SOME ASPECTS OF THE BIOLOGY OF THE RAGWORT FLEA BEETLE LONGITARSUS JACOBAEAE (WATERHOUSE) RELATING TO ITS ROLE AS A BIOLOGICAL CONTROL AGENT IN NEW ZEALAND

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Some aspects of the biology of the ragwort flea beetle Longitarsus jacobaeae, (waterhouse) relating to its role as a biological control agent in New Zealand.

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Keywords: Longitarsus jacobaeae, Coleoptera, Chrysomelidae, Alticinae, Senecio jacobaea, biology, biological control, New Zealand.

An Italian biotype of L. jacobaeae (Waterhouse) was introduced in to New Zealand in 1981 as a biological control agent for ragwort Senecio jacobaea, a problem weed in dairy farms. This thesis investigated various aspects of the biology of L. jacobaeae and its interactions with its host plant that could be helpful in furthering the aims of the biological control programme.

Laboratory experiments showed that the black spot stage and larval movement in the egg are two distinct characters which can be useful for determining the imminent hatching of eggs. The finding of Cullen (1981) on storing ability was confirmed. Eggs of L. jacobaeae can be stored at 4 ^{o}C and used for incubation to obtain healthy larvae even after 16 weeks. Experiments under controlled temperature conditions showed that the eggs have the ability of to overwinter in the field and then hatch with the return to favourable conditions. Mean incubation periods at 15 ^{o}C and 18 ^{o}C were 25 days and 18 days respectively.

Analysis of head capsule widths of laboratory hatched and field collected L. jacobaeae larvae confirmed that this species possesses three larval instars and supported the description of Newton (1933). Larval development at different constant temperatures showed that at lower temperatures tested (10 ^{o}C ; 12 ^{o}C), larvae fed and developed slowly in the root crowns whereas at higher temperatures tested (18 ^{o}C; 20 ^{o}C), larvae fed heavily on lateral roots and developed at a higher rate. According to the temperature conditions of different areas of New Zealand, eggs which hatch before the winter, may produce adults in December, but eggs that do not hatch before the winter may overwinter in the field and with warm spring and summer temperatures hatch and produce adults in January.

Laboratory experiments using controlled day lengths showed that short days (8 hours light + 16 hours dark) initiated heavier feeding, reproductive maturation, mating and oviposition of L. jacobaeae, whereas long days (16 hours light + 8 hours dark) maintains them in diapause. Therefore, there will not be a time lag in egg production between early emerged adults (adults that emerge during a long day period) and late

emerged adults (adults that emerge during short days). Measurements of flight muscles of reproductive and non- reproductive females showed that there was a complete resorption of flight muscles as the insect produced eggs. It is argued that whether the insect use flying for dispersal or insect use jumping for dispersal.

Experiment on oviposition of L. jacobaeae with relation to different temperature conditions showed that the range 15-20 ^{o}C were the most suitable temperatures for oviposition. Insect may oviposit for a longer time at a higher rate in North Island of New Zealand than in South Island of New Zealand.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Ragwort Senecio jacobaea (L.) is a poisonous pasture weed, specially on dairy farms. It is native to wide areas in Europe, Asia, and North Africa and spreads extensively as a weed. Since it was introduced into North America it has given considerable trouble. In Australia it established itself in several States, and has been included in schedules of the Noxious Weed Acts of Victoria and Tasmania. The first record of this plant in New Zealand was near Dunedin in 1874 (Thomson, 1922). Since that time it has continued to invade new areas and to increase the severity of infestation on land already infested. This rapid establishment was due to its ecological suitability; it is a plant well equipped to spread with great rapidity, which in turn helps it to persist in an area when it is once established.

Ragwort has invaded most pasture land to a varying degree. In New Zealand, the worst infestations were generally found on large holdings of 300 acres to 2,000 acres (Pool and Cairns, 1940). Many of these farmers have had to convert from dairying to sheep farming due to ragwort infestation. Small farm holders have had to purchase sheep to control ragwort where the weed has got out of control. On good dairying pastures, control is carried out by chemical means. However, many farmers considered that the use of sheep was cheaper than herbicide application. Schmidl (1972) emphasized that control by grazing is temporary because ragwort tends to recover following the removal of sheep, even after 5-7 years of intensive grazing.

In ragwort infested countries, there were attempts at biological control using insects imported from Europe as early as the 1920s (Syrett, 1983). Following the work of Cameron in the late 1920s in England, cinnabar moth Tyria jacobaeae(L.) and the seed fly Phegohylemyia jacobaeae (Meade) were selected for prospective introduction (Miller,1970). Unfortunately success was limited. Frick (1969) reported that though cinnabar moth larvae feed heavily on leaves, some larger plants were still alive even after 3 years. This behaviour was also described by Cameron (1935) as the root crowns supporting regrowth, even after severe damage. Newton (1933) and Cameron (1935) briefly discussed a flea beetle that would feed in the root crown during the winter and spring. They realised that the combination of summer defoliation by cinnabar moth larvae plus winter and spring feeding on the primary storage tissues of the root crown by flea beetle larvae might cause death of the plant.

In 1964, a population of flea beetles Longitarsus jacobaeae (Waterhouse) was collected at Delamont in Western Switzerland on ragwort; another was found near Rome, Italy, feeding on the closely related S. erraticus (Bert), and another was collected in France. This flea beetle has since been shown to be highly specific to ragwort and, in fact, almost entirely restricted to ragwort and a few closely related species of Senecio (Syrett, 1983).

From the early establishment of the flea beetle in California, Hawkes and Johnson, 1976 recorded that it controlled the ragwort problem in 3-4 years. It had a high dispersal rate, and it reduced the plant rosette density by 90% at the original California release site. They observed that the flea beetle in combination with the cinnabar moth provided excellent control of the weed at many sites.

1.2 BIOLOGICAL CONTROL OF RAGWORT IN NEW ZEALAND

Insects recorded from the plant in New Zealand are: the magpie moth Nyctemera annulata Boisduval (Lepidoptera:Arctiidae), the blue stem borer, Homoeosoma farinaria Turner (Lepidoptera:Pyralidae), another stem borer, Melanagromyza senecionella Spencer (Diptera:Agromyzidae), the leaf miner Phytomyza syngenesiae Hardy, an aphid Brachyaudus helichrysi Kaltenbach, and cut worm larvae (Noctuidae). Of these insect species, the magpie moth is the most conspicuous and the only one to do any significant damage to the plant. Unfortunately, the effectiveness of this species is reduced by high levels of parasitism by a braconid, Microplitis sp. (Syrett, 1983).

New Zealand has imported the most promising insect species used elsewhere, the cinnbar moth, the seed fly and the flea beetle. Between 1929 and 1932, cinnabar moth was distributed on ragwort throughout New Zealand. Although good initial establishment occurred, after 1932 establishment was limited only to a few areas (Syrett, 1983 and 1984). Syrett (1989) reported that, the moth persist in abdundance only through the southern North Island from Wellington to Pahiatua and Manawathu. The reason for poor establishment in New Zealand is not known, but overseas workers have reported parasitism, diseases, unfavourable climatic conditions, and limited power of dispersal as limiting factors.

Initial release of ragwort seed fly in New Zealand was made in February 1936. Syrett (1983) reported that since most reproduction of ragwort in pasture is vegetative, a seed feeding insect will have little impact and also it is unlikely to survive in association with cinnabar moth since the moth larvae consume flowers. Dymock (1987, 1988) reported that lack of synchrony between emergence of adult insect and availability of oviposition sites (ragwort flowering) was the cause of poor establishment in New Zealand. Due to poor synchronisation a high proportion of seed heads escaped predation, and the high germinating capacity and longevity of these seeds and ragwort's ability to reproduce vegetatively mean that the seed fly's impact on ragwort was considered

negligible (Dymock, 1987). The current distribution of the ragwort seedfly is only in some areas of central North Island (Syrett, 1989).

Ragwort flea beetle was imported in 1981, and the first release in the field was in 1983. The beetles were released at 95 sites throughout New Zealand, and are known to have established in 18 sites (Harman and Syrett, 1989), tough it would have established at some other sites. The reason for the poor rate of establishment has not been clearly identified in New Zealand. Thus, further understanding of the biological, ecological and climatic factors which interact to produce the final level of establishment of the insect is essential.

1.3 DESCRIPTION OF THE STUDY

The main objectives of the research studies described in this thesis were:

(a) Determination of the influence of temperature on the development of life stages of L. jacobaeae.

(b) Some aspects of reproductive diapause of L. jacobaeae.

(c) Determination of the influence of temperature on the oviposition of L. jacobaeae.

Chapter 2 is a review of the general information gathered about the weed Senecio jacobaea, and the beetle L. jacobaeae. Chapter 3 deals with the morphological changes of the egg during the incubation period and the effect of temperature on the development of egg. The most important section of the study is addressed in Chapter 4. This Chapter consist of : methods used for obtain small ragwort plants for rearing vessels, a laboratory rearing technique for L. jacobaeae larva, larval instar analysis, and effect of temperature on the development of different larval instars and pupa. Chapter 5 deals with the adult reproductive diapause. In Chapter 6 effect of temperature on oviposition is discussed. Each chapter contains a separate conclusion. An overall summary and conclusion on suitability of L. jacobaeae as a biological control agent in New Zealand and necessity for further studies are given in Chapter 7.

CHAPTER TWO

REVIEW OF LITERATURE

2.0 INTRODUCTION

This literature review deals with two subjects. Part one consists of the general description, history, ecology, important botanical characters, life history and control methods of ragwort. The second part of the review considers the taxonomic position, life history, type of damage, and seasonal variation of the ragwort flea beetle.

2.1 RAGWORT

2.1.1 General Description

Ragwort Senecio jacobaea L. belongs to the daisy family, the Compositae or Asteraceae (Pool and Cairns, 1940). It is a biennial or perennial herb. The stem of a normal mature plant whose growth has not been interfered with, is stout, upright, with few or no branches in the lower part, and averaging about 50 cm in height. The lower leaves are stalked, dark green above and paler underneath, and are almost glabrous. The upper part of the stem is much branched and produces numerous flowers, up to 2,500. The flowers form a flat topped or convex inflorescence with a short axis. If growth is interfered with, it usually causes the plant to convert to a perennial with a multiple crown and a number of flowering stems.

2.1.2 History of Ragwort

Ragwort is native to wide areas of Europe, Asia, and North Africa and has also been spread extensively as a weed (Pool and Cairns, 1940). It was introduced early into North America, where it has given considerable trouble. It has spread to many other parts of the world and is reported as a weed in Australia, Canada, New Zealand, and the United States of America (Pool and Cairns, 1940; Meijden, 1970; Syrett, 1983; McEvoy and Cox, 1987).

In New Zealand, ragwort was first recorded near Dunedin in 1874. Ragwort has rapidly increased, especially in the South Island, and in parts of Auckland, Wellington and Taranaki. Ragwort was declared a noxious weed in the Second Schedule of the Act of 1900, but was placed in the First Schedule in the Act of 1908 (Pool and Cairns, 1940). Bird (1977) reported that it was illegal for land owners to allow ragwort to flower on their properties. This plant was classified as a class B noxious weed, and the Noxious Plant Act (1986) required farmers to maintain a ragwort free strip at least 20 metres wide inside their farm boundaries (Wardle, 1987). Since the species is widespread throughout New Zealand it is considered as a serious problem (Harman and Syrett, 1989).

2.1.3 Ecology of Ragwort

2.1.3.1 Weather

Pool and Cairns (1940) described the main ecological factors governing the distribution of ragwort as sufficient rainfall and the presence of medium to light soil types. It was reported by Friend (1980) that the distribution of ragwort did not appear to be limited by soil physical or chemical factors. Wardle (1987) reported that ragwort has a wide pH tolerance (from 3.95-8.20). Syrett (1983) reported that ragwort is found in most areas of New Zealand which receive more than 800 mm rain annually. Wardle (1987) agreed that rainfall was the principal factor controlling ragwort distribution. Even though rainfall was the main limiting factor, ragwort could not survive under water for long periods (Wardle,1987). It has also been reported by Wardle (1987) that frost was not a limiting factor in undamaged healthy plants, but defoliated plants were killed by frost. In the Dutch dunes, ragwort generally occurred in clusters or pockets in all types of vegetation, ranging from very poor communities with interrupted cover of mosses, lichens and very short grasses to tree covered areas (Meijden, 1970). After a two year study, Meijden concluded that weather factors are the most important factors in determining the number of plants in a particular area.

A high relative humidity was also considered as a significant factor in helping seedling plants to become established (Pool and Cairns, 1940). Though prolonged dry spells during the summer limited the growth of seedlings, temperature was not considered as a limiting factor for the distribution of ragwort (Wardle, 1987).

2.1.3.2 Other Factors

Apart from weather, the most important factor in the establishment of ragwort was the incidence of grazing. Significant numbers of sheep kept the ragwort grazed and this prevented it from completing its life history, while cattle encouraged ragwort growth by feeding on potentially competing pasture and at the same time encouraged vegetative propagation through disturbance (Wardle, 1987). It has also been reported by Wardle (1987) that disturbance by grazing would encourage dormant seeds to germinate. Therefore, continuous disturbance would enhance the persistence of ragwort.

Another factor which limited its spread was the presence of a continuous pasture cover. Wardle (1987) reported that pasture cover was considered as an inhibiting factor for ragwort seed germination and also for the establishment of seedlings.

Thompson (1985) reported that management practices such as removing of flowering stalks had a great influence on ragwort population where there were no other disturbances such as grazing, because the seedlings were the principal source of replacing plants. Steep hills heavily infested with ragwort provided a very good source of seeds which readily spread into neighbouring areas (Syrett, 1983). Therefore, it can said that location also has an effect on the distribution of ragwort.

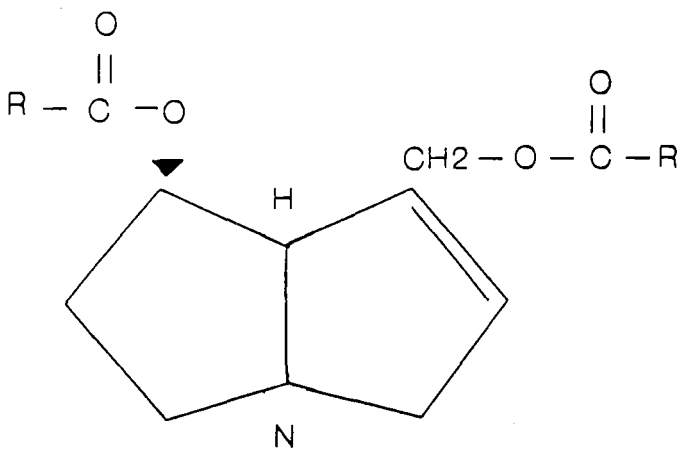
2.1.4 Ragwort Poisoning

Generally ragwort plants are unpalatable to livestock. However, where the pasture is very poor, or where ragwort plants have been mowed or sprayed with a herbicide, plants became more palatable and cause trouble to livestock.

Ragwort is known to be connected with winton disease of cattle in New Zealand and some disease conditions in cattle (pictou disease)) and horses in Canada (Connor, 1951 and 1977; Mortimer and White, 1975). Ragwort alone caused more annual loss of livestock in Britain than all other poisonous plants (Mortimer and White, 1975).

Ragwort poisoning was generally a chronic condition due to the cumulative effect of the toxic alkaloids which include senecionine, seneciphylline, jacobine, jaconine, jacoline, and jacocizine. The most toxic of these were senecionine and seneciophylline (Howatt, 1989). Lethal doses were 77 mg of the former and 85 mg of the latter per kg of body weight. All six of these alkaloids were cyclic diesters of the 1,2 dehydrophyrrolizidine ring system. These ring systems contain a double bond in their structures (Fig.2.1). These alkaloids usually occurred partly as their amine oxides, but this did not alter the toxicity. Ragwort contains 0.2 to 0.4 % alkaloid (on a dry weight basis) in the softer green material (Mortimer and White, 1975). The whole plant is toxic in both fresh and dry state, but the concentration of alkaloids is considered to be highest in the flowers followed by leaves, roots and stems. These alkaloids are not toxic themselves but, in the presence of certain liver enzymes, are converted to "pyrroles" which are toxic to mammals.

Figure 2.1 The ring structure of p yrrolizidine (obtained from Deinzer et al. (1977))



When the plant is eaten by cattle, symptoms of poisoning are: indefinite unthrift and loss in condition, diarrhoea, nervous disturbance, and irritability extending over a period of weeks. In cows, the milk was tainted. In calves, diarrhoea, jaundice, loss of weight, photosensitization, and typical alkaloid liver injury were observed by Mortimer and White (1975). In horses, dullness, unsteadiness, aimless wandering and slow deliberate eating of food are the symptoms. In postmortem examination, the carcasses were yellow, and cirrhosis of the liver was the most prominent symptom (Connor, 1951 and 1977). Sheep are considered to be more resistant to poisoning than cattle or horses. The chronic lethal dose of dried ragwort for cattle was 0.14 kg/kg of body weight compared to more than 2 kg/kg of body weight for sheep. The greater resistance of sheep may be due to differences in liver alkaloid metabolism. Mortimer and White (1975) suggested that symptoms like facial eczema and death of sheep occurring many weeks after they had been removed from infested pasture may be due to ragwort poisoning.

Since these alkaloids have cumulative effects, and these alkaloids are known to taint milk and honey, contaminated milk and contaminated honey may not be safe for humans. Certain liver diseases in humans in developing nations have been attributed to the consumption of foods, prepared from plants containing pyrrolizidine alkaloids (Deinzer and Thomson, 1977).

2.1.5 Propagative Power of Ragwort

2.1.5.1 Seed Propagation

McEvoy and Cox (1987) distinguished two achene types in the flower, and they recorded that the ragwort fruits are heteromorphic. Central (disk) florets yield achenes that are lighter, more numerous, and bear a pappus which helps wind dispersal, and are released shortly after they mature. Marginal florets have achenes that are heavier, less numerous and remain on the parent plant for a period of months following maturity. These characters show the dispersal ability of ragwort in different conditions. Though this system shows the theoretical possibility of dispersal, dispersal distances were short under the conditions of humidity, wind and vegetative structure in which ragwort infestations were to be found (Pool and Cairns, 1940; McEvoy and Cox, 1987).

2.1.5.2 Vegetative Propagation

Ragwort roots are vigorous vegetative propagators. The root system arises from the sides of the root crown. An average sized rosette (30 - 40 cm across) has about 50 - 100 roots. Plants more than one season old have large multiple crowns and up to 600 or 700 roots.

Roots are fleshy, especially in the young stage. The central woody cylinder is covered with a cell layer known as "endodermis". The "pericyclic cells" which have the power of active division lie just inside the endodermal cells. When the shoots were cut or injured these pericyclic cells used their power to produce new shoots (Pool and Cairns, 1940). This regeneration results in a number of new shoots. Pulling up plants in the rosette stage has the same effect, since a high proportion of damaged roots remains in the soil (Pool and Cairns, 1940; Meijden, 1970). Pool and Cairns (1940) reported that the following conditions were important in effective shoot production:

(1) The cut surface must heal over.

(2) The roots should be young with ample food reserves. In their experiments they found that from a rosette (young plant), recovery was 37.5% and from a flowering plant (old plant), the recovery was only 10%.

(3) A vascular cambium should be present.

(4) The cell walls of the dividing tissue (pericyclic cells) should be rich with phloem sap which is furnished when the plant is divorced from the roots. Higher concentrations of phloem sap around pericyclic cells might cause rapid production of new shoots.

At Lincoln, roots of potted plants were damaged on 16th May 1989. In early July, after about seven weeks, small shoots of about 2.5 - 5.0 cm could be observed.

2.1.6 Life History

In New Zealand, plants shed ripe seeds as early as the end of December. Peak fall is in March and April (Pool and Cairns, 1940). Under natural climatic conditions, potted plants (after a year) at Lincoln produced flowering stems in November, started to flower by the end of December and seeds fell in early February (pers.obs.).

Pool and Cairns (1940) reported that during less favourable times such as a dry summer or cold wet winter, seeds remained two or three months without germination. It has been reported by Thompson(1985) that ragwort seed has no inherent dormancy and will germinate at any time of the year.

The first true leaf appears about a month after germination and a few further leaves develop in the winter months. The formation of the root crown begins at an early stage, when the top is about 5 cm in diameter, and commences from a thickening of the top portion of the first root. If the plant grows without interference, a single crown is formed, generally about 5 cm in diameter and about the same deep. By December, rosettes have formed up to 30 cm in diameter. Depending upon the conditions, some plants then produce flowering stems while others remain until the next flowering season.

