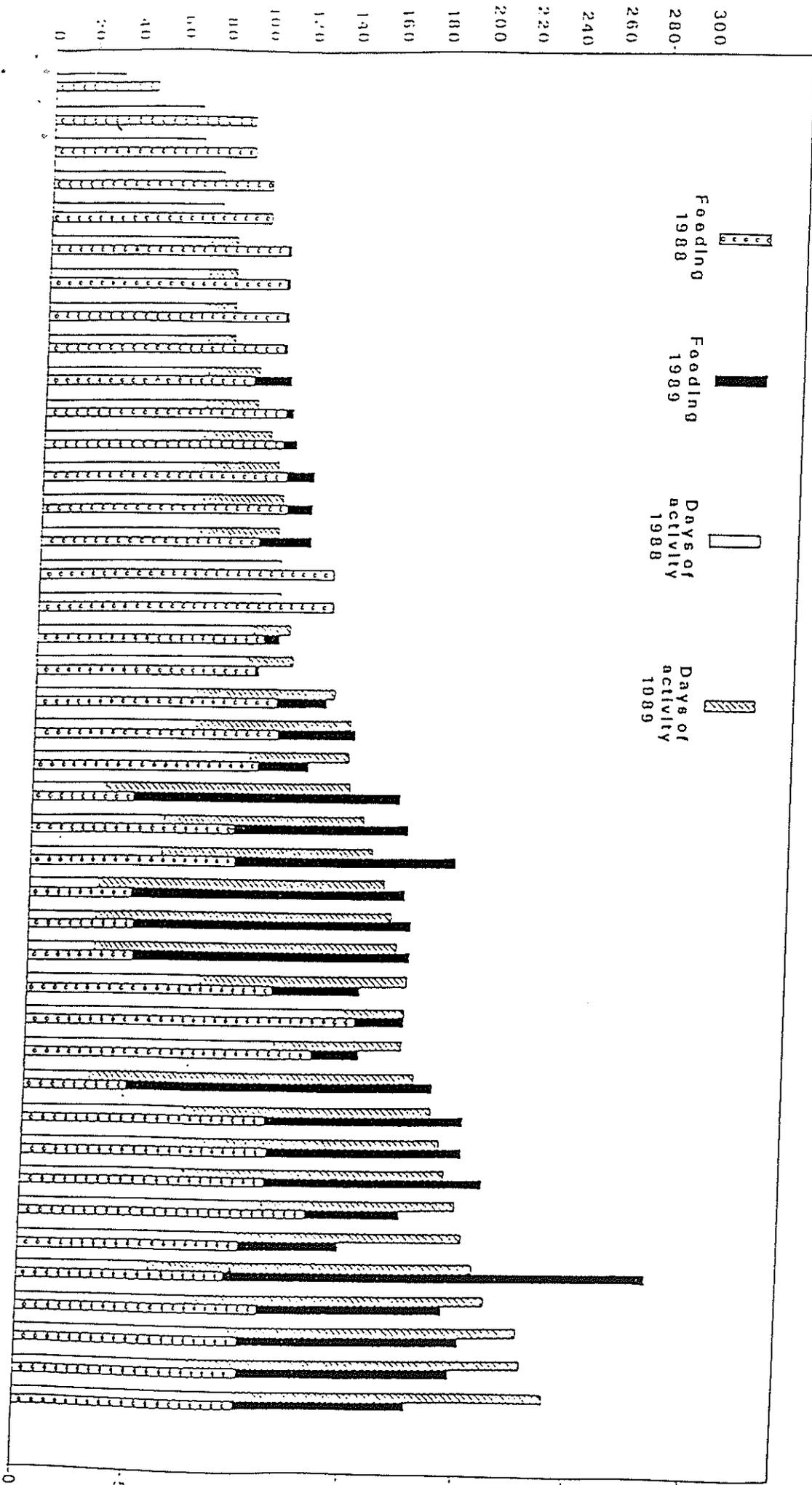