2.1.7 Control of Ragwort

In New Zealand, ragwort is not usually a severe problem on well managed productive land, but it is a successful weed on most of the steep hill country farms, dairy farms, and larger holdings of about 125-800 ha., where chemical or mechanical control methods are often uneconomic. Therefore, the choice of control measures is governed by the area, location, the economic value of the land and the potential cost of control.

2.1.7.1 Modified Farming Method for Ragwort Control

Pool and Cairns (1940) reported that many ragwort infested dairy farms had to be converted from dairying to sheep farming to get rid of the ragwort infestation. In some small dairy farms, sheep were purchased and run as a small flock with the dairy herd where the weed had got out of control. However, these methods reduced the dairy herd production by almost half, and caused loss of sheep from chronic ragwort poisoning. This method of control is only temporary since plants recovered following removal of sheep, even after 5-7 years of intensive grazing (Pools and Cairns, 1940; Schmild, 1972).

Schmild (1972) reported that change of land use in more remote hill country from agriculture to forestry was another method of successful control. However, this method wasn't practicable in all cases. According to Friend (1980), if the initial population of ragwort was high, adequate control was unlikely by using pasture and stock management control strategies alone. Wardle (1987) reported that stock manegement practices might encourage vegetative propagation and dormant seed germination through disturbance, and this might enhance the persistence of the ragwort population.

2.1.7.2. Mechanical Control

2.1.7.2.1. Cutting or Mowing Plants Before Flower Formation

Plants can be cut or mown before flower formation as a control method. This method prevented growth of seedings, but plants reacted to cutting by regenerating either stem buds or root buds (Radcliffe, 1969).

2.1.7.2.2. Pulling Flowering Plants and Burning or Burying

Another mechanical control method is pulling flowering plants and burying or burning them. If almost all the roots were pulled up, this method may give good control, since firing may destroy seeds and many plants. However, if root fragments remained in the soil, this method is not effective, as regeneration could occur. When shallow burying was practised instead of pulling up plants, there was a considerable regeneration from seeds and root fragments after ploughing (Radcliffe, 1969). Thompson (1985) reported that removing of flowering stalks had a great influence on ragwort population in undisturbed populations.

2.1.7.3. Chemical Control

In the past, sodium chlorate was widely used but it was not effective since it killed only foliage on contact. Unless the initial treatment was followed by repeated spraying, control was only temporary. Radcliffe (1969) has reported that good pasture management, supplemented by chemical spraying was the best method for paddock-scale control. She also reported three chemicals: 2,4-D (2,4-dichloro phenoxy acetic acid), picloram (4-amino-3,5,6-trichloro picolinic acid) and dicamba (3,6-dichloro-2-methoxy benzoic acid) as suitable since these chemicals have systemic action and killed both roots and shoots. It was reported by Friend (1980) that good control of ragwort has been obtained from autumn spraying with both 2,4-D ester (1.4 kg a.i. per ha) and 2,4-D amine (1.6 kg per ha). He also reported that 2,4-D ester at 1.6-2.4 kg a.i. per ha and 2,4-D amine plus dicamba at 1.6 and 0.4 kg a.i. per ha had given good control in the spring. According to Friend, though spot spraying with 2,4-D ester + diquat (2.0 g a.i. of each per litre) prevented seed set, it failed to reduced the rosette population in the following year. Friend (1986) reported another chemical, clopyralid (3,6-dichloropyridine-2-carboxylic acid), as very effective at (0.3 g a.i. per litre) for spot spraying at the rosette stage. At the flowering stage, clopyralid at the rate of 0.6 g a.i. per litre provided an excellent control. Though the seed viability of treated plants was poor, some seeds could produced seedlings. Martin et al. (1988) reported that spot treatment of ragwort plants with a mixture of picloram or dicamba with either 2,4-D or MCPA (4-chloro-2-methyl phenoxy acetic acid) usually provided adequate control.

2.1.7.4. Biological Control

Three insects species have been released in four countries for biological control of ragwort. Details of the releases summarized in Table 2.1 .

2.1.7.4.1 Biological Control of Ragwort Overseas

2.1.7.4.1.1 Canada

In the lower Fraser Valley in British Columbia, ragwort remains a serious socio-economic problem where there are many small farms with few cattle. The small field size and lack of specialized equipment makes chemical and mechanical control difficult and expensive. Another important thing is that the death of one or two cattle from ragwort poisoning was a serious loss to these family farms. In 1968 ragwort seed fly was introduced and it has widely established in south-western British Columbia but not establish in Prince Edward Island (Julien, 1987). Cinnabar moth was

introduced in 1963 and it has established throughout the ragwort infested regions of Canada (British Columbia, Nova Scotia, Prince Edward Island and New Burnswick) including Newfoundland where no releases were made (Harris et al. 1984), but not established in Ontario (Julien, 1987). Two species of flea beetle were introduced into Canada in 1971. Eventhough there was no control of weed, L. flavicornis has established on Vancouver Island. L. jacobaeae has established in Prince Edward Island and Nova Scotia providing no control of the weed (Julien, 1987). In British Columbia L. jacobaeae has established and increased in number to attack 95% of plants with 5-13 larvae plant. Pemberton and Turner (1990) reported that biological control of ragwort by flea beetle and cinnabar moth has been achieved to a lesser degree in Canada.

2.1.7.4.1.2 Australia

Ragwort is a serious problem in the southern high rainfall areas of Victoria and Tasmania. Nearly 400,000 ha are affected by ragwort in Victoria, ranging from high fertility dairy pastures to forest and abandoned farm land (Schmild, 1972). The worst ragwort areas were Gippsland (average annual rainfall ranges from 760-1520 mm) and the Otway ranges (average annual rainfall ranges from 760-1900 mm). In these areas ragwort occurred at elevations from sea level to 700 m on a variety of soil types.

Ragwort seed fly and cinnabar moth have not established in Australia (Julien, 1987). Heavy predation of larvae by scorpion flies and birds and disease problems were considered to be the reasons for the lack of success of cinnabar moth.

A species of flea beetle, L. jacobaeae was released in 1979 to control ragwort. It has well established causing high reduction in weed density at some sites. Another species of flea beetle L. flavicornis was introduced in 1985 and since its introduction, it has been released at approximately eighty different sites in Tasmania and is considered to be established at seven sites (Friend, 1986). In the south of the State at Lachlan a large population has built up occupying an area of approximately five ha. According to Julien (1987) effect of this species is still under evaluation.

2.1.7.1.3 United State of America

Ragwort is a range weed along the north west Pacific coast of the United States. This area of infestation extends from near Fort Bragg, California, to the vicinity of Centralia, Washington (Frick, 1972).

Table 2.1 Biological control agents used against Senecio jacobaea.

Biological control agent	Country obtained	Country released	year
Longitarsus flavicornis	Britain	Canada	1971
(Stephens)	Spain	Australia	1985
(Coleoptera:Chrysomelidae)			
Longitarsus Jacobaeae	Italy	U.S.A	1969
(Waterhouse)	Italy	U.S.A	1971
(Coleoptera:Chrysomelidae)	France	Australia	1979
	Oregon	New Zealand	1983
Pegohylemyia jacobaeae	England	New Zealand	1936
(Hardy)	England	Australia	1959
(also referred to as	France	U.S.A	1966
Hylemyia jacobaeae	Italy	Canada	1968
(Meade)			
(Deptera:Anthomyiidae)			
Tyria jacobaeae	England	New Zealand	1928
(L.)	England	Australia	1930
(also referred to as	England	Australia	1936
Callimorpha jacobaeae	England	Australia	1955
(L.)	Italy	Australia	1955
(Lepidoptera:Arctidae)	France	U.S.A	1959
	England	Australia	1960
	Switzerland	Australia	1962
	Austria	Australia	1962
	France	Canada	1963
	France	Australia	1978

According to Julien (1987), ragwort seed fly was released in California, Oregon and Washington. Though the insect established in California, the release area was destroyed. Insect is well established in Oregon and in Washington. Cinnabar moth was released in California, Oregon and Washington. In California the insect helped to reduce the weed to low levels at one site. In Oregon and Washington, though the effect on density was less, the insect defoliated the weed annually at release sites (Julien, 1987). Two European biotypes of ragwort flea beetle were introduced into California, Oregon and Washington. It has reduced the weed to less than 1% at one site in California and the beetle increased rapidly in number at other sites in California, Western Oregon and Washington (Frick, 1972; Julien, 1987). Pemberton and Turner (1990) reported that biological control programme in nothern California was very successful due to the excellent complementary actions of the cinnabar moth and the ragwort flea beetle. According to Pemberton and Turner (1990) significant biological control of ragwort by flea beetle and cinnabar moth has also been achieved in Redwood National Park, Del Norte Co., California.; Oregon; and Washington.

2.1.7.4.2 Biological Control of Ragwort in New Zealand

Table 2.1 shows the biological agents used for the biological control of ragwort and the years of release. Two species of ragwort seed fly, Botanophila (Pegohylemyia) seneciella (Meade) and B.(=P.) jacobaeae (Hardy), were released. Syrett (1989) reported that only B. jacobaeae had established. It was found in a small area of the central North Island. Dymock (1987 and 1988) reported that ragwort seed fly could not be considered as an effective biological control agent for ragwort in New Zealand. It has also been reported (Syrett, 1989) that attempts to transfer this insect to other areas were unsuccessful.

Initially 3.5 million cinnabar moth eggs were distributed throughout the country and in some areas initial establishment was very good, though the population failed to be maintained for more than two or three years (Syrett, 1989). To date, cinnabar moth persists in abundance only throughout the southern North Island. Insects have been collected from this area and re-releases were made in one area in the North Island and numerous areas in the South Island. No records could be obtained about long term establishment of cinnabar moth from new releases.

Establishment of ragwort flea beetle in the northern part of the North Island and in some areas of South Island (Hanmer, Inangahua) has been very successful, though the first releases on the South Island were disappointing (Syrett, 1989). In most of the established sites, populations have increased successfully in the second and the third season. In cages at Lincoln, Canterbury, insect numbers increased five times and three times over two successive years (Syrett, 1989). During my

study, some biological evaluations have been undertaken in artificially manipulated environmental conditions.

2.2 RAGWORT FLEA BEETLE Longitarsus jacobaeae(Waterhouse)

2.2.1 General Description

The current taxonomic position of ragwort flea beetle is as follows:

Order: Coleoptera
Suborder: Polyphaga
Superfamily: Chrysomeloidea
Family: Chrysomelidae (leaf beetles)
Sub family: Alticinae/Halticinae (flea beetles)
Genus: Longitarsus
Species: L.jacobaeae (Waterhouse)

There are about eighty Longitarsus species in South America (Scherer, 1988). These species are difficult to determine even with the aid of a microscope. In addition, several species can be found simultaneously on the same host plant (LeSage, 1988). Due to this reason, L. jacobaeae and L. flavicornis were always mis identified in the field. Although these species are indistinguishable externally, thare are some genitalic differences to identify these species (Shute, 1975). Newton (1933) reported that though there were seven species of Longitarsus in Europe, only four were associated with Senecio, and L. jacobaeae was one of the most abundant. Harris et al. (1984) reported that L. jacobaeae was one of the six species of Longitarsus known from Senecio in Europe. LeSage (1988) reported that eight European species of Longitarsus had been introduced into North America, either by accidentally or intentionally.

The adult beetle is a tan colour at emergence, but becomes light brown when it is mature and dark brown when it is old. The elytral suture is not darkened; the tibial spur is short; and pronotal and elytral punctation is faint (LeSage, 1988). The females are about 2.8-3.75mm long and the males 2.5-3.25mm long. In females, the last sternite is convex, and in males a circular concave depression is obvious in the last visible abdominal sternite (Frick, 1971).

The egg is elongate, oval with rounded ends 0.66 mm long and is rather less than half as broad as long. The egg is light yellow just after laying but becomes dark brown with time (Newton, 1933).

Eggs are laid either on the root crown or near them in the soil (Frick, 1970). The surface of the egg consists of a network of polygonal pits.

The following larval description was obtained from Newton (1933):

> *The young larva just after hatching is about 1.5 mm long and 0.25 mm in width. The head capsule, prothoracic shield and anal plates are dark greyish brown, the head capsule being darkest. The legs and segmental plates are faintly brown. The fully grown larva is about 6 mm long and just over 1 mm broad. It is white in colour, elongate in form. The head capsule is dark brown, anal plate and prothoracic shield brown and legs light brown. The three short stumpy pairs of thoracic legs show the usual five segments. The first eight abdominal segments are similar, the ninth carries the anal plate above, and beneath the anal proleg containing the anal opening on the reduced tenth segment. One thoracic and eight abdominal spiracles are present. The head capsule is well chitinised above, weakly beneath and shows the fronto-lateral and epicranial sutures. Dorsally the prothoracic shield covers the prothorax. Generally the anal plate is broad, rounded and slightly depressed apically. In sorting larvae, Newton found that larvae fell into two groups of approximately equal numbers depending on a slight difference in the shape of the anal plate. In one group the anal plate was slightly broader and in the other group it was narrower and more elongate. It has been thought that this could be a sexual difference.*

The pre-pupa assumes the normal recurved shape and builds up the earthen cell in which pupation takes place. The pupa is white and resembles the typical flea beetle pupa. The seventh abdominal segment is shield shaped with the eight and ninth segments reduced and the ninth bearing the brown anal horns.

2.2.2 Distribution

The world distribution of L jacobaeae includes the British Isles, Europe, Nothern Africa, Siberia, Kazakhstan, Kirgizia, Dagestan, Morocco, British Columbia in Canada and California and Oregon in the United States (LeSage, 1988).

2.2.3 Life Cycle

Literature for this topic is reviewed from Frick (1970), Frick (1971), Frick and Johnson (1973), Harris et al. (1984), and Syrett (1986).

There are several biotypes of L. jacobaeae, distinguished by their life cycles. The Swiss biotype was found at Delamont in Western Switzerland on S. jacobaea. The Italian biotype came from near Rome, Italy, on closely related S. erraticus. Another biotype was from France.

The eggs of L. jacobaeae are laid either on the root crowns or near them in the soil. Over 100 eggs per female may be produced over a period of several months. Most of the eggs hatch during late autumn. Before the winter, larvae enter the root crown where they feed throughout the winter. They do not diapause but the larval period can be prolonged by cold weather (Frick, 1970). Syrett (1986) reported that some larvae were observed in the spring and even in early summer. The larvae require a biennial or short-lived perennial root crown in which they complete their feeding. The larvae feed primarily on the outer tissues of the root crown, specially the epidermis and cortex which are primary tissues. The phloem tissue of the vascular system is eaten to a lesser extent, and the xylem is eaten only to reach the central parenchyma tissues, which are extensively mined. When they are very numerous, larvae bore upwards into the petiole of the lower leaves and cause leaves to collapse and die. If the larvae feed externally on lateral roots, brown grooves can be seen on root surface.

Pupation occurs in early summer in the soil near the plant. The pupal stage requires about eighteen days. The adult appears in mid summer and they continue emerging till early autumn.

Adults of the Italian biotype have a oligopause type of dormancy. Oligopause is described as "a response to prolonged though moderate environmental adversity which is associated with seasonal climatic changes" by Mansingh et al. (1972). Adult dormancy begins about two weeks after emergence when feeding drops, activity ceases and beetles react negatively to light by seeking darkened places to hide. Due to the oligopause of adult beetles, though the beetles emerge in the spring, they do not oviposit until the autumn. Once laid, eggs hatch in two to three weeks. In contrast, the Swiss strain does not oligopause; adults start egg laying about 13 days after emergence in the spring, but the egg has an aestival diapause that delays hatching until suitable climatic conditions occur. This duration varies from one to nine months. Normally eggs hatch in autumn. The ultimate result of both these strategies is to overcome hot dry conditions which are

unsuitable for eggs and ensure the hatching of eggs when root reserves are high, which in turn provides adequate food sources for larvae.

2.2.4 Adaptation to Different Climates

In Britain, Newton (1933) found that adult emergence started at the end of July. Peak emergence was in late August. The first eggs were seen in mid August. Those laid in late summer hatched in about one month, but later ones did not hatch till the following spring, so that the winter was passed in both egg and larval stages. Frick (1971) suggested that the Swiss biotype would not survive in a climate with a dry summer, as the eggs are vulnerable to desiccation. Since the Italian biotype originated from a dry summer type of climate they survived well in areas with dry summer climatic conditions. A very good example was the Pacific coast of the United States. In California, Frick and Johnson (1973) reported that adult females of the Italian biotype delayed oviposition due to the dry summer conditions in June, July and August, so the Italian biotype has survived and increased in number in these areas. Syrett (1986) noted that in New Zealand, Italian biotype adults started to emerge in early summer (December) and continued emerging until late February; they fed actively for two or three weeks and then aestivated. They became active again in autumn (March), fed heavily on foliage for several weeks and started egg laying in April. Larvae fed mainly in the root crown during winter (from May onward) and pupation occurred in the spring (November and December). Timing of the life cycle was observed to vary slightly from year to year and it was thought that this might be due to readjustment to the varying climatic conditions.

CHAPTER THREE

INFLUENCE OF TEMPERATURE ON EGG DEVELOPMENT

3.1 INTRODUCTION

Suitable temperatures are necessary for all insect eggs to hatch and there is a threshold temperature below which hatching does not occur (Chapman,1982). This temperature varies for different insects but they may remain viable below this temperature for a considerable time.

A thorough understanding of development of the egg was essential before starting studies on larval stages. Understanding morphological changes and development time at different temperatures would help to obtain healthy first instar larvae whenever necessary without depending upon the season and peak period. At the same time these studies would give some information about the life cycle at various temperatures. Lower threshold temperature might help to get some idea about overwintering ability of eggs of L. jacobaeae.

Newton (1933) described the size, shape and the colour of the ragwort flea beetle egg. That description is included under the general description of the beetle(section 2.2.1 in chapter two). Frick and Johnson (1972) reported on the effect of higher and lower temperatures on egg development. They found that at 24 degrees it took 3 weeks for 50% hatching and at 10 degrees it took 9 weeks for 50% hatching. When eggs were kept near freezing they survived for 6 months and still showed 51% hatching. Cullen (1981) has reported that hatching of ragwort flea beetle eggs could be delayed for 4-5 months by keeping at 4 degrees.

The situation is potentially complicated by the fact that 3 different strains of L. jacobaeae from different parts of Europe have been used for biological control purposes and different experiments have been conducted with each of them. However, all the data on egg development has been collected on the Italian strain. The purpose of this part of the study was to investigate morphological changes occurring throughout the development of the egg, to find the development rates of the egg at different temperatures and to develop a lower threshold temperature for the egg of L. jacobaeae.

3.2 MATERIALS AND METHODS

3.2.1. Obtaining Newly Deposited Eggs

During the peak egg laying period, twenty pairs of male and female adults were collected from the insectory. A bouquet of six fresh ragwort leaves was held in a vial stoppered with cotton wool and filled with water. This vial and the collected insects were kept in a 4-liter plastic cylinder 250mm tall and 160mm in diameter which was lined with moist filter paper on the bottom and covered with a piece of white terylene gauze (Figure 3.1). This method was described by Cullen (1981) and Syrett (1985) for adult oviposition and feeding. Syrett (1985) also mentioned that with this method leaves remained fresh even after 10 days. However, the bouquet of leaves was removed every other day and a fresh bouquet of leaves was provided. Removed leaves and the cotton wool were inspected carefully for eggs, and eggs were removed using a fine moist camel hair brush (000 Haydn 100 series finest sable).

3.2.2 Egg Development Studies

Newly deposited eggs were carefully placed in hatching dishes. Hatching dishes were 5cm diameter pyrex glass petri dishes and lids, lined with moist, 5.5cm, no.2, Toyo filter paper circles. Thirty newly deposited eggs were placed in each dish. Eight of these dishes were placed in "Calvert" plastic food container which was lined with moist filter paper sheet and covered with a lid (Figure 3.2). One of these food containers was kept at each of 10, 15, 18, 20, and 23 ^{0}C in Contherm Scientific constant temperature cabinets, and another one was kept at 4 degrees in a refrigerator. Eggs were observed daily for colour change and hatching.

Figure 3.1 Ovipositing cage for adults as used in egg collecting experiment

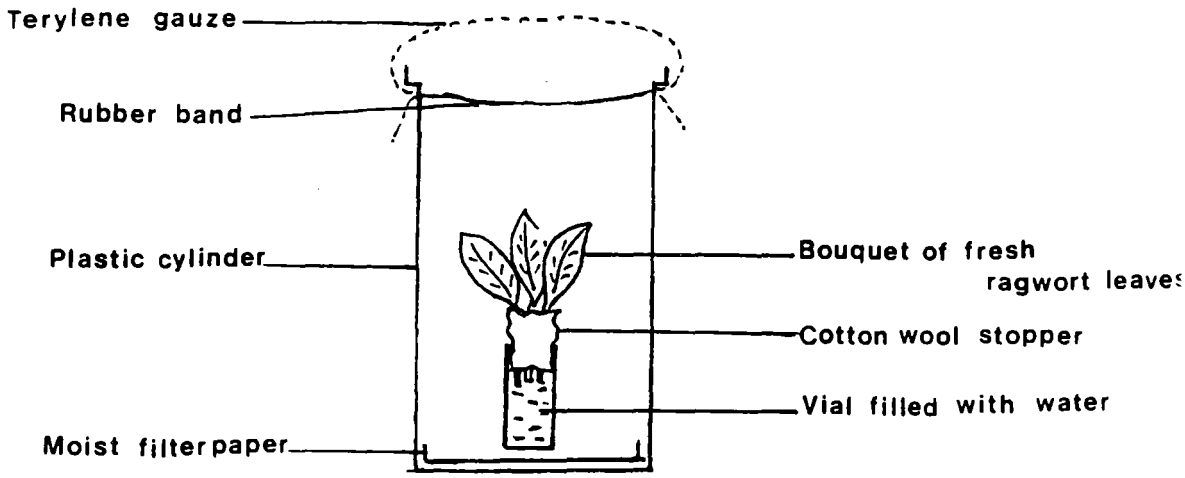
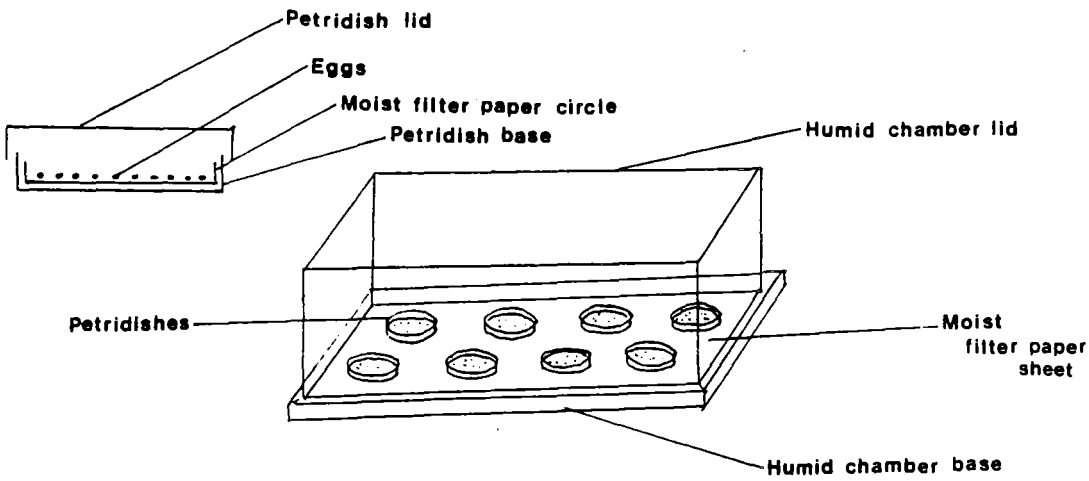


Figure 3.2 Hatching dishes and the humid chamber as used in egg development experiment.

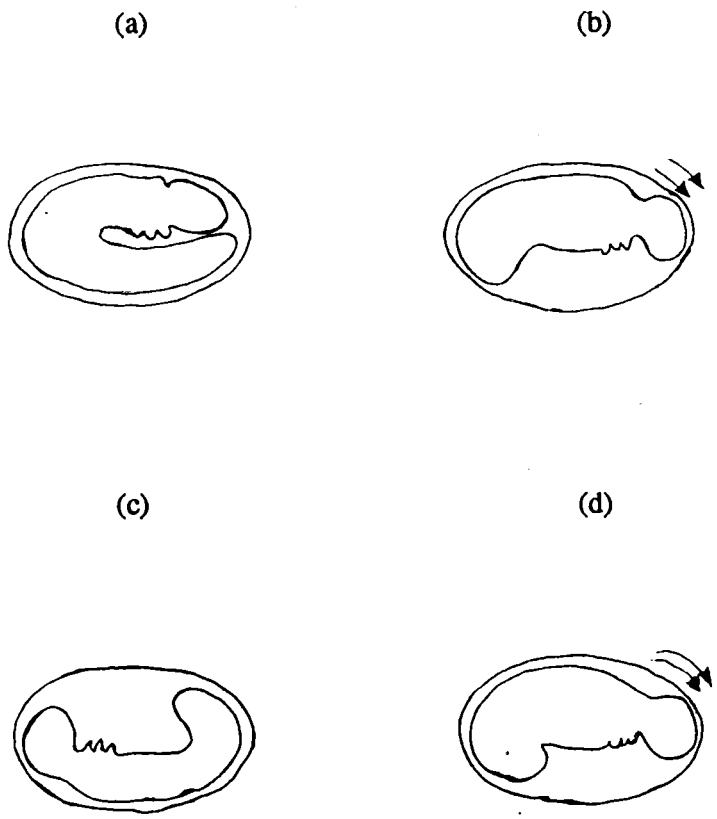


3.3 RESULTS AND DISCUSSION

3.3.1 Changes During Development

Plate 3.1 shows the colour variation from laying to hatching. At the time of laying only the yolk is visible, but when it is maturing the yellow colour changes to brown. Within 1-2 days of hatching a black spot appears on the egg. This is the head capsule and with careful observation the larval head and thoracic legs can be observed through the egg wall using a binocular microscope. At the time of hatching larval movement in the egg can also be observed. Figure 3.3 presents the stages of movement of larvae. In this movement, the larvae rotates inside the egg, tending to turn downward and upward while pressing the egg wall with its head. After few such movements, the egg wall ruptures and the larvae comes out (plate 3.2).

Figure 3.3 Larval movement during egg hatching



a) resting.

b) first downward movement while pressing the wall.

c) upward movement without pressing the wall.

d) second downward movement while pressing the wall.

Plate 3.1 Morphological changes in ragwort flea beetle eggs from deposition to maturity (from left to right).

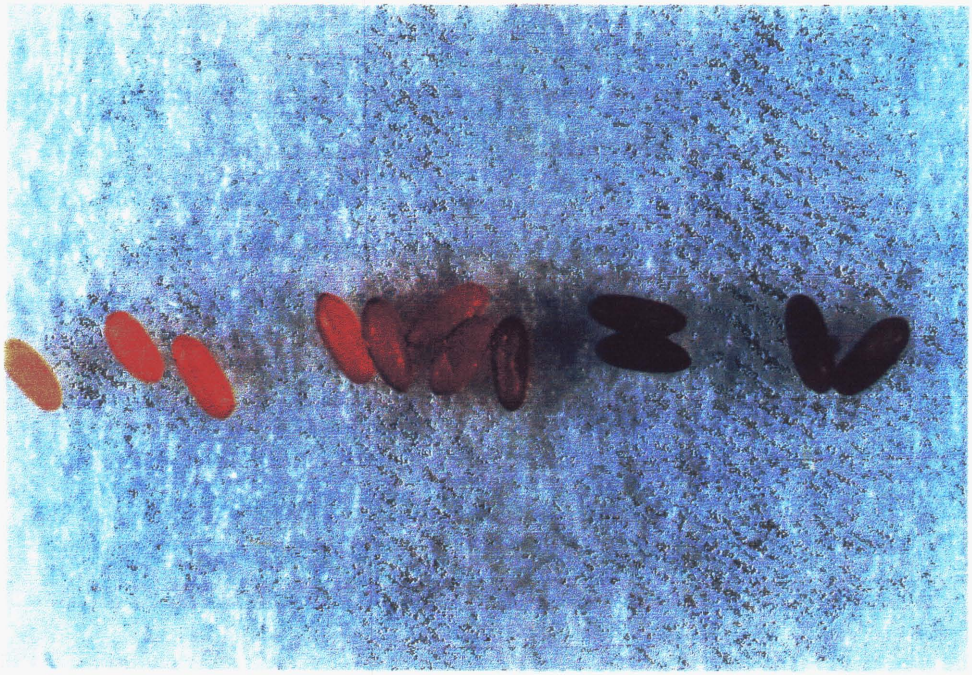


Plate 3.2 Egg eclosion and newly hatched first instar larvae.

3.3.2 Temperature and Development

For each insect species, the temperature below which no measurable development occurs is its threshold of development (Campbell et al., 1974). The relationship between the rate of development and temperature is usually of the type shown in Figure 3.4. Over a range of temperatures B, the relationship can be represented by a straight line which, when extended cuts the x-axis at the temperature threshold t (Campbell et. al.,1974). The number of degree-days above threshold required by an insect to complete its development can be obtained by the reciprocal of the slope b of the straight line.

Figure 3.4 The relationship between the rate of insect development and temperature, showing the non-linear portions A, C, and the linear portion B, used to estimate the threshold of development (t) by extrapolation. (Obtained from Campbell et. al., 1974).

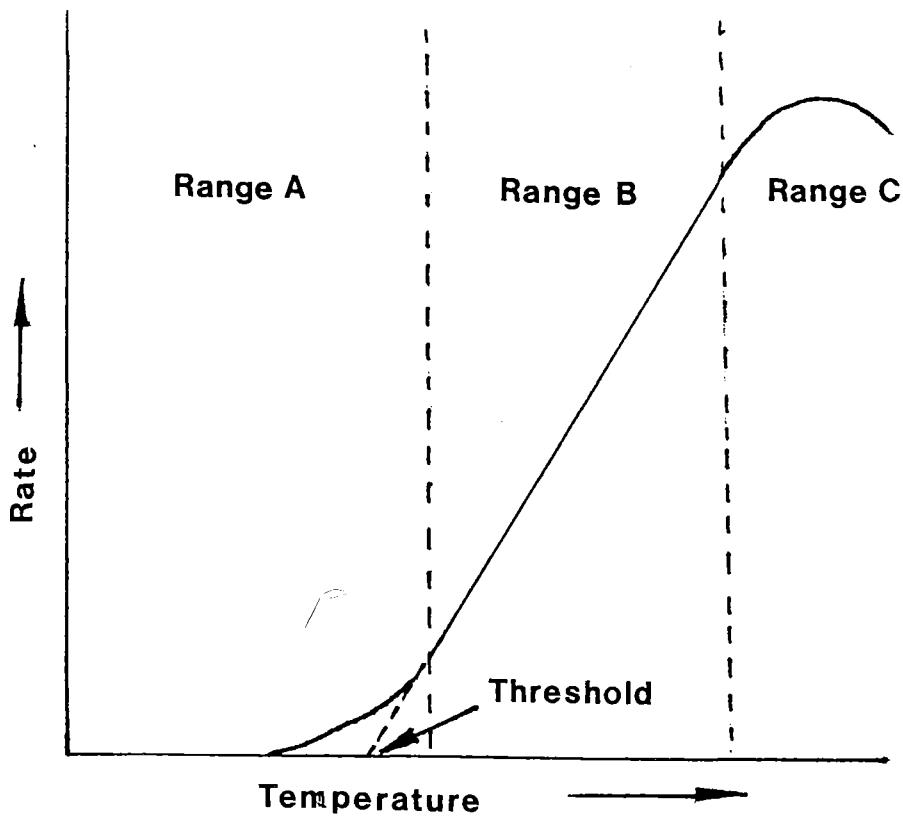


Table 3.1 presents the mean nuber of days taken for 50% hatching of L. jacobaeae eggs, and the rate of development.

Table 3.1 Effect of different temperatures on egg development of ragwort flea beetle.

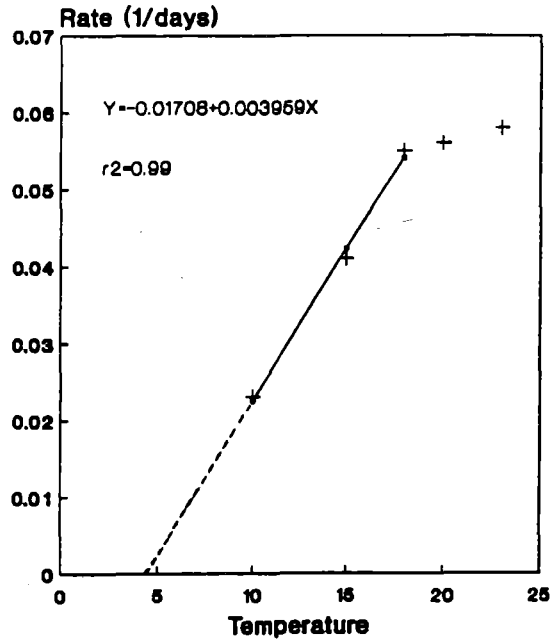
Temperature	No. of days taken to 50% hatching (mean of 8 units)#	Rate (1/mean no. of days)
23	17.12	0.058
20	17.62	0.056
18	18.13	0.055
15	24.25	0.041
10	42.38	0.023
4	no hatch*	

* These eggs were transferred to the 20 ^{o}C after 110 days and more than 50% hatching occoured after 15-20 days.

\# One unit contained 30 eggs.

Using the Campbell et al. (1974) method linear regression analysis was done for the rate of development (obtained from mean no. of days) versus different temperatures. Regression line is presented in Figure 3.5. This line did not include the values at 20 ^{o}C and at 23 ^{o}C which did not appear to be part of the linear function. The lower threshold temperature for development was determined by using the regression equation $Y = -a + bX$, where, the rate Y is equal to 0 and temperature (X) is equal to (-a/b) which is 4.3 ^{o}C. The number of degree days required for egg development was determined by calculating the reciprocal of the slope (1/b) which is 252.5 degree days. Value of the coefficient of correlation is 0.99.

Figure 3.5 Development rate for the egg of L jacobaeae under various constant temperatures showing the estimated lower threshold temperature(base temperature).



3.4 CONCLUSION

Observations of colour variation of the egg and the larval movement confirm the exact timing of hatching. These results could be used to obtain newly hatched first instar larvae for larval development experiments. Results of development in relation to temperature confirm that the eggs of the ragwort flea beetle can be stored at 4 ^{o}C and used for incubation even after 16 weeks. A lower temperature threshold of 4.3 ^{o}C showed that they have the ability to over winter in the field and then hatch with the return to favourable conditions. These results would help to timing of obtaining first instar larvae. At the same time these results show the capability of the egg to overwinter in the field, and its ability of hatch in favourable climatic conditions.

CHAPTER FOUR

LARVAL REARING

4.1 INTRODUCTION

Larval feeding in the root crown by the ragwort flea beetle is considered to be the most destructive damage to ragwort. So for biological control purposes it is important to have a good understanding of the larval biology. Frick (1970) reported that there were three larval stages, and the activity of larvae was slowed by cold weather. He also reported that in the laboratory at temperatures of 24 degrees for 12 hours and 12.75 degrees for 12 hours, the larval and pupal periods lasted from 8-20 weeks. Frick,1971 reported that the 1st and 2nd larval stages each required an average of 16-17 days, and the 3rd instar required about 1-3 months. No other reports were found on studies of larval development and behaviour with respect to different temperatures. A more detailed study could give information about the duration of different larval instars and at the same time would give a measure of root destruction by different instars at different temperatures. This type of study requires a suitable larval rearing method to enable the insect to develop while allowing observations to be made on the immature stages without disturbing them.

The first experiment described below, was designed to find a suitable rearing technique. The second experiment was designed to observe behaviour of different larval instars, to find out development times and rates and feeding behaviour with respect to different temperatures.

4.2 EXPERIMENT ONE, REARING TECHNIQUES

4.2.1 Plant Material

4.2.1.1 Root Cuttings

Pool and Cairns (1940) reported that if the crown is cut off and the roots are left in the field,new green shoots appeared in about six weeks, and an individual root could produced several shoots. When the root was cut or injured, the pericyclic cells increased their power of active division and produced new shoots.

4.2.1.1.1 Materials and Methods

Small ragwort plants collected from the field were potted up with potting mix and kept under shade. Plants were maintained to get healthy plants about a year old. Roots of these plants were thoroughly damaged with a sharp knife. New green shoots could be obtained after six to seven weeks. These shoots were carefully separated and grown in metal trays filled with potting mix. After about a month these plants were removed and washed to remove soil particles, placed in 10% alcohol solution for five minutes and rinsed several times with distilled water.

4.2.1.2 Seedlings

4.2.1.2.1 Materials and Methods

Twenty four ragwort plants were maintained in pots until they flowered. At Lincoln flowering stalks appeared in November, and flowering started by the end of December. When flowers began turning brown, flower heads were covered with muslin bags to prevent seed from blowing away. Seed fall occurred in early February. In mid February a germination test was carried out. By the end of February almost 100% germination had occurred. At that time ragwort seeds were separated and placed on metal trays filled with potting mix. These trays were kept in the insectory and moistened whenever necessary. After about a month seedlings were at a proper size to transfer to rearing vessels. Healthy and well grown plants were selected and treated as before, before being used in experiments.

4.2.1.3 Results and Discussion

With both methods healthy plants could be obtained continuously. In my experiments I observed several problems in the root cutting method. One problem was the development of sciarid larvae on decaying mother root portions; most of the time they tended to go on to damage the new root system. Another problem was the development of saprophytic fungi on decaying root portions. Seedlings therefore, provided a better root system than root cuttings.

4.2.1.3 Conclusion

For my experiments, my main objective was to obtain a good healthy root system without pest organisms, so I selected the seedling method to obtain plant material.

4.2.2 Rearing Vessel

4.2.2.1 Survey of Rearing Methods for Root Feeding Laevae

A thorough literature survey of methods used in rearing root feeding insect larvae for experimental purposes was carried out. Table 4.1 shows the rearing methods found in the literature survey.

Table 4.1 Some rearing techniques developed for root feeding larvae

Rearing technique	Insect used	Author/Year
1. Thin slices of root crowns	Longitarsus jacobaeae	Frick, 1970
2. Bare root crowns	Longitarsus jacobaeae	Cullen, 1981
3. Storage portions of other crops	Prionoxystus robiniae	McFarland, 1970
4. Roots of living plants		
a) grown in water culture	Diabrotica vittata	Searls, 1927
b) grown in soils	root feeding insects of Pineapple	Illingworth, 1964
	Longitarsus jacobaeae	Frick, 1970
		Cullen, 1981
	Sitona humeralis	Sue et al.,1980
c) grown in moist filter paper or absorbent cotton	Diabrotica vittata	Robinson and Arant, 1931
	Longitarsus albineus	Hurber, 1979
	Sitona humeralis	Aeshlimann, 1986
	Longitarsus jacobaeae	Cullen, 1976
	Diabrotica vigifera	Jackson and Davis, 1978

From these methods, I have chosen the methods described by Seals (1927), Hurber (1979), and Aeshlimann (1986) to observe larval instars and their behaviour without disturbing the system. I made some modifications in materials used due to convenience and availability.

4.2.2.1.1 Modified Seals(1927) Method

In this method, plastic petri dishes containing plaster of paris were used to grow plants on. Both halves of the 8.9cm diameter petri dishes were used, and a V-shaped groove was made in the side wall of each half. The cut surface was made smooth using a dissecting blade after heating over a flame. Plaster of paris mixed with distilled water was poured in to the bottom half of the petri dish as far as the bottom of the V-shaped groove, and permitted to set. Small ragwort plants obtained as in 3.2.1 were placed in petri dishes with the roots spread out upon the plaster of paris, and the leaves extending outward through the groove(Plate 4.1 and 4.2). Nine cm, no.2, Toyo white filter paper with a small curved cut was laid over the roots, fitted to the sides of the dish and saturated with distilled water. Top half of the petridish was covered with black paint to keep the roots in darkness. Then the bottom half was covered with the top half of the petridish in such a way to allow the shoots to grow out through the groove. Six of these petri dishes were kept in a "Calvert" plastic food container lined with moist filter paper and covered with the plastic lid. Four of these containers were kept at 20 degrees Celceus in a Contherm Scientific Controlled Temperature Cabinet. Plants were observed daily and whenever necessary distilled water was supplied to the plants and to the filter paper lining the plastic container.

4.2.2.1.2 Modified Hurber(1979) and Aeshlimann(1986) Method

Hurber (1979) and Aeshlimann (1986) reared Longitarsus and Sitona larvae on plants grown in 800ml unmarked lipless beakers. Due to unavailability of these beakers in the quantities required, I used 1000ml, marked beakers. A disk of 11.0cm, no.3, Whatman filter paper with slightly upturned edges was fitted inside the bottom of the beaker. The beaker was then lined on the inside with a close fitting cylinder of thick blue denim cloth stitched at the join and ending at the same height as the beaker. Before lining the beaker, the denim cylinder was soaked in distilled water. One third of the beaker was filled with sand to position the denim cloth, and 3-4 plants (depending on the size) were arranged around the beaker between the glass and the denim cylinder with their root collars about 20mm below the top edge of the denim cloth. Since it was necessary to avoid the marked area of the beaker, most of the time 3 plants per beaker was quite adequate. Once the plants were positioned, the other 2/3 of the beaker was filled with sand. Then the sand was

Plate 4.1 Larval rearing chamber as described in the modified Searls (1927) method.