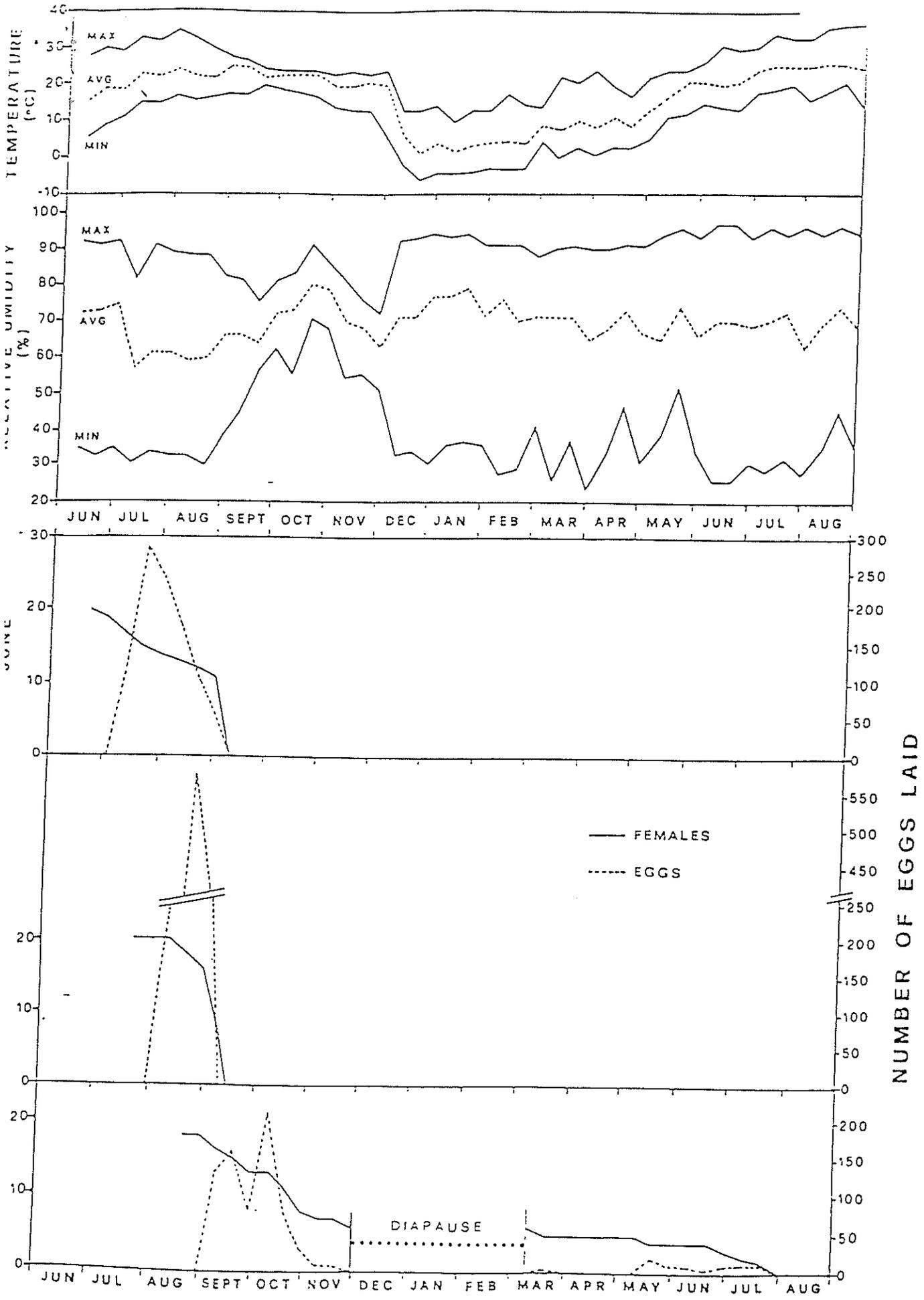
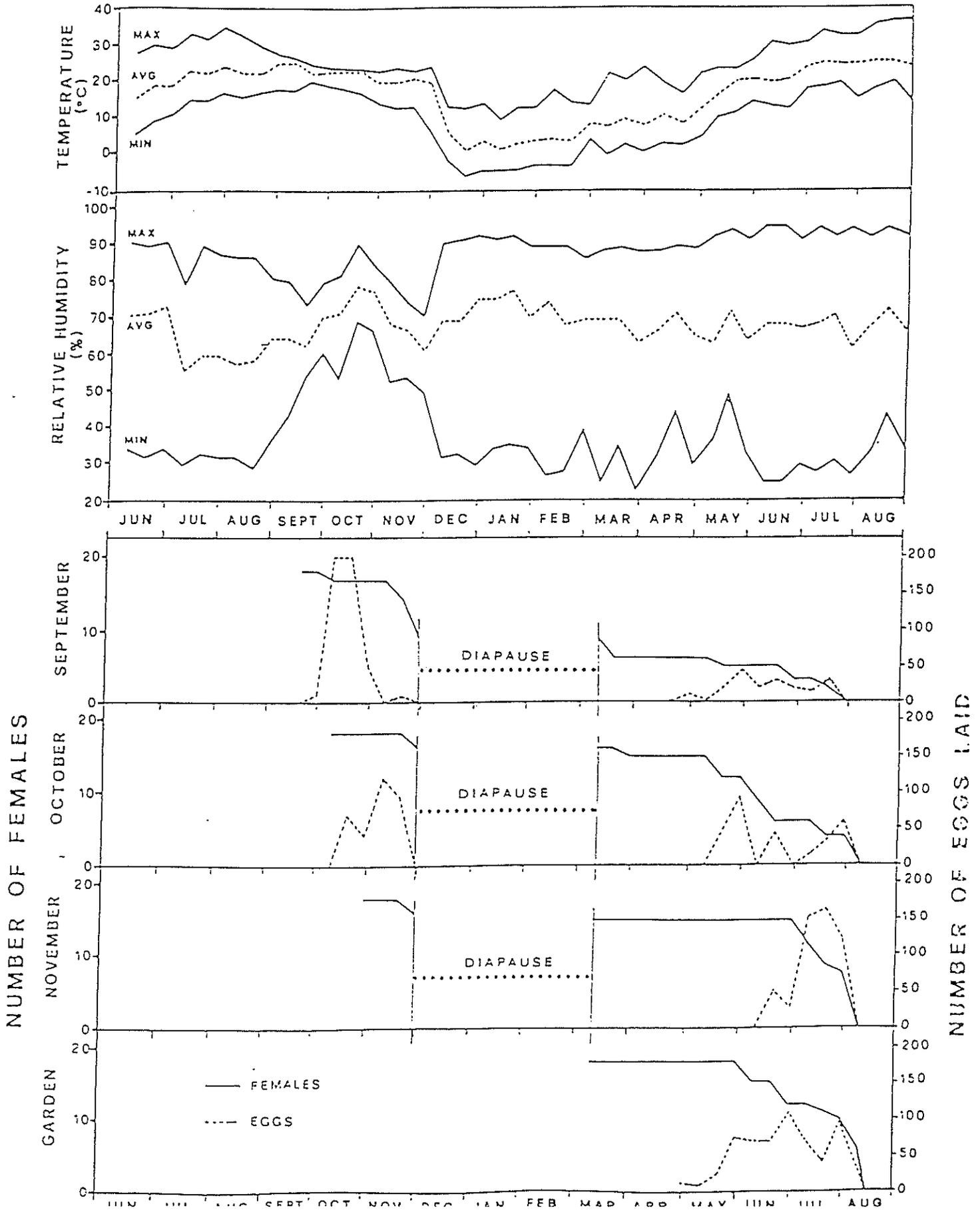