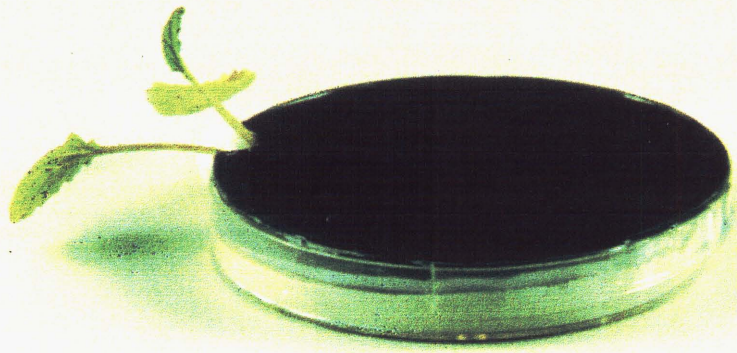
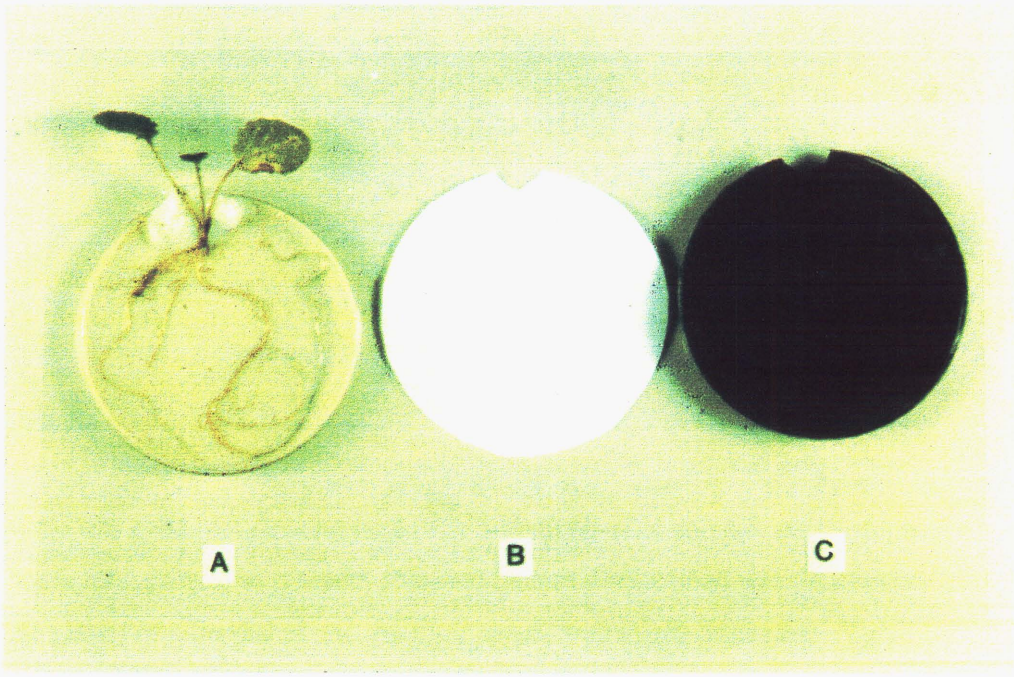
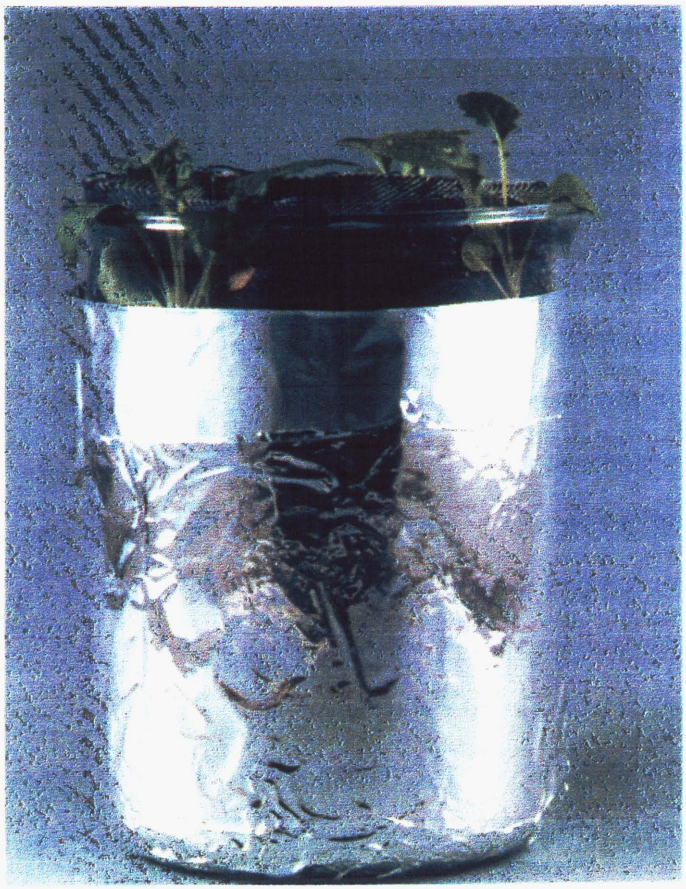


Plate 4.2 Larval rearing chamber as described in the modified searls (1927 method; top half of the petridish has been removed and placed beside.



moistened with distilled water. The beaker was kept in a slightly bigger cylinder made out of 450mm wide catering aluminium foil, to exclude light (plate 4.3 and 4.4). To avoid development of pest organisms, the beakers were kept in 60x60x60cm fine terylene cages supported by steel stakes. The sand was moistened with distilled water whenever necessary. After about two months there was sufficient root development to permit larval introduction (plate 4.3 and plate 4.4 and 4.5).

Plate 4.3 Larval rearing vessel as described in modified Hurber (1979) and Aeshclimann (1986) method.



4.2.2.2 Results

Though there was good root development in both methods after about 8 weeks, the beaker method showed better root growth. A problem observed in petri dish method was the contamination of most of the plaster of paris dishes and the filter papers with micro-organisms. These contaminations sometimes affected the plants. Compared to this method, the beaker method provided a healthier and better developed root system (plate 4.5).

Plate 4.4 Larval rearing vessel as described in modified Hurber (1979) and Aeshclimann (1986) method; aluminium cap has been removed from the beaker with growing plants and placed beside.

Plate 4.5 Beaker with ragwort plant to show well grown root development after 2 months.

4.2.2.3 Conclusion

Since my main objective was to rear good healthy larvae till they came up to the pupal stage (2-4 months), I required a method which would provide a good sanitation with adequate food material for larvae over an extended time period. Therefore, I selected the beaker method for my rearing technique.

4.3 EXPERIMENT TWO, LARVAL DEVELOPMENT

4.3.1 Obtaining First Instar Larvae

4.3.1.1 Collecting Eggs

Eggs were collected as described in Chapter 3, section 3.2.1 .

4.3.1.2 Storage of Eggs

To find the development time of larvae at different temperatures, it was necessary to obtain first instar larvae at the same time for all the treatments. In the egg development experiment it was observed that the eggs could be stored at 4 ^{o}C and used for incubation even after 16 weeks. Therefore, collected eggs were placed in polystyrene boxes lined with a moist filter paper sheet and stored at 4 ^{o}C in the refrigerator. The filter papers were moistened with distilled water whenever necessary.

4.3.1.3 Obtaining First Instar Larvae

In the egg development experiment it was found that at 20 degrees eggs took about 15-20 days for more than 50% eclosion. Therefore, 20 days before the day I planned to introduce larvae to plants in rearing containers, the polystyrene boxes containing eggs were removed from the refrigerator and placed in a constant temperature room at 20 ^{o}C and 65-70% relative humidity. Filter paper was moistened daily with distilled water.

4.3.2 Rearing Laevae on Roots of Ragwort Plants

4.3.2.1 Maintaining Rearing Vessels in Different Temperatures

Two weeks before the larvae were introduced, rearing vessels were kept in constant temperature rooms to make sure that the plants could evenly survive over the whole range of temperatures. The temperatures used were 10, 12, 15, 18, and 20 ^{o}C. In all rooms humidity was maintained at 65-70 percent and the photoperiod was maintained at 10 hours light and 14 hours dark using anglepoise lamps with 160w blended mercury bulbs and timers. Since the water requirement varied with different temperatures plants were observed daily and distilled water was provided whenever necessary.

4.3.2.2 Introduction of First Instar Larvae to Ragwort Plants

At the time of egg hatching, newly hatched first instar larvae were removed using a fine camel-hair brush (same as described in 3.2.2.1). Each larva was carefully placed on a lateral root, each plant being infested with one larva. Each temperature involved 8 vessels each containing three plants, i.e. 24 plants and 24 larvae. When there was no more root material for feeding, larvae were transferred to new plants

4.3.3 Observations

Observations were carried out every other day. Larvae and the feeding damage were observed by removing the aluminium cup which covered the beaker. More detailed observations were made as follows: 11cm, no.3, Whatmann filter paper circle with upturned edge was fitted inside the denim cylinder to cover the sand without disturbing plants. The beaker was then turned horizontally and placed under a Wild M4A binocular microscope. The observations made were: head capsule width, larval length and width at rest, moulting (whenever possible), and the feeding behaviour. When larvae were ready to pupate they were transfered to petri dishes with moist soil. Coloured photos were taken at different stages whenever possible.

4.3.3.1 Head Capsule Width

Newton (1933) reported that there were 3 larval instars in the ragwort flea beetle. However, to confirm each instar the head capsule width of newly hatched first instar larvae and field collected larvae throughout the year (obtained from DSIR,Lincoln) was measured using an eyepiece graticule

in a Wild M4A binocular microscope. Results of these measurements were used to confirm the development time of different instars at different temperatures.

4.3.3.2 Larval Length and Width of different Instars

When the larvae were not moving their length and width were measured.

4.3.3.3 Moulting

Whenever possible, colour change of the head capsule and the anal plate, cracking of the head capsule, and moulting movements were observed and noted. These observations made it possible to confirm the exact date of moulting.

4.3.3.4 Feeding Behaviour

Though it was difficult to get exact measurement of feeding, feeding behaviour was observed to gain a general impression of feeding in different instars.

4.3.4 Results and Discussion

4.3.4.1 Head Capsule Width and Larval Instars

The head capsule widths of newly emerged first instar larvae and field collected larvae are plotted on the frequency histogram in fig.4.1. Mean measurement of instar sizes and inter instar ratios are presented in table 4.2.

It is obvious that head capsule width of L. jacobaeae larvae fall into three distinct groups. The ratios between the mean measurements of successive instars are 2:1 = 1.35 and 3:2 = 1.36. The similarity of these ratios between successive instars indicates that no intermediate instar had occurred. Therefore, it could be said that the Dyar's constant for L. jacobaeae at each moulting is 1.35 (the mean value of I-I ratios) and there are only three instars during the larval period. Coloured photographs were taken to show the size difference of three instars and shown in plate 4.6.

Table 4.2 Instar sizes and inter instar ratios of head capsule width of L. jacobaeae.

Instar	Modal width (mm)	Mean width (mm)	Range (mm)	I-I ratio
1	1.3	1.4	1.2 - 1.6	
2	2.0	1.9	1.6 - 2.2	1.35
3	2.5	2.6	2.2 - 3.0	1.36

Figure 4.1 Frequency histogram of head capsule widths of L.jacobaeae larvae to show the three larval instars.

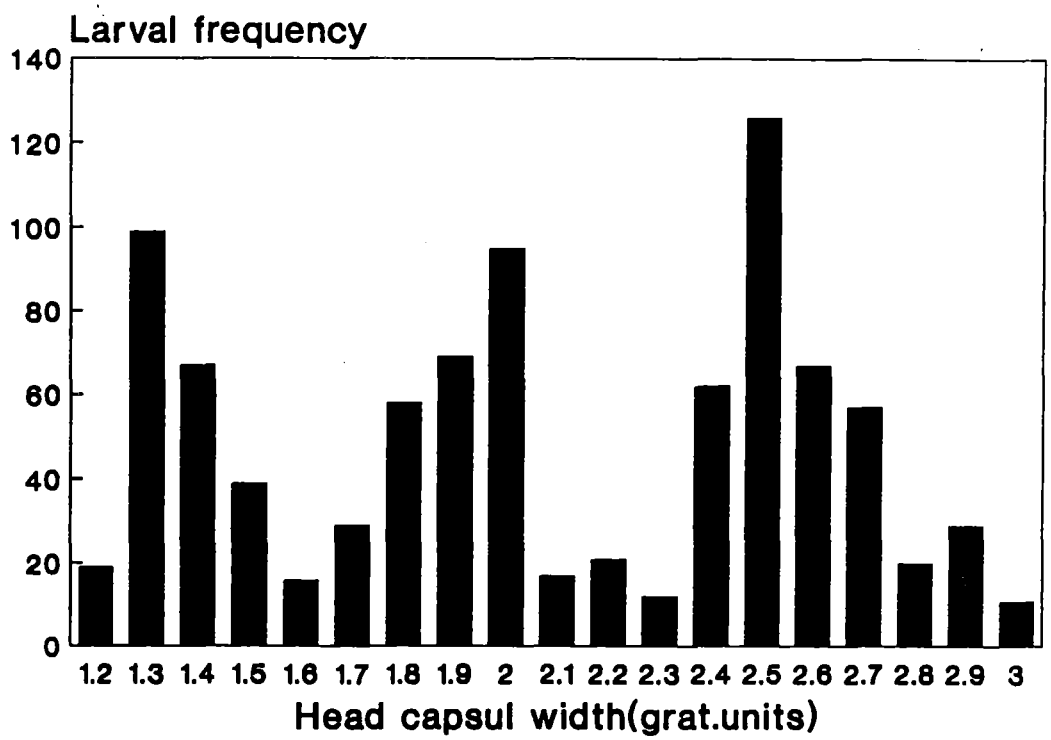
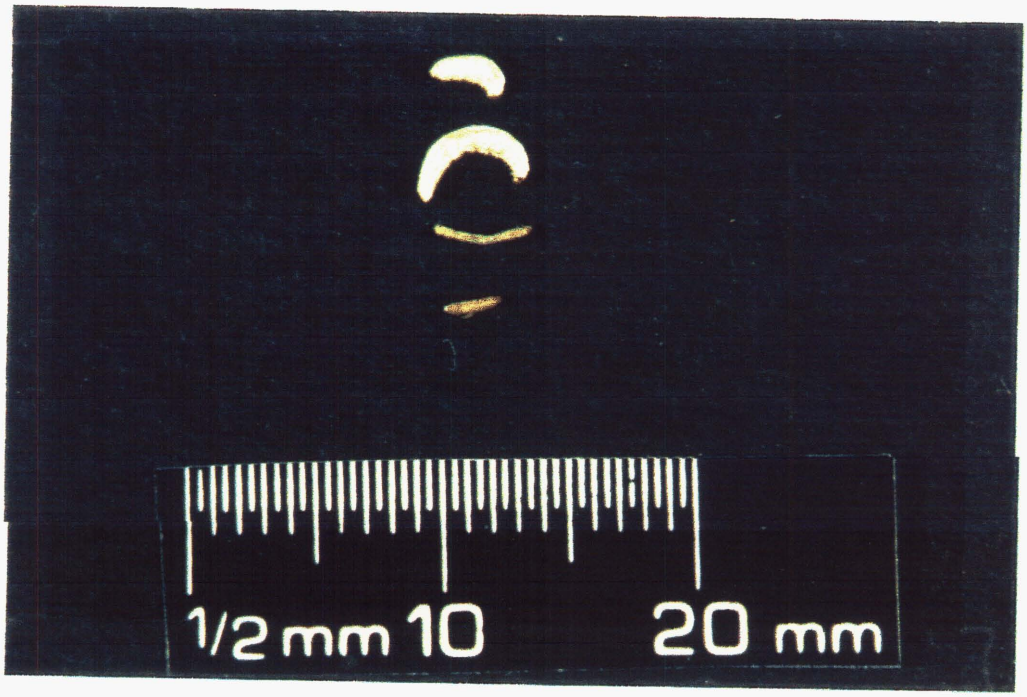


Plate 4.6 Larvae of L. jacobaeae to show the three different instars.



4.3.4.2 Larval Length and Width

Larval length and width of different instars are presented in table 4.5. There is a progressive increase in length and width throughout the instars of L. jacobaeae. From a hatching length and width of about 1.32mm and 0.23mm respectively the larva grows to a length and width of about 5.10mm and 0.86mm at the 3rd instar.

4.3.4.3 Moulting

4.3.4.3.1 Duration of In-Active Period

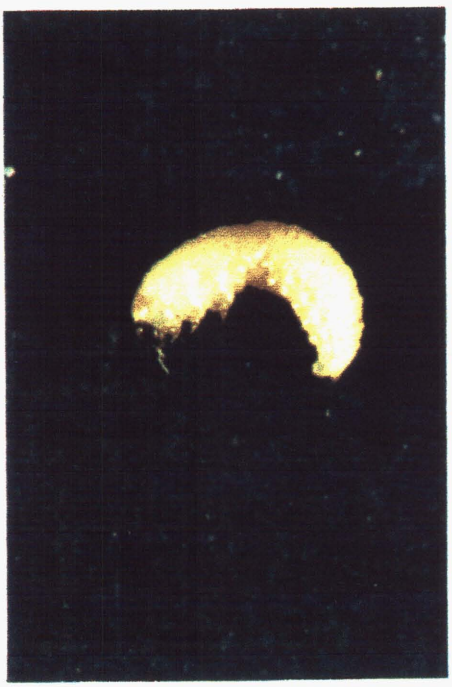
At 10 ^{o}C and 12 ^{o}C fully grown 1st instar larvae stayed about 12 days without feeding or moving before moulting to second instar. At 15 degrees this period was observed as 5-8 days. At higher temperatures (18 and 20 ^{o}C) the resting period of 1st and 2nd instar larvae was not apparent and it was therefore thought to be less than 2 days since they were observed every other day. At the

higher temperatures third instar larvae spent an in-active period of about 11 days as pre pupae. At this stage the body shape of the larvae changed from its elongate shape to a recurved shape (plate 4.7).

Table 4.3 Length and width of L. jacobaeae larvae in relation to different instars.

Instar	Mean length(mm)	Range(mm)	Mean width(mm)	Range(mm)
1st instar (just after hatching)	1.32	1.2 - 1.7	0.23	0.2 - 0.3
2nd instar	3.46	3.0 - 4.5	0.46	0.3 - 0.9
3rd instar	5.10	4.0 - 7.0	0.86	0.6 - 1.2

Plate 4.7 Pre-pupae of L. jacobaeae to show the recurved shape.



4.3.4.3.2 Moulting Movement

At the time of moulting larvae attached to the substrate with their anal end and moved the body around that point. After sometime, splitting off the old cuticle started with cracking of dark brown coloured head capsule through the frontal and coronal sutures. After shedding the head capsule, larvae moved forward, leaving the old cuticle. Just after moulting, the new head capsule was creamy white in colour and it changed into light brown after about 24 hours. This behaviour was observed in about 25 per-cent of the larvae tested. The colour change of the head capsule was a good indication of the exact moulting date.

4.3.4.4 Feeding Behaviour

4.3.4.4.1 Perferance of different instars at different temperatures

Table 4.4 shows the tendency of different instars of larvae to bore into the root system at different temperatures. These observations show that at lower temperatures, larvae prefer to bore in to the root system and feed internally rather than staying out and feeding externally. This behaviour was thought to be due to lower activity of larvae at lower temperatures, compared to higher temperatures. The percentage of the larvae observed in lateral roots and in root crowns (Table 4.5) show that larvae which bore in to the root system at lower temperatures (10^0C and 12^0C) prefer to bore in to root crown rather than in to lateral roots. This behaviour of boring and feeding internally in the root crown may help to destroy the plant easily.

Table 4.4 Tendency of different larval instars to bore in to root system, at low and high temperatures.

Instar	High t^o (18;20 ^{o}C)	Low t^o (10;12 ^{o}C)
1	**	****
2	**	***
3	*	*

**** : very high, *** : high, ** : low, * : very low

Table 4.5 Percentage of L. jacobaeae larvae observed in root crowns or in lateral roots in four different destructive samples.

Sample	Root crown	Lat.roots
1	62.5	37.5
2	66.6	33.3
3	50	50
4	60	40

4.3.4.4.2 Damage cause by different instars at different temperarures

The first damage symptom was the destruction of a small portion of root as shown in plate 4.8.a. First instar larvae tended to feed very slowly compared to 2nd and 3rd instars. Second instar larvae fed heavily and destroyed large portions of tender roots. The 3rd instar was the most damaging since it entirely consumed individual roots (plate 4.8.b). It was necessary to transfer them to new plants twice or thrice during the larval period. When there were no more palatable roots larvae tended to feed on leaf petioles and apex of the plant. This ultimately caused plants to wilt and die (plate 4.8.c). These observations show that a single L. jacobaeae larvae may sufficient to destroy a small ragwort seedling.

4.3.4.5 Duration of Instars

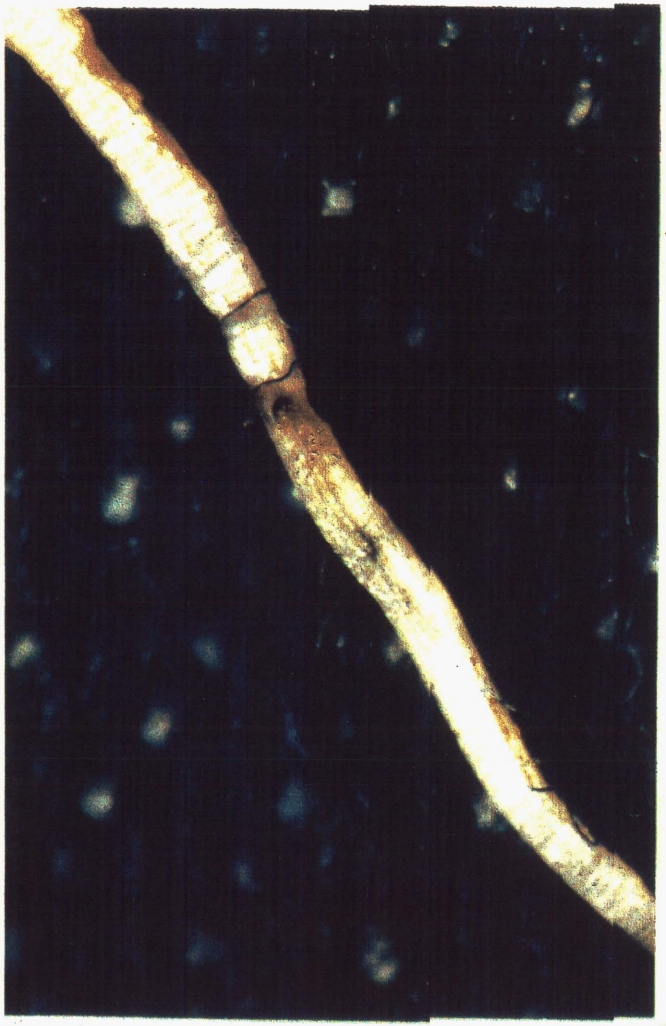
Table 4.6 presents the instar duration (days), in relation to five different constant temperatures.

On the 94 th day a fault developed in the operating system causing drastic high temperatures in the constant temperature rooms. The experiment could not be continued beyond that stage due to plant and larval death. One way of over coming the problem caused by the breakdown of the constant temperature rooms has been to seek generalizations from the data collected and from the literature, in order to make predictions of what would have happened if the experiment had been run to its conclution.

(a) From the 15 ^{o}C, 18 ^{o}C, and 20 ^{o}C experiments it can be seen that the first and second instars took approximately the same time to complete at 15.5 and 15.0 days, 18.1 and 18.0 days and

Plate 4.8 Damaged root system of ragwort plants to show damage symptoms caused by different larval instars.

(a) First visible damage symptoms



(b) Damage caused by 3rd instar lavae

(c) Plant death due to severe damage.

37.2 and 38.2 days respectively. This agrees closely with the finding of Frick (1971) who reported an average instar length of 16 - 17 days for the first and second instars at an average laboratory temperature of 18.3 ^{o}C.

(b) At 18 ^{o}C and 20 ^{o}C first and second instars took about half the time to complete their development that the third instar took (15.25 versus 30.1 days, 18.05 versus 34.5 days respectively. Frick (1971) again reported a similar finding at 18.3 ^{o}C.

Table 4.6 Mean duration of different life stages of L. jacobaeae at 5 different constant temperatures.

T^{o}C	Mean development time(days)[a]					
	Larvae 1	Larvae 2	Larvae 3	Prepupae	Pupae	Total[b]
20	15.5 (0.294/24)	15.0 (0.337/17)	30.1 (1.163/11)	11.0 (0.447/5)	20.0 (0.881/3)	76.1
18	18.1 (0.478/24)	18.0 (0.492/22)	34.5 (1.473/11)	11.7 (0.629/4)	21.0 (1.5/2)	85.2
15	37.2 (1.097/20)	38.2 (1.47/7)				
12	79.0 (1.357/14)					
10	81.6 (1.220/12)					

a = mean (S.E./n)

b = established from mean development time of all life stages.

From these results and from additional less complete information provided by Frick (1971) for average temperature of 18.3 ^{o}C and some breief observations of Newton (1933), I predict:-

(1) at 15 ^{o}C constant temperature, the third instar would have taken about 75 days (twice the first or second instar time).

(2) at 10 ^{o}C and 12 ^{o}C the second instar would have taken 82 and 79 days respectively (similar to first instar).

(3) at 10 ^{o}C and 12 ^{o}C the third instar would have taken 164 and 158 days respectively (twice the first instar.

These predicted values together with the measured values as well as total expected larval duration are shown in Table 4.7.

Table 4.7 Predicted mean durations of 3 larval instars of L. jacobaeae at different temperatures.

T ^{o}C	Instar 1	Instar 2	Instar 3	Total period
10	81.60	82.00*	164.00*	327.60
12	79.00	79.00*	158.00*	316.60
15	37.25	38.20	75.00*	150.45
18	18.10	18.00	34.50	70.60
20	15.50	15.00	30.18	60.68

* predicted values

Depending on these data development rates were calculated and presented in table 4.8. Since most of the pre-pupae escaped, and only few could be observed, non feeding prepupal period was not take into account to get a better estimate. Similar method has followed by Logan et al. (1985) to get a better estimate for development rate of the larva of Colorado potato beetle. Since there was high mortality in the non feeding time in the final instar larvae Logan et al. ommited prepupal stage of Colorado potato beetle.

Table 4.8 Rate of development of 3 larval instars of L. jacobaeae at different temperatures.

T °C	Development rate (1/days)			
	Instar 1	Instar 2	Instar 3	Total period
10	0.0122	0.0121*	0.0060*	0.0030
12	0.0126	0.0126*	0.0063*	0.0032
15	0.0269	0.0262	0.0130*	0.0066
18	0.0552	0.0556	0.0289	0.0141
20	0.0645	0.0667	0.0331	0.0164

* predicted values

Using the Campbell et al. (1974) method (see section 3.3.2 and Figure 3.4), the threshold temperatures for L. jacobaeae larvae were determined as 9, 9.1, 9.1 and 9.0 for the first instar, second instar, third instar and for the total larval period respectively (figure 4.2). Considering these thresholds, degree days were calculated for three different instars at different temperatures and presented in table 4.9.

Figure 4.2 Development rates for the 3 larval instars of L. jacobaeae at different temperatures showing the estimated lower threshold temperatures.

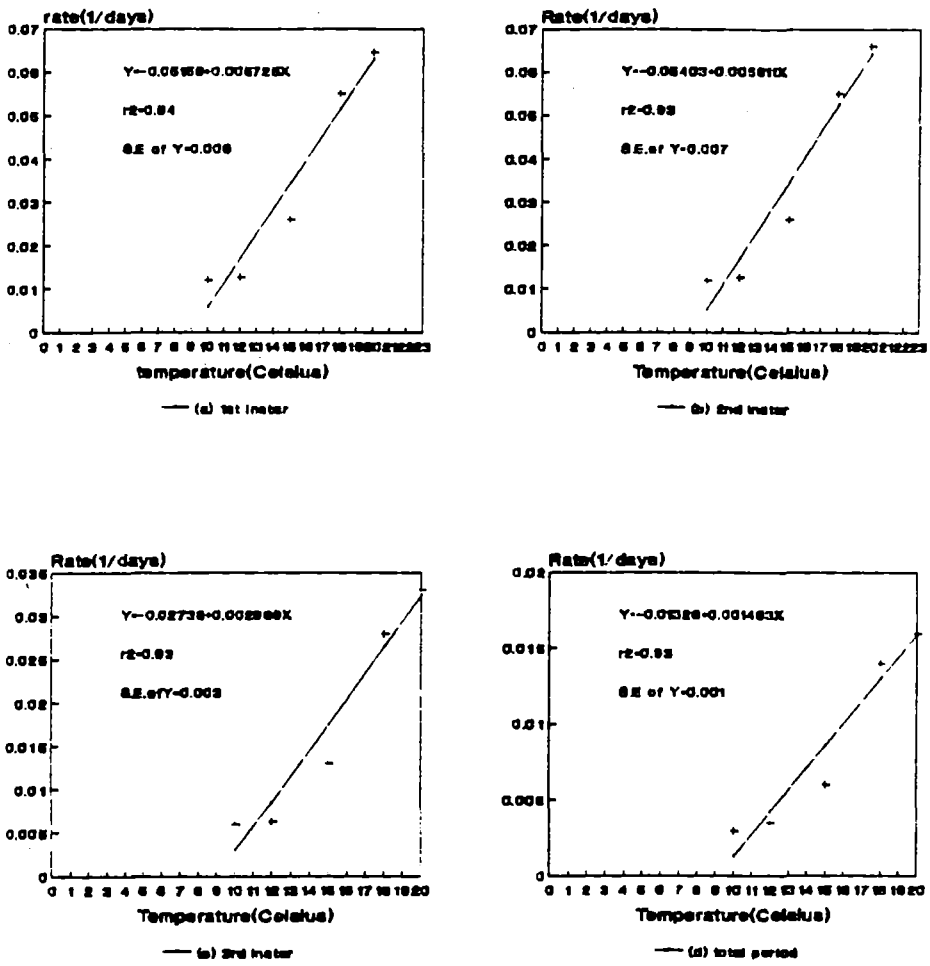


Table 4.9 Degree-days for 3 larval instars of L. jacobaeae under five different constant temperatures.

Instar	Degree-days *					
	10	12	15	18	20	Mean(1/b)
Instar 1	81.6	237.0	223.5	162.9	170.5	174.67
Instar 2	73.8	229.1	225.4	160.2	163.5	169.17
Instar 3	147.6	458.2	442.5	307.1	328.9	334.56
Total	327.6	948.0	902.7	635.4	667.5	683.52

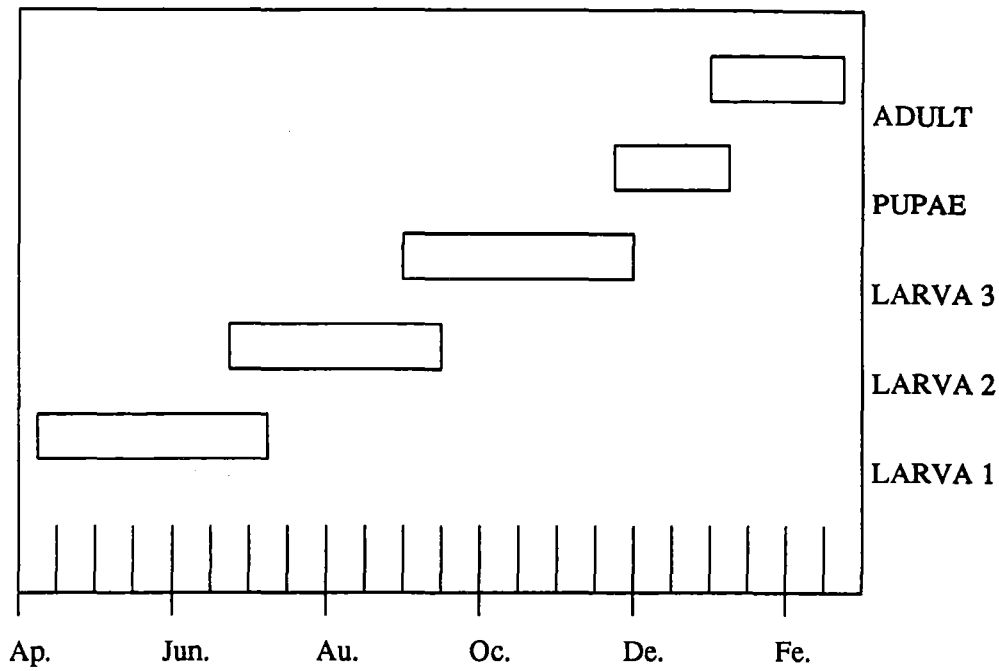
* Degree-days = (relevant temperature-lower threshold t) X no. of days taken to development)

At Lincoln, in 1989 first egg was observed on 13 th March. Since the egg might take about 3 weeks for incubation, first instar larvae should appear in April. Syrett (1986) suggested that pupae first appeared in November and second generation adults in 1989 were observed from 20 th December, third instar larvae should have moulted to pupae by late October. Therefore, calculation of accumulated degree-days was started from 1 st April and ended 31 st October. Accumulated degree-days for air temperature and soil temperature at Lincoln were calculated using the lower threshold temperature for total larval development (9 ^{o}C). These values were 330.85 and 146.2 respectively. These results show that the calculated thermal constant 696.6 is much higher than the accumulated degree-days. Therefore, it could be suggested that the lower threshold temperature for total larval period is too high. Degree-days for 1 st and 2 nd instars (175.5 and 170.6) are closer to accumulated degree-days for soil temperature (146.2) where as the required degree-days for 3rd instar (336.8) are closer to accumulated degree-days (330.85) for air temperature.
By the time of completion of three larval instars at 15 ^{o}C and first two larval instars at 12 and 10 ^{o}C it could be assumed that the mean air temperature in the field should be higher than 15 ^{o}C since the winter time has already passed. Therefore, similar durations as at 18 and/or 20 ^{o}C could be expected from pre-pupae to adult.

From April to October mean monthly air temperature of 3 areas of North Island (Gamon rd, Bay of Island; Kaihu, Hobson; and Whakatane) where the insect has established well is more than 9 ^{o}C (Appendix 1). The temperature in these areas during this period is around 10 and 12 ^{o}C.

Therefore, duration of 3 larval instars can be expected to be similar to the results obtained at 10 and 12 ^{o}C in the laboratory. According to this expectation, duration of different instars can be show as in Figure 4.3.

Figure 4.3 Expected durations of different life stages of L. jacobaeae in the field.



At Whakatane, adults were released in May, 1985 and second generation adults were emerged in January,1986. At Gamon Road, adults were released in April,1985, second generation adults could be seen from March, 1986, but early emergence of adults was observed in November,1986. At Kaihu, adults were released in April,1985, and adult emergence was observed in December,1985. There are some places in South Island where the mean monthly temperature is lower than the lower threshold temperature, but insect establishment is successful. Three good examples are Upper Takaka, Nelson; Hanmer, North Canterbury; and Inangahua, West Coast. At Inangahua and Hanmer the mean monthly air temperature from May to September is less than 9 ^{o}C. At Upper Takaka, adults were released in April, 1985, and second generation adults were observed in February, 1986. Again, fifth generation adults were observed in January, 1989 (P.Syrett, pers. comm.).

All these observations show that the time taken for development in the field is less than in the laboratory under constant temperature conditions. This can be explained in two ways.

(1) The duration under constant temperature conditions could differ from the actual field conditions, because there is a temperature fluctuation in the field (Appendix 1).

(2) Not only the temperature but also there should be some other factors affecting development in the actual environment.

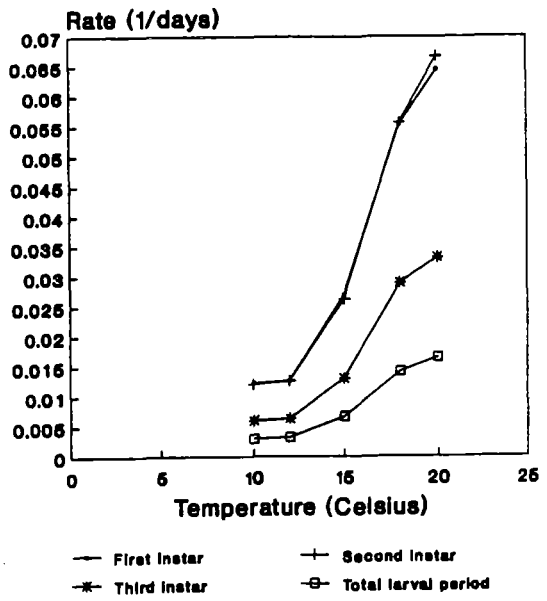
Successful establishment of the insect in some places of South Island where the mean monthly temperature is lower than the threshold, suggest two points:

(1) If the calculated threshold temperature is correct,there could be an unmeasurable development of insect larvae at lower temperatures below the threshold, as described by Campbell et. al. (1974).

(2) The calculated threshold temperature is an overestimate. Therefore, the calculation approach (Campbell et al.,1974) with the above data may involve considerable error.

If there is no linear temperature-rate relationship, there should be a non linear model which could well fit the data. There are many models available that describe mean development rate as a function of temperature. These include those of Stinner et al. (1974), Logan et al. (1976), Sharpe and DeMichele (1977), and Hilbert and Logan (1983). Among those non linear models, sigmoid model described by Stinner et al. (1974) could be used for further studies since there is no decline in rate at the highest temperature tested (20 ^{o}C). As well, there is an almost similar development rate at the lower temperatures tested (10 and 12 ^{o}C) and the rate Vs temperature curve (Fig.4.4) is sigmoid.

Figure 4.4 Mean development rate for different larval instars and for total larval period of L. jacobaeae under constant temperature conditions showing sigmoid relationship.



Therefore, the following nonlinear model given by Stinner et al. (1974) was fitted to the data using the Statistical Analysis System (SAS, 1985):

$$R_t = \frac{C}{1 + e^{k1 + k2.t}}$$

Where, R_t = rate of development (1/days) at temperature t. C, k1 and k2 = temperature dependent development parameters.

The parameters, C, k1, k2 were calculated by the Marquardt method which is available in the NLIN procedure of SAS (The Marquardt method is a numerical technique to fit parameters of a nonlinear regression model). Table 4.10 present the values obtained for C, k1, k2, the correlation co-efficient, and the standard errors for the non linear regression.

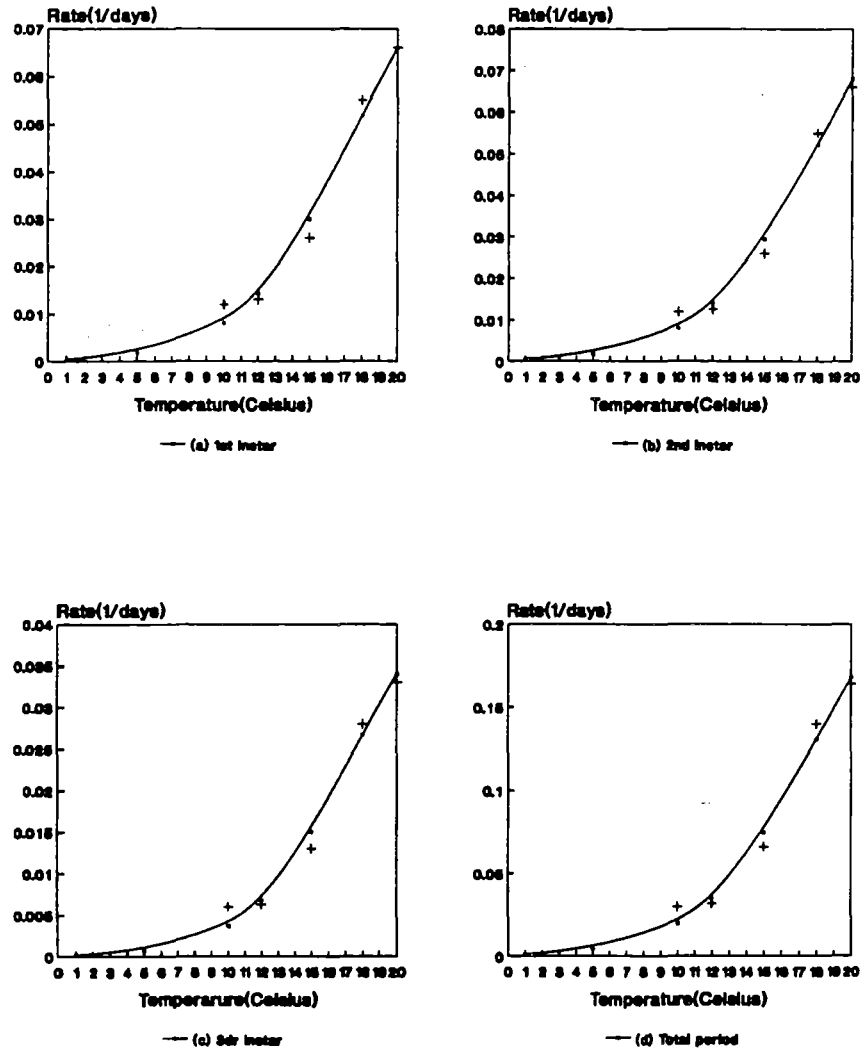
Table 4.10 Parameter values, regression coefficient, and standard errors for the temperature dependent development rates for L. jacobaeae larval instars.