Figure #3





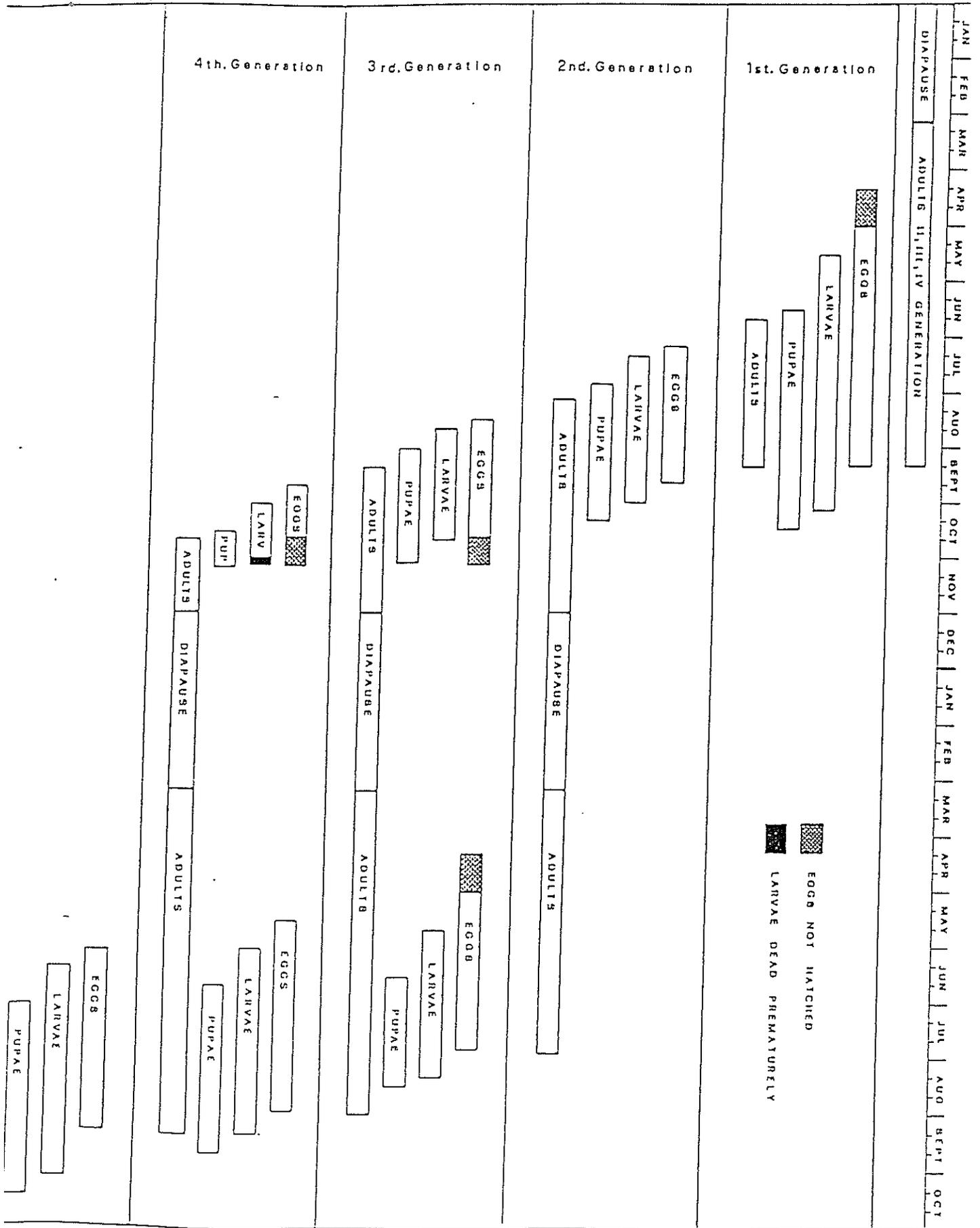