Stage	Development parameters			r^2	S.E.
	C	k1	k2		
Instar 1	0.095	5.567	-0.319	0.994	0.00002
Instar 2	0.105	5.598	-0.310	0.995	0.00002
Instar 3	0.047	5.886	-0.342	0.992	0.000007

High r^2 values (always more than .99) and very low standard errors clearly show the closeness of this model to data. Figure 4.5 shows the sigmoid curves obtained by fitting the model to different instars. Assuming that the developmental rate-temperatures relationship is nonlinear and that all estimates of development are reasonable, this model indicates that development of larval instars can proceed at temperatures below the lower threshold of 9 ^{o}C estimated by the linear model. It also explain the capability of L. jacobaeae larvae to withstand extremely cool conditions (less than 1 ^{o}C) and overwinter in the root crowns where they feed. The cooler temperatures would slow the larval development so that it would be unmeasurable but larvae can survive the cooler winter time. When the environment becomes suitable again they would be able to develop in higher rate. Based

on the above results, I would choose the sigmoidal model of Stinner et al. (1974) for the temperature dependent development relationship of L. jacobaeae larvae.

Figure 4.5 Mean development rate for L. jacobaeae larvae under constant temperatures under the assumptions of Stinner et al. (1974).



4.3.4.6 Pupae and Adult

4.3.4.6.1 Pupae

Newly formed pupae were observed to be stuck to soil particles with some sticky material. When they are disturbed, pupae tended to roll or showed slow movement apparently to escape the disturbance. Plate 4.9 shows a newly formed pupa. The newly formed pupa was creamy white and about 3-4 mm long. After about 14 days, the white eyes turned brown. This stage was called the red eye stage or partially mature pupa as described by Frick (1970). At that stage, femurs of the developing hind legs were brown. After 16 days the whole pupa was tan, the legs and mouthparts were fully developed, but wings were only partially developed. After 18-21 days new adults emerged.

4.3.4.6.2 Adult

Plate 4.10 shows the adult just after emergence. By the time of emergence, the adult was tan, and with the wings and elytra folded, the insect was unable to move. The dark brown colour of the hind femurs, observed in the partially developed pupa was still present on adult femurs.

4.3.4.7 Survival Rate

With the rearing procedure described in 4.2.2.1.2 an average of 46% survived to the third instar (See Table 4.11).

Table 4.11 Mean per-cent survival of L. jacobaeae when rear in modified beaker vessels.

Instar	Survival percentage*
Upto 3rd instar	45.8
Upto adult	10.4

* mean percentage of survival at 20 and 18 degrees.

This results indicates that the method is satisfactory for observational studies. Pupa to adult survival was about 44%. Mortality of pupae was observed to be due to two reasons; The first was desiccation; the other was fungal infection (Plate 4.11). It was observed that there were some mites associated with desiccated pupae but it was not quite certain whether the mites were responsible for desiccation because mites were observed only after the desiccation.

Plate 4.9 Newly formed pupae of L. jacobaeae showing its creamy white colour.

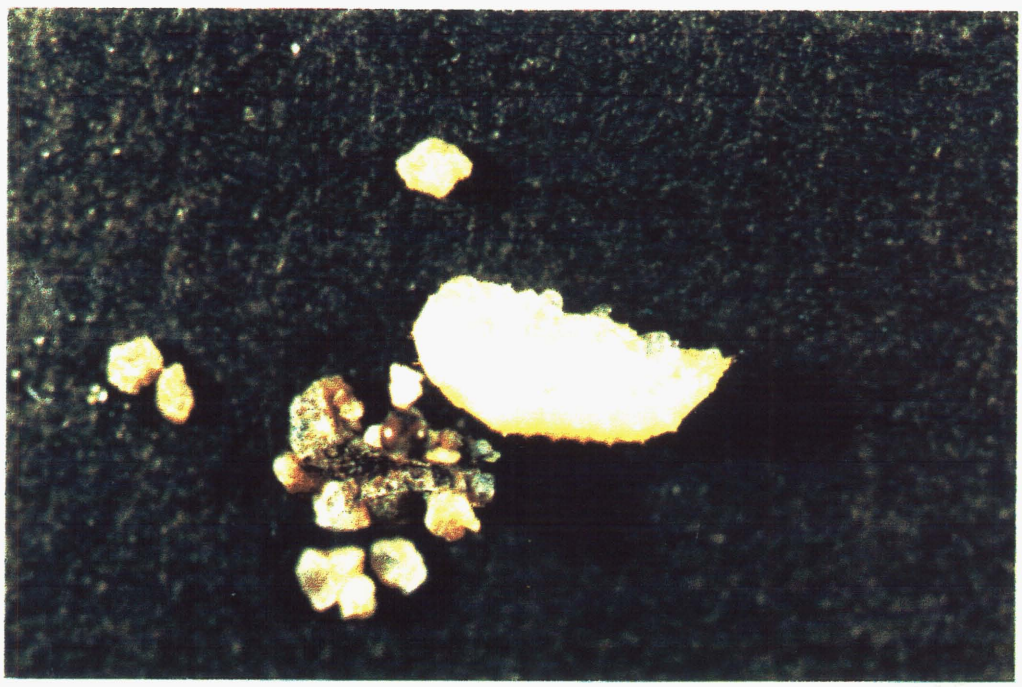
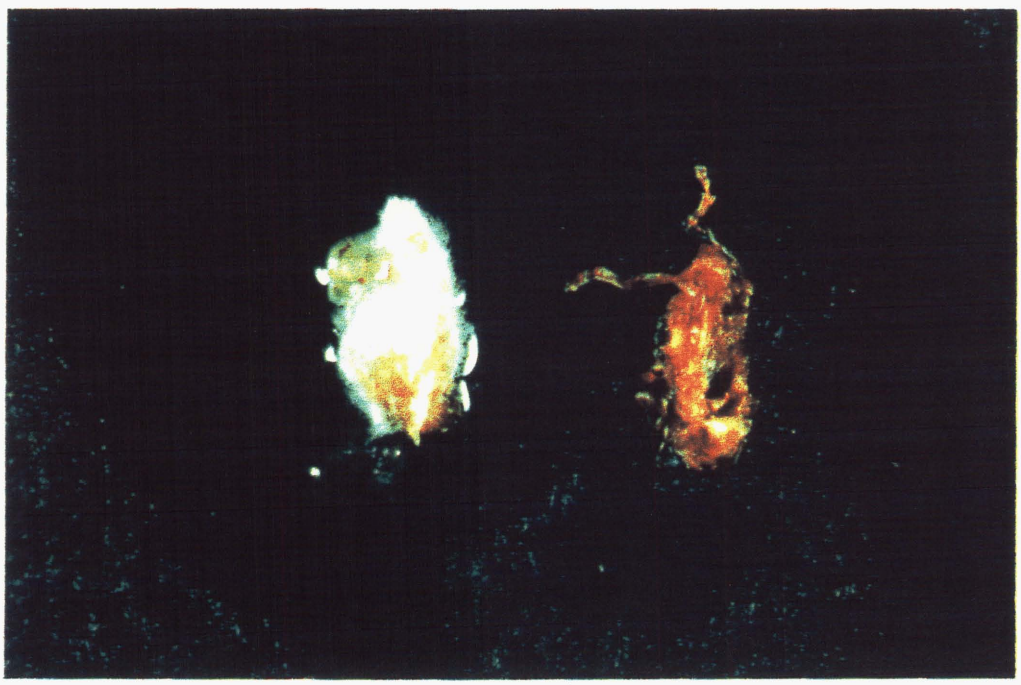


Plate 4.10 L. jacobaeae adult just after emergence showing its tan colour.

Plate 4.11 Dead carcuses of L jacobaeae pupae showing (a) fungal infection and (b) desiccation.



4.3.5 Conclusion

Results from measurement of head capsule widths of L. jacobaeae larvae confirm that there are three larval instars which supports the description of Newton (1933). The head capsule width classes are distinct enough to be used to allocate larvae to each of the three instars. Colour change of the head capsule at moulting can be used to detect the exact date of moulting.

The beaker method used to rear larvae is a successful method for laboratory rearing, which enable the insect to develop while allowing observations without disturbing them. However, this method can not be considered a good rearing method for pupae or adults, because only some of the prepupae could be obtained from the bottom of the beaker and most of them were escaped during that time.

It is obvious from the results, that at lower temperatures larvae of L. jacobaeae feed and develop slowly in the rootcrowns whereas at higher temperatures larvae feed heavily on lateral roots and develop at a higher rate. Even though the first and second instars feed on the root system of ragwort plants, they do not cause much damage to the plant. The third instar larvae feed heavily and cause the most destructive damage to the plant. It could be said that the higher feeding rate of third instar may be due to the greatest growth occurring during this period.

Very low threshold temperatures for development of of larvae suggest that the larvae can continue to develop during the winter in New Zealand. Under field conditions at release sites in New Zealand, adult emergence was observed to range from December to February. This can be explained as follows: the first instar larvae that enter to the root system before the winter, may develop slowly in the roots throughout the winter, pupate in the spring and produce adults in December. The other thing that can happen in the field is that the first instar larvae that enter the root system after the winter may develop at a higher rate and develop into pupae in December or in January and produce adults in January or in February.

At the emergence of the adult, appearance of dark brown portion of the hind femur is thought to be due to the metafemoral spring (plate 4.10).

CHAPTER FIVE

EFFECT OF PHOTOPERIOD ON DEVELOPMENT OF REPRODUCTIVE ORGANS AND FLIGHT MUSCLES

5.1 INTRODUCTION

5.1.1 Reproductive Diapause of L. jacobaeae

There are two main ways in which insects can adapt their life cycles to different climatic conditions. One is the development of locally adapted physiological races. This phenomenon is not well documented in the Coleoptera. The other is by interpolation of diapause at suitable stages in the developmental cycle and of suitable lengths to local conditions (Crowson,1981). This second type of adaptation has been noted in ragwort flea beetle by Frick and Johnson (1973). They have reported that in the laboratory eggs of the Swiss biotype of this species entered into the diapause and took 15 weeks to hatch, whereas in similar conditions eggs of Italian biotype hatched within 3 weeks. Similarly the time from female emergence to oviposition in the Swiss biotype averaged 13 days whereas the Italian biotype adults began a facultative aestival diapause 1-2 weeks after emergence. Frick (1970) also reported that non laying females didn't have reproductive organs but contained only fat bodies.

In the Pacific Coast States adult flea beetles emerged from pupae in early summer (late May and early June) fed for a few weeks and then became inactive and remained inactive throughout the summer. During this period feeding dropped, activity virtually ceased, and the beetles reacted negatively to light by seeking dark places in which to hide. This period of aestivation was taken to be an adaptation of the insect to the relatively hot, dry summers of the mediterranean region from where they had come. Any eggs produced during the dry months would probably desicate. In Rome, this summer period (June,July,and August) has an average rainfall of 84mm , 10% of the annual precipitation. With the coming of shorter days, cooler temperatures and late summer, beetles became active and feed (these conditions also simulated fresh growth on the host ragwort plants) (Frick and Johnson,1973).

Frick and Johnson (1973) have also reported that the summer dormancy of the Italian biotype of ragwort flea beetle appeared to belong to the oligopause type of dormancy described by Mansingh (1971). He described oligopause as " a response to prolonged though moderate environmental adversity which is associated with seasonal climatic changes". Under conditions of appropriate photoperiod, temperature and food supply ragwort flea beetles developed continuously without the intervention of diapause (Frick and Jonson,1973). The dormancy was induced by long days (Frick and Johnson, 1973; Philip and Syrett, 1988). However, at 24 degrees Celsius whether it is long or short photophase the females generally went into a typical dormancy (Frick and Johnson, 1973). Syrett (1988) reported that the diapause was induced by long days and was not affected by two different rearing temperatures, 17 and 26 degrees.

In New Zealand, at Lincoln beetles can be observed from January but the peak emergence is in February (late summer). They enter aestivation about 1-2 weeks after emergence. Eggs can be observed from mid March (Autumn) onwards (Syrett,1986 and personal observation). Larvae were found from May (early Winter) onwards. Pupation occurred mainly in November and December (Syrett,1986).

All the above literature and my personal observations of the insect encouraged me to carry out an experiment to find out the effect of photoperiod on the development of reproductive organs of both male and female of the Italian biotype.

Frick (1971) described a way to identify the sexes of L. jacobaeae externally. According to him, the females are 2.8-3.75mm long and the males are 2.5-3.25mm long. In females, the last sternite is convex and bluntly obtuse angulate posteriorly. In males, a circular concave depression is obvious on the last visible abdominal sternite. Little information was found on the internal reproductive system of male or female L. jacobaeae. It was important to understand the structure of the reproductive systems before starting studies on photoperiodic effects on these organs.

5.1.2 Flight Muscle Resorption

Crowson (1981) reported that in adult beetles, particularly females the flight muscles may used as food reserve and they may undergo considerable resorption, presumably supplying protein materials for the ripening eggs. Fraser (1987) suggested that one reason for degeneration of flight

muscles, accompanied by reproductive maturation, may be to vacate thoracic space and to make room for development of the reproductive organs. Therefore, flight muscles of newly emerged males and females as well as those influenced by different photoperiods were measured during dissections.

5.2 MATERIALS AND METHODS

5.2.1 Collection of L. jacobaeae Adults

Adult beetles were collected from the insectary just after emergence from pupae, using an aspirator. These insects were kept in a cage with fresh ragwort leaves till they were used in the experiment.

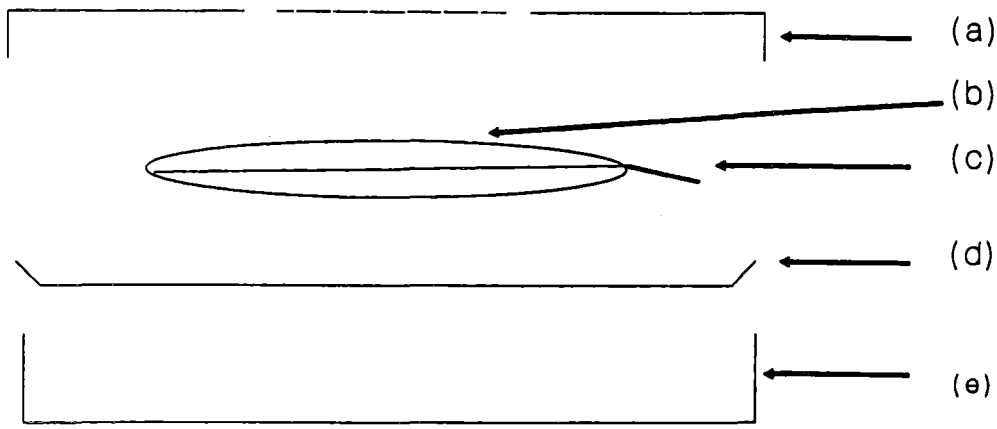
5.2.2 Treatments

Forty eight, 99mm x 15mm clear plastic petridishes were used as cages in this experiment. Lids of these petridishes had 50mm gause covered hole for ventilation. The bottom of dishes were lined with TQYO,No 2, 90mm moist filter paper circles (Figure 5.1a). Fourty eight male/female pairs were randomly selected and placed in individual cages. Fresh ragwort leaves almost similar in size and age were carefully washed. Petioles were wrapped with moist cotton wool and covered with parafilm to maintain freshness. One leaf was placed in each cage. These cages were then placed in humidity chambers made of "Calvert" white plastic dishes and close-fitting clear plastic covers. Twelve petridishes were kept in each humid chamber as shown in Figure 5.1.(b). A similar method was previously reported by Fraser (1987) for Chrysolina hyperici.

Two humidity chambers (24 male and female pairs) were kept at 20 ^{o}C and long days (16 hrs of light and 8 hrs of dark) and other two humidity chambers were kept at the same temperature and short days (8 hrs of light and 16 hrs of dark). Long and short day lengths were provided by placing caged insects pairs in Contherm Scientific Controlled temperature cabinets.

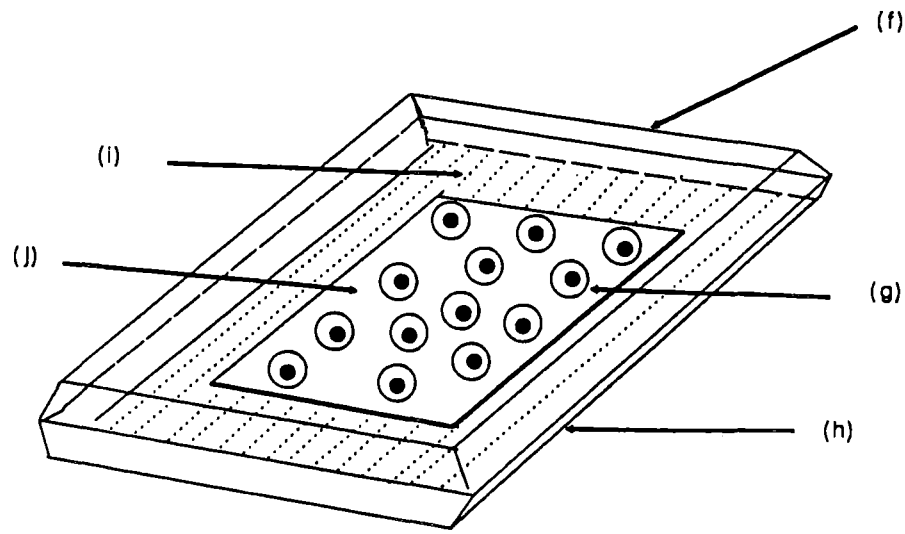
Figure 5.1 Disign of cages and humidity chambers as used in photoperiod experiment

[a] Cage used for oviposition and feeding



(a)- Petridish lid with gause ventilation, (b)- Ragwort leaf, (c)- Leaf petiole covered with moist cotton wool and parafilm, (d)- Filter paper circle, (e)- Petridish base

[b] Plan view of the humid chamber



(f)- Transparent humidity chamber lid, (g)- Petridishes, (h)- White Humidity chamber base, (i)- Saturated NaCl solution, (j)- Tray supporting cages

5.2.3 Observations of Oviposition and Feeding

The presence or absence of eggs and feeding rate were recorded every two days. On each occasion new leaves, as described in 5.2.2. were provided, all eggs were removed and filter paper linings were changed. Since the leaves used were always similar in age and size, feeding rate was scored by placing them in one of the feeding damage classes shown in Figure 5.2. Since the number of pairs of beetles maintained decreased throughout experiment as beetles were dissected for morphological studies the number of observations made was calculated according to the plan shown in figure 5.3. Results of the feeding rate observations are presented as the percentage of the damage classes (N, L, M, and H) compared to the total number of observations.

5.2.4 Dissection

Before the start of the experiment 10 adult beetle pairs were collected and mounted dorsal side uppermost in a petridish that had been half filed with black carbon suspended parafin wax. Invertebrate physiological saline (7% NaCl in distilled water) was used to cover insects while dissecting. Mounted insects were dissected under a Wild M4 binocular microscope. The elytra and wings were carefully removed and an incision made along both sides of the abdominal terga using "Circon" dissecting scissors (Medtel Australian Propriorty, NSW). The metanotal and abdominal terga were peeled back to the last segment of the abdomen. Then fat bodies were carefully removed till it was possible to observe the reproductive organs.

In the treated insects dissections were planned at 30, 60, and 90 days after begining treatment, because insects under natural conditions usually start egg laying about 70 days after emergence. The number of insects dissected in each treatment at each time was 8 pairs.

5.2.5 Measurements

In males, testis diameter and the length of the anterior lobe of the right accessory gland were recorded. In females, length of the right ovary, ovarian development time (youngest age at which an egg is found in oviducts), number of eggs in the abdomen and the size of the largest egg were measured. In both sexes the diameter of the right flight muscle bundle was measured. All these measurements were taken under Wild M4 A binocular microscope fitted with an eyepiece graticule.

Figure 5.2 Feeding damage classes

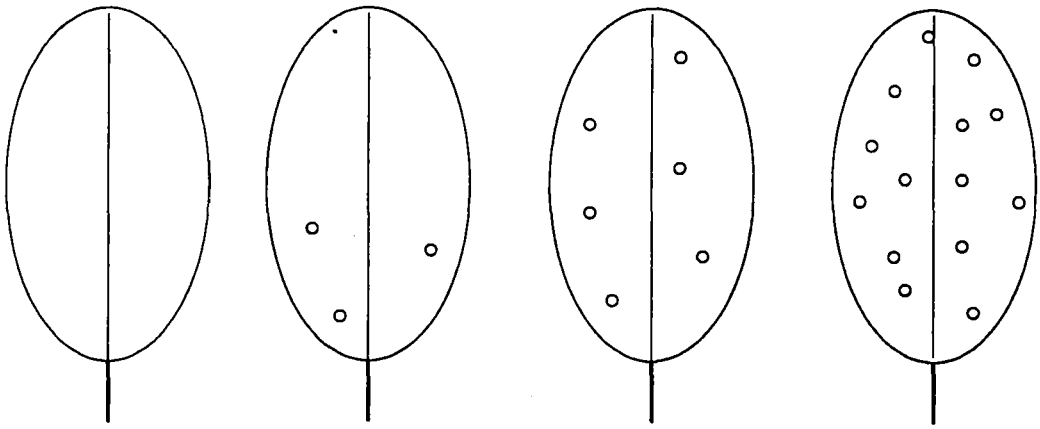
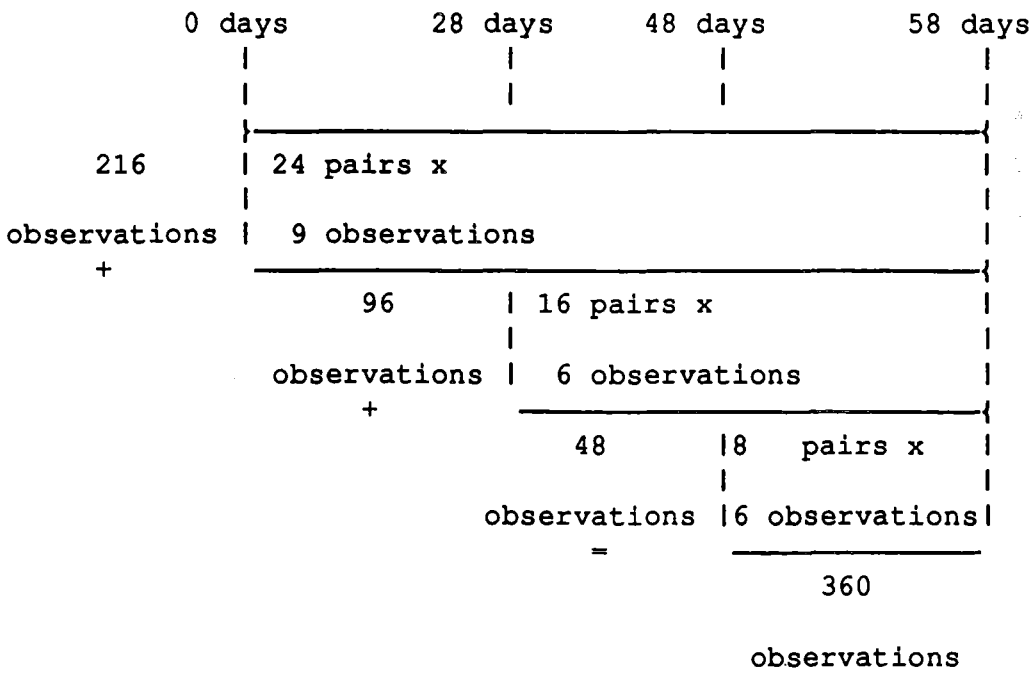


Figure 5.3 Observations of oviposition



5.2.6 Experimental Design and Analysis of Data

When a character of an experiment is measured over time, the main objective is to examine the rate of change from one time period to another. So, it is important to determine the interaction effect between treatment and stage of observation. Hence, the common approach is to combine data from all stages of observation and obtain a single analysis of variance (Gomez and Gomez, 1984). Therefore, experimental design used in this experiment was split-plot design. Since my main objective was to find the effect of photoperiod, different photoperiods was assigned to the main plot. The second factor, timing was assigned to the sub plot. Results were analysed with pooled analysis of variance for measurements over time based on a split plot design.

5.3 RESULTS AND DISCUSSION

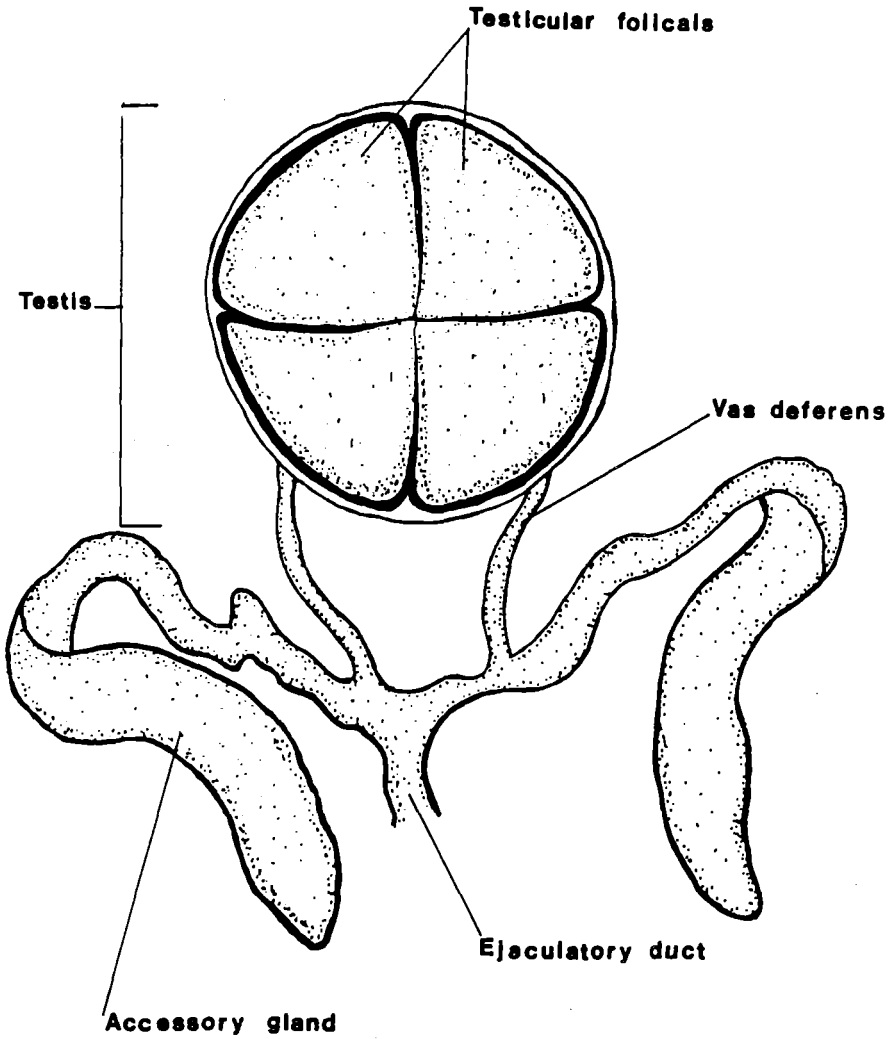
5.3.1 Reproductive Organs

5.3.1.1 Male Reproductive Organs

In the order Coleoptera, appearance of unusual type of testes appears to be a characteristic of 3 of the 4 sub orders. Some of these these types are single long tube like testis, the fillicular type of testis, and testis fused into a single median structure. Members of the sub family Halticinae to which ragwort flea beetle belongs, have a single fused median testis, giving rise to two vasa diferentia (Crowson, 1981; Suzuki, 1988). Thus, this single structure contain 4 large sperm tubes, 4 vas efferens, and swollen anteririor extremities of 2 vasa diferantia. The accessory gland is very variable in length, width, and shape in different species. The anterior part of the gland is occasionally distinctly expanded (Suzuki, 1988).

In ragwort flea beetle, the median testis was bright yellow in colour. Goldson (1979) reported similar orange-yellow coloured testis in Argentine stem weevil and concluded that the colour may be due to the adherence of fat globules. The colour of testis of ragwort flea beetle similarly could be due to carotinized fat deposits. Deposits of fat were carefully removed and it was found that there were 4 testicular lobes and these lobes were white in colour. The lobes were bound by a very thin membrane to form the single median testes. Two vasa diferantia started from the testes and joined to the ejaculatory duct, which also received the openings of the accessory glands. The accessory glands were slightly club shaped and folded backwards half-way along (Figure 5.4).

Figure 5.4 Dorsal View of dissected reproductive organs of male L. jacobaeae.



Accessory glands such as those observed are believed to function mainly in providing material for spermatophores. In groups where no spermatophores are produced accessory glands are reduced or altogether absent (Crowson, 1981). Crowson (1981) also reported that there was some evidence in the super family Cucujoidea that the irritant substance cantheridin was produced in the accessory glands of males and transferred to the female reproductive tract during copulation. Fraser (1987) mentioned that insect accessory glands were variously said to provide spermatophore material, spermatophore coat material, and substances to prevent second mating by the female.

LeSage (1988) decribed the aedeagus of L.jacobaeae as having the median lobe slightly narrowed at the middle, and nipple shape at the apex, with short oblique wrinkles basally, in ventral view; appearing weakly curved at the middle, pointed and slightly recurved downward at the apex in lateral view.

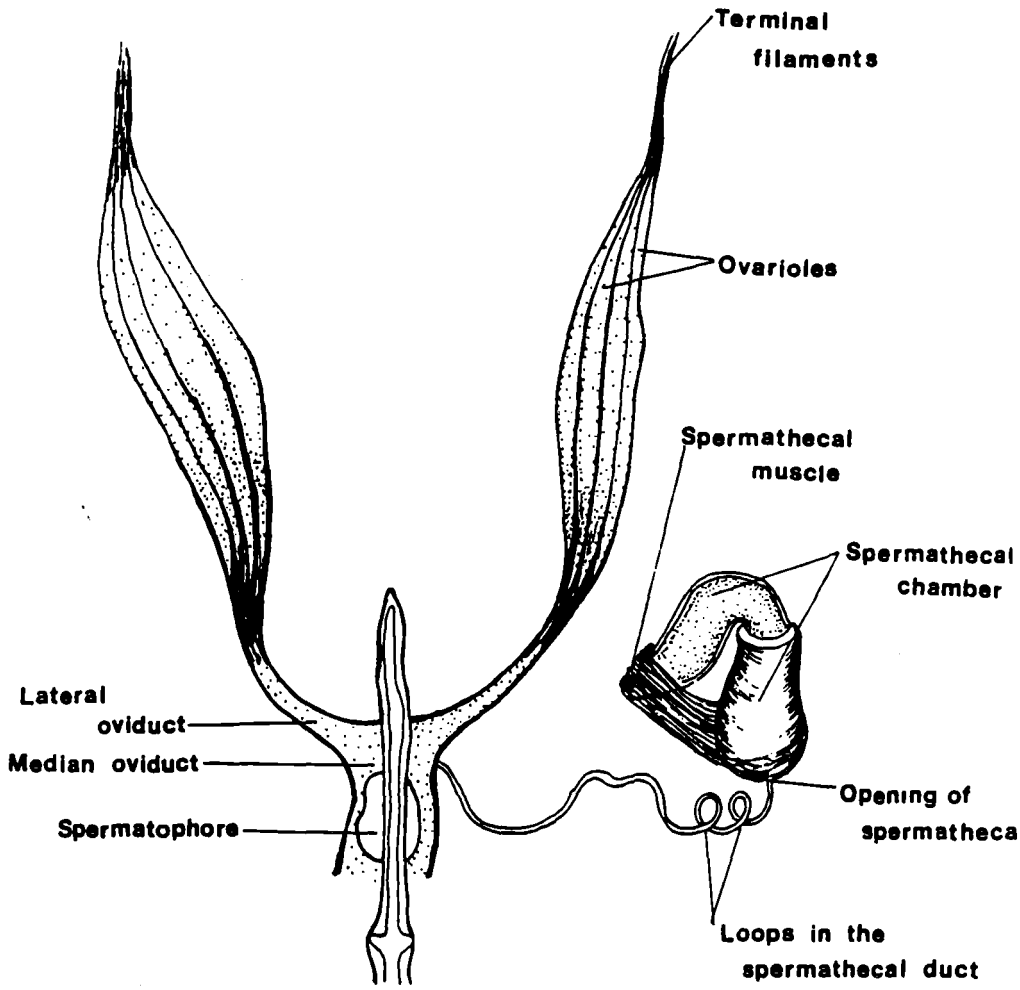
5.3.1.2 Female Reproductive Organs

Female reproductive organs of Chrysomelidae were described by Suzuki (1988) as follows: The system consists of 4 essential parts. These are; a pair of ovaries, a pair of lateral oviducts, a median ectodermal tube, the common oviduct and the genital chamber, and a spermathacal capsule with spermathacal duct and spermathacal gland. The ovary is a collective organ consisting of many ovarioles. The number of ovarioles per ovary (varies from 3 to 114), and the shape of the ovarioles varies in different groupes. The spermathecal gland is the most diversified organ in the female reproductive system. In the sub family Halticinae the spermathecal capsule is very variable in size, and shape. LeSage (1988) decribed the spermatheca of L. jacobaeae as a bladder shaped one, wider in the basal third, with long neck, fusiform pump, and 2.5 loops in the spermathecal duct.

The female reproductive organs of a newly emerged ragwort flea beetle are illustrated in Figure 5.5. These comprised two ovaries located in the dosolateral portions of the abdominal cavity. Each ovary consisted of 15 ovarioles. Apically each ovariole was produced into a long terminal filament. Individual filaments from each ovariole combined to form a suspensory ligament. Mean immature ovary length was 0.034mm. Posteriorly all ovarioles of each ovary fused to form the lateral oviducts (Figure 5.5). The paired lateral oviducts fused posteriorly to form the short median oviduct. The spermatheca was usually located on the right hand side of the abdominal cavity. It consisted of a sac like gland from which the spermathecal duct started and a curved structure to which the compressor muscles attached (Figure 5.5). Both the curved structure and the spermathecal gland were highly sclerotised. The spermathecal duct connected the spermathecal

gland to the median oviduct. As described by LeSage (1988), loops are very clear in the spermathecal duct.

Figure 5.5 Dorsal view of the dissected reproductive organs of newly emerged female of L.jacobaeae.



5.3.2 Oviposition

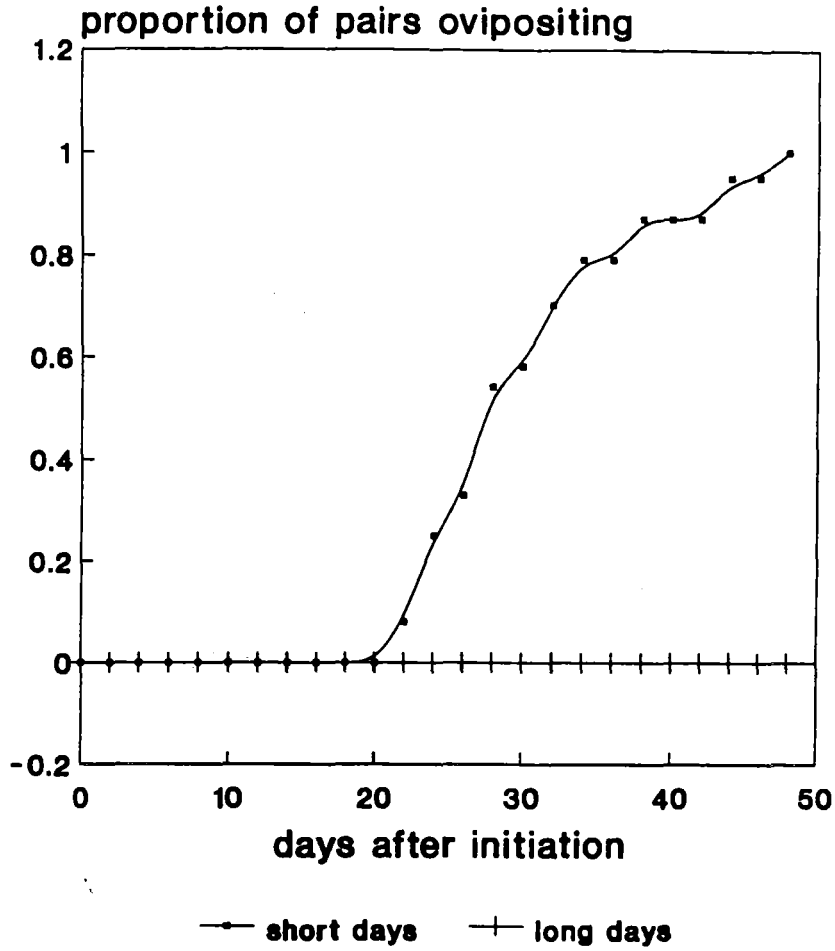
Females that were exposed to short days started egglaying from the 22nd day and the last pair started 48 days after initiation (Table 5.1 and Figure 5.6). None of the females exposed to long days ever laid eggs.

Table 5.1 Number of females starting egg laying after exposure to short days and long days.

Number of days of treatment	number of females starting oviposition	
	short days	long days
0 - 21	0	0
22	2	0
24	4	0
26	2	0
28	5	0
30	1	0
32	3	0
34	2	0
36	0	0
38	2	0
40	0	0
42	0	0
44	2	0
46	0	0
48	1	0

Since the insects in the short day treatment started egg laying before they were expected and the insects under natural conditions started egg laying 56 days after emergence, dissection dates were changed to 28 days, 48 days and 58 days after initiation.

Figure 5.6 Oviposition of females that receiving long days and short days.



5.3.3 Ovarian Development

5.3.3.1 Length of the Ovary

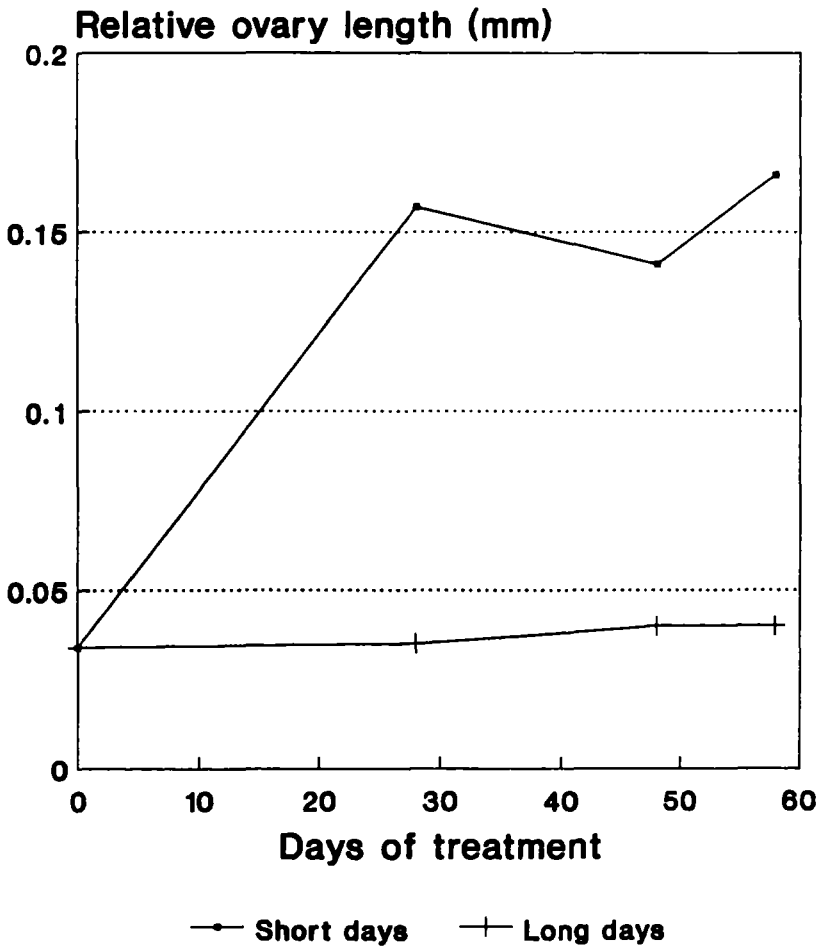
The ovaries of females that were exposed to short days developed to a mean length of 0.156mm by 28 days from the begining of the experiment, while ovaries of insects exposed to long days grew very slowly to a mean length of 0.047mm after 58 days (Table 5.2 and Figure 5.7). Difference between short day and long day treatments were significantly different ($p<0.05$).

Table 5.2 Mean ovary length of females exposed to short days and long days

days after treatment	mean ovary length (mm)*	
	short days	long days
- 1	0.034 (0.002)	0.034 (0.002)
28	0.157 (0.013)	0.035 (0.001)
48	0.141 (0.015)	0.040 (0.002)
58	0.166 (0.012)	0.048 (0.001)

* mean of eight insects, standard errors are given in parentheses.