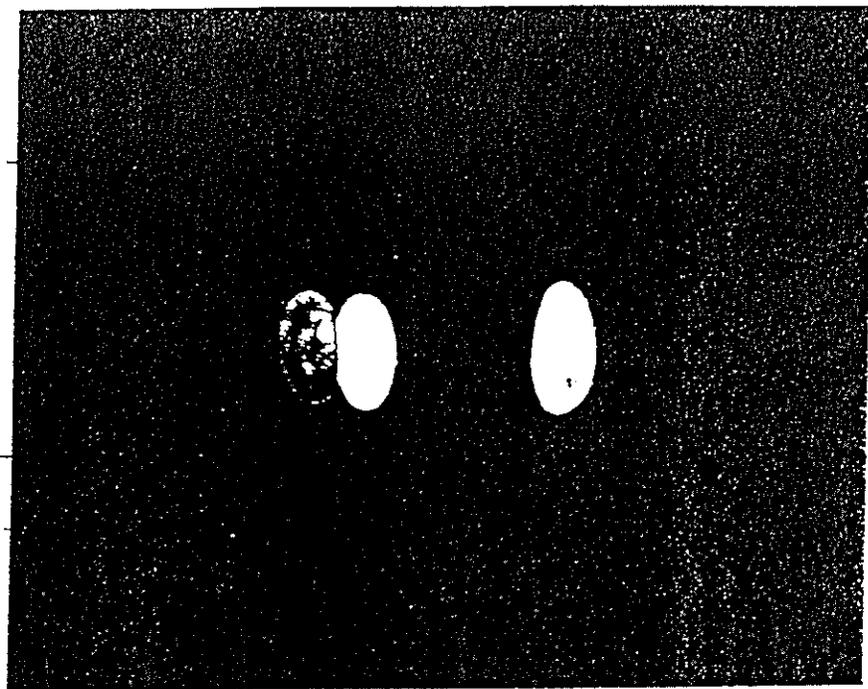


Figure #6



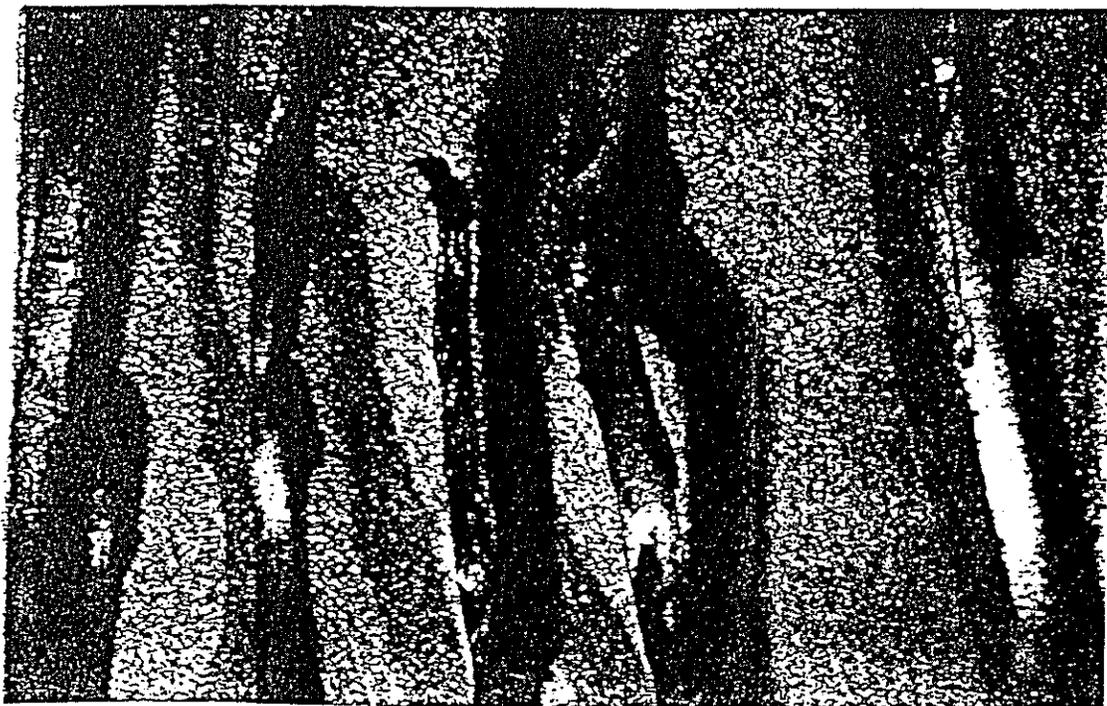


Figure #8

Figure #9

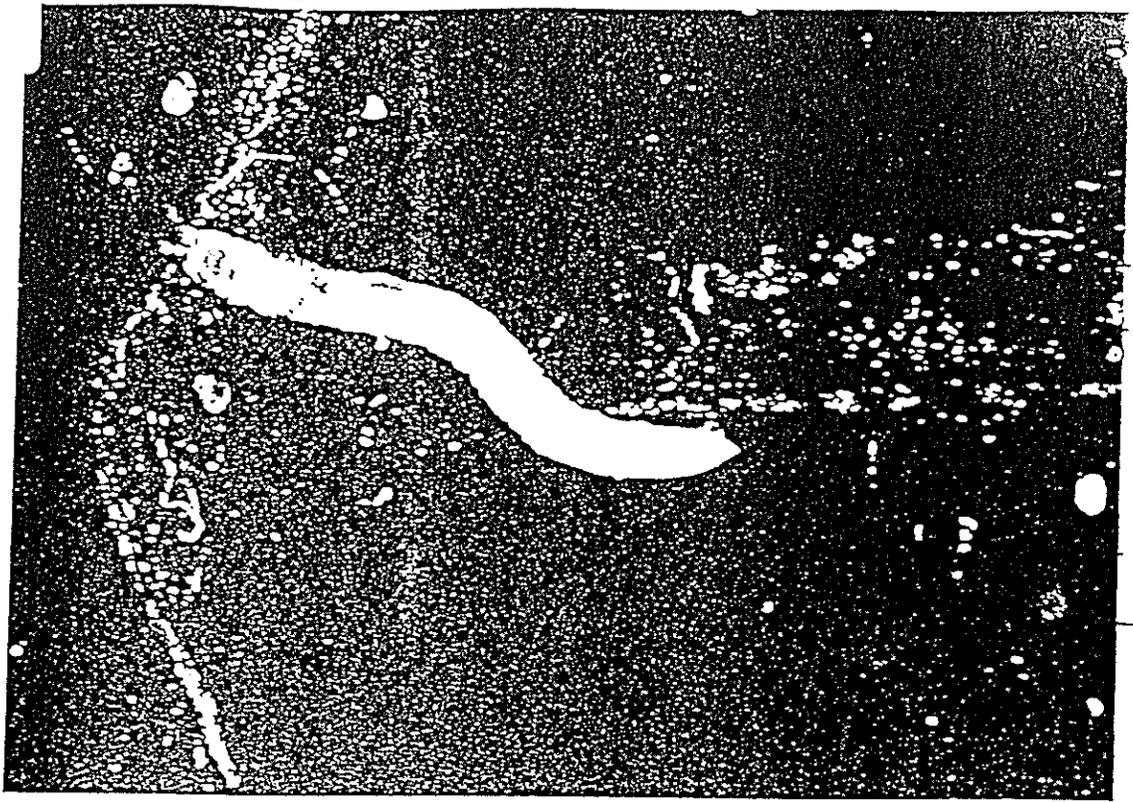


Figure #7 & 10