Figure 5.7 Ovary length of female L. jacobaeae exposed to short days and long days.



5.3.3.2 Number of Eggs in the Abdomen

Table 5.3 shows the number of eggs observed in the abdomen of L. jacobaeae exposed to long days and short days.

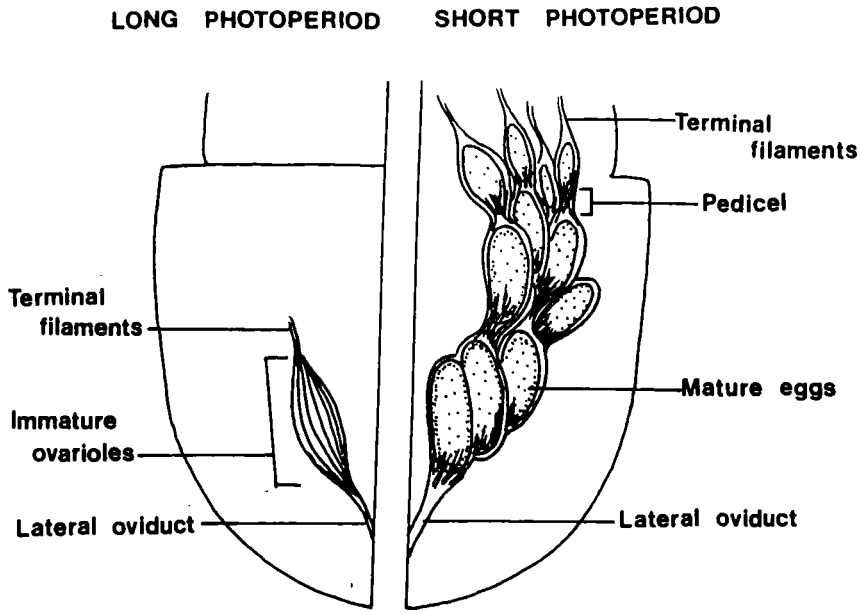
Table 5.3 Effects of short and long days on the number of eggs in the abdomen of L. jacobaeae.

days after treatment	mean no.of eggs in the abdomen*	
	short days	long days
- 1	0	0
28	12.7 (1.6)	0
48	13.0 (1.64)	0
58	14.5 (0.90)	0

* mean of eight insects, standard errors are given in parentheses.

The results of these experiments show that females that were kept in short days developed functioning ovaries, containing numerous eggs, while the ovaries of insects exposed to long days remained undeveloped. Figure 5.8 shows a comparison of the size relationship of ovaries of L. jacobaeae females after long and short day treatments. In insects, exposed to short days, ovary length increased up into the metathorax and often filled most of the abdomen, while the ovaries of insects exposed to long days did not need extra space since they were un developed and remained almost the same size throughout the experiment.

Figure 5.8 Comparison of size relationship of ovaries of L.jacobaeae after long day and short day treatments.



5.3.4 Testis Development

Table 5.4 and Figure 5.9 (a) and (b) show the respective sizes of testis of L. jacobaeae that exposed to long days and short days.

Table 5.4 Mean testis diameter of L. jacobaeae exposed to short days and long days.

Number of days of treatment	mean testis diameter(mm)*	
	short days	long days
- 1	0.027 (0.0007)	0.027 (0.0007)
28	0.036 (0.0009)	0.031 (0.001)
48	0.037 (0.0008)	0.036 (0.0008)
58	0.040 (0.001)	0.037 (0.001)

* mean of eight insects, standerd errors are given in parentheses.

Figure 5.9 (a) Effect of short days and long days on the testes size of L. jacobaeae.

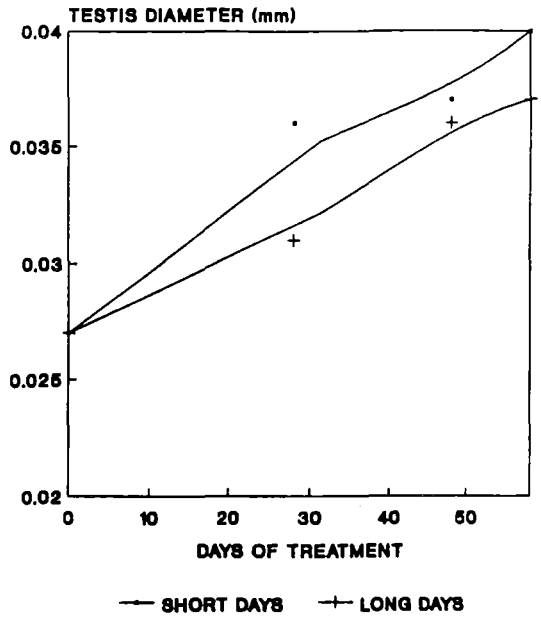
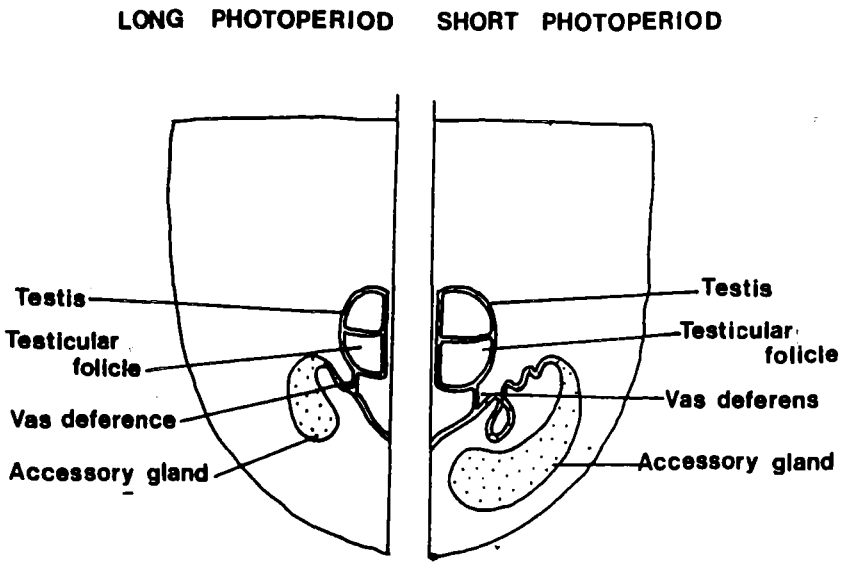


Figure 5.9 (b) Comparison of the size relationship of testis and accessory gland of L. jacobaeae in short day and long day treatments.



These results shows that the testis developed in size with time in both treatments but the development rate is faster under short days than long days. The difference between short day and long day treatments was significant at 5% level ($P<0.05$).

5.3.5 Accessory Gland Development

Table 5.5, and Figure 5.9 (b) and 5.10 show the size relationship of the male accessory gland of L. jacobaeae exposed to long days and short days.

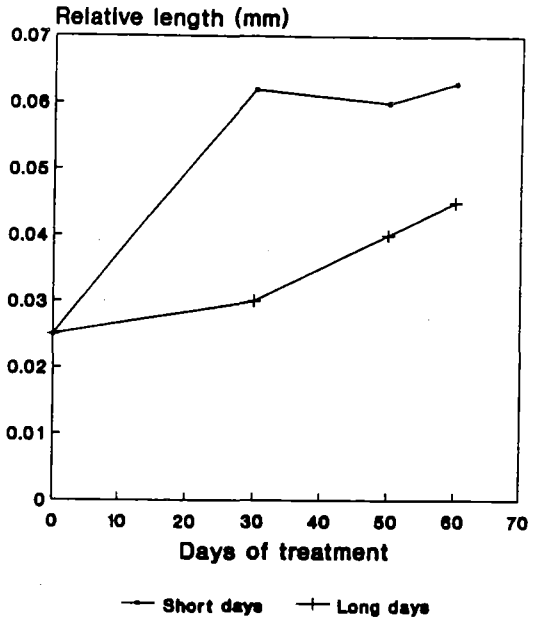
Table 5.5 Mean length of the anterior lobe of the male accessory gland of L. jacobaeae exposed to short days and long days.

Number of days of treatment	mean length of accessory gland* (mm)	
	short days	long days
- 1	0.025 (0.0003)	0.025 (0.0003)
28	0.062 (0.0008)	0.03 (0.001)
48	0.060 (0.001)	0.040 (0.003)
58	0.063 (0.001)	0.045 (0.0007)

* mean of eight insects, standerd errors are given in parentheses.

These results shows that males that received short days had larger accessory glands and males that received long days had smaller and ill developed accessory glands. The difference between short day and long day treatments was significant ($P<0.05$).

Figure 5.10 Effect of short days and long days on the accessory gland size of L. jacobaeae males.



5.3.6 Flight Muscle Changes

Table 5.6 and Figure 5.11 presents the flight muscle bundle diameter for male and female L. jacobaeae, exposed to long and short days.

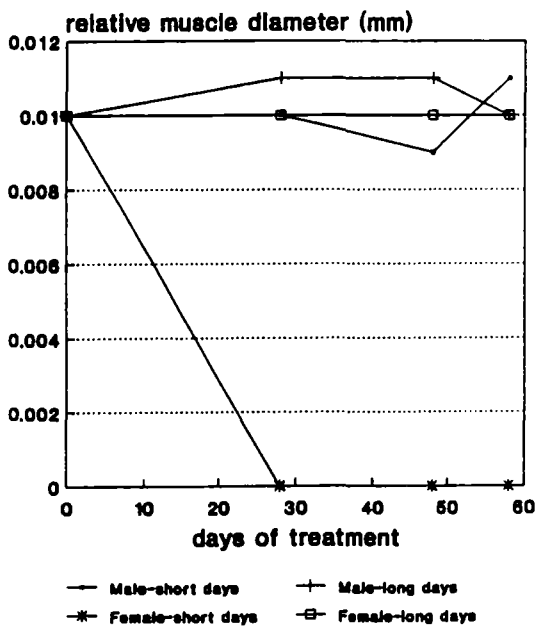
Table 5.6 Mean diameter of the right-side flight muscle bundle of male and female L.jacobaeae, that received short and long days.

days (#)	mean flight muscle bundle diameter(mm)*			
	males		females	
	short days	long days	short days	long days
- 1	0.01 (0.0003)	0.01 (0.0003)	0.01 (0.0007)	0.01 (0.0007)
28	0.01 (0.0007)	0.01 (0.0007)	0	0.01 (0.0005)
48	0.009 (0.0007)	0.01 (0.0007)	0	0.01 (0.0006)
58	0.01 (0.0007)	0.01 (0.0006)	0	0.01 (0.0007)

* mean of eight insect, (#) days after treatment, standerd errors are given in parentheses.

The difference between short and long day treatments on male flight muscle is not significant, but the difference between the two treatments on female flight muscle is very clear and no statistical proof is necessary. The difference between male and female flight muscles exposed to long days was not significant. These results very clearly show that the effect of photoperiod on female flight muscle is high and short days resulted the resorption of the muscle, but there is no such effect on male flight muscle. Fraser (1987), and Frampton (1987), have done similar work on St. John's wort beetle Chrysolina hyperici and Sitona weevil Sitona humeralis respectively and both authors reported that there were small thread like flight muscles remain in above insect after they have been exposed to prefered photo period for egg development. In L. jacobaeae flight muscles were totally undetectable and the metathoracic space was filled with the anterior end of the developed ovary. Crowson (1981) has reported that, in beetles losing the capacity for flight, flight muscles may disappear completely. From the results and from the observations of Crowson (1981), it is clear that at the time of ovarian development L. jacobaeae females do not fly. Instead, they may use protein material from flight muscles for ripening eggs and at the same time, it use the vacated thoracic space for the developing ovaries.

Figure 5.11 A comparision of the size relationship of flight muscle of L. jacobaeae males and females after long and short photophase treatments.



5.3.7 Feeding Rate

Table 5.7 and Figure 5.12 compares the occurrence of different feeding rates of L. jacobaeae adults that were exposed to long days and short days.

Table 5.7 Frequency of different feeding rates of L. jacobaeae adults exposed to short and long days.

score	Percentage of observation	
	short days	long days
N	05	28
L	12	65
M	23	05
H	54	0

Damage classes:

N = no feeding

L = low feeding

M = medium feeding

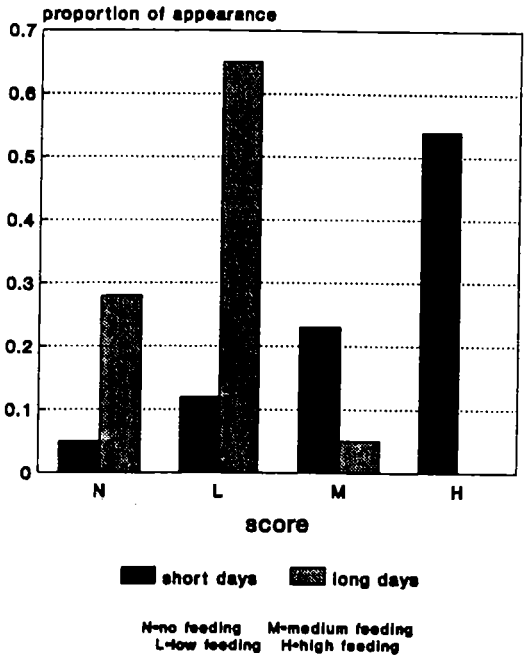
H = high feeding

(rating procedure is described in section 5.2.3)

The percentage of observations is the number of leaves in a particular damage class devided by the total number of observations (360) x 100.

These results show that when adult L. jacobaeae beetles were exposed to short days they feed on leaves aggressively (more than 75% high and medium feeding rate). When they were exposed to long days they do not feed at all (more than 25% no feeding) or even if they feed feeding rate was very low (65% low feeding rate).

Figure 5.12 Comparison of frequency of occurrence of different feeding rates of L. jacobaeae adults exposed to short and long days.



5.4 CONCLUSION

It is obvious from the results that short days permitted maturation of reproductive organs of male and female L. jacobaeae. Complete maturation was reached after 28 days. Long days did not permit maturation(Tables 5.1, 5.2, 5.3, 5.4, 5.5, and Figures 5.6, 5.7, 5.8, 5.9 (a) and (b), 5.10).

Under field conditions Italian biotype L. jacobaeae was observed to be in aestivation about 3-4 weeks after emergence. This dormancy can be considered to be a reproductive diapause. From the results, it is also clear that long days did not completely stop development of reproductive organs, instead these organs continued development very slowly. However, even after 58 days of exposure to long days, reproductive organs of both males and particularly females did not develope to their maximum size, whereas under short days, reproductive organs of both males and females had developed to a productive state after 28 days.

Under natural conditions, adult emergence was observed mainly at the end of the summer (January, February). The first egg was observed 56 days after the first appearance of adults. Therefore, in nature, it is probable that long days restrain the development of reproductive organs in late summer and the shorter days of autumn activate their development.

Changes in flight muscles show that short days cause females to loose their flight muscles but with long days females retain large white flight muscles. There was no effect of photophase on male flight muscles (Table 5.6 and Figure 5.11). Since there was a significant effect of photophase on female flight muscle it could be assumed that:

(a) as Crowson (1981) reported, flight muscles of females may supply protein materials for ripening eggs; or/and,

(b) at reproductive maturation ovaries enlarged up to the metathorax (Figure 5.8). So, as Fraser (1987) reviewed, resorption of flight muscles may be to vacate thoracic space for developing ovaries.

In males, though their reproductive organs develop, there was no enlargement of these organs up to thorax (Figure 5.9.b). This suggests that males do not need extra thoracic space on the reproductive maturity. On the other hand, the protein requirement of females is probably higher than the males, so this may be the reason why only female flight muscles degenerated. It is also clear that the female L. jacobaeae are unable to fly in the reproductive state, so dispersal flights, if they occur, should happen before reproductive diapause.

The above results combined with field observations could be used to test two hypotheses::
(a) L. jacobaeae uses flying for dispersal; and
(b) L. jacobaeae uses jumping for dispersal

(a) L. jacobaeae disperses by flight

At Rotokauri, one of the L. jacobaeae release areas in New Zealand, insects were released in March, 1986 and in the following year larvae and pale new adults were found 40-70 metres away from the release site (Syrett, pers. comm.). This observation gives a clue that, if dispersal flight occurs, most of the released beetles may have dispersed before they entered diapause and oviposited in new areas after they came out of diapause.

At Whakatane, insects were released in May, 1985, and by January, 1986, beetles could only be observed at the release site itself (Syrett,pers.comm.). This could be explained as follows: by May, the female beetles had already started egg laying and they could not disperse due to degenerated flight muscles. By 1987, beetles were found 25 metres away and in 1988 beetles were found 150 metres away indicating that newly emerged beetles in 1986 and 1987 may have dispersed to new areas before they came to the reproductive state.

Similar behaviour was observed at Upper Takaka. Insects were released in April, 1985, and in February, 1986, adults could only be observed at the same place but, by April, 1987, adults have crossed a river and moved up to more than 50 metres and in January, 1989 insects dispersed up to 100 metres.

These results emphasise the importance of timing in the release of beetles as a biological control agent, if they use only flying for locomotion. After release of a biological control agent there may be an advantage if it produces its offspring at the same place as the release, rather than dispersing to other areas. The following generations are then expected to disperse and produce their offspring. If this is the case, L. jacobaeae should be released late in the season, if it is considered that flight is the main means of dispersal.

(b) L. jacobaeae disperses by jumping

Although the jumping behaviour of those beetles which jump is considered to be an escape reaction, jumping serves as an efficient method of locomotion, especially for flightless insects. The jumping ability of flea beetles is activated by the metafemoral spring and the presence of metafemoral spring is defining character of the Sub family Alticinae. Flea beetles can move long distances relative to their body length. One Longitarsus species has been observed to jump considerably more than 100 times of its body length (Fruth, 1988). Observations on several Longitarsus species in the field suggested that there was little tendency for the the beetles to disperse by flight even when the food plant was heavily infested. However, individuals were found to jump actively and readily distribute themselves between patches of host plant (Shute, 1980).

In L. jacobaeae, jumping was observed but flying was never observed during the experiments. Because of these observations and because Shute (1980) and Furth (1988) have already reported that Longitarsus may use jumping as its locomotion, it is possible that L. jacobaeae is basically a non-flying species. If it doesn't fly, it doesn't need flight muscles to provide energy for flying. So,

the protein material stored in muscles may be used completely for egg development. At the same time, the empty thoracic space may provide enough space for the developing reproductive organs.

Field observations of insect dispersal showed that insects released late in the season (insects that have already started egg laying) seem unable to disperse whereas insects released early in the season (those that haven't yet started egg laying) have the ability to disperse. If jumping is the main method of dispersal, then there should be a change in the jumping muscles during the season. To test this theory further study is required to determine whether any such changes occur.

The feeding rate results showed that insects exposed to short days fed aggressively on leaves whereas insects exposed to long days fed very little or not at all. This feeding behaviour can be seen as the gaining of nutritional requirements for their reproductive activities by the insects exposed to short days.

Dispersal capacity and synchronization of life cycle are two of the major requirements of a successful biological control agent. In the case of ragwort flea beetle, it has been shown to be well synchronized with its host, capable of withstanding the same environmental conditions as its host and of searching out new host plants either in the immediate vicinity or by local dispersal. For these reasons it has the potential to be an effective biological control agent.

CHAPTER SIX

INFLUENCE OF TEMPERATURE ON EGG LAYING

6.1 INTRODUCTION

Generally, insects power of producing their off-spring is mainly depend out on longevity and rate of egg production. Within a given population, the rate of oviposition is strongly influenced by temperature (Southwood, 1978). Longevity is usually greatest at the lowest temperatures at which an insect can feed normally. Most of the time egg production is maximum at about the middle of the prefered range of temperature for oviposition (Chapman,1982).

One of the most important characteristic of an effective biological control agent is its power of producing its offsprings. Female L. jacobaeae start egg laying in the field during autumn. The Mean monthly temperature of selected release sites of L. jacobaeae in New Zealand (listed in Appendix) varied from 7-18 ^{o}C. To be an effective control agent L. jacobaeae should be able to maximize its egg production whilst maintaining a high longevity in this range of temperature. Therefore, the experiment described below was designed to test the oviposition rate, total number of eggs and longevity of L. jacobaeae at 5 different temperatures, approximating to the range shown above.

6.2 MATERIALS AND METHODS

6.2.1 Obtaining Ovipositing Adults

Newly emerged adults of L. jacobaeae were collected with a mouth aspirator and kept in a cage over potted ragwort plants in the laboratory. The cage was 50cmx50cmx50cm in size, constructed with nylon mesh supported by an aluminium framework. The mesh could be opened on three sides by a zipfastner. Beetles were kept in the cage for several weeks to confirm that they were mating and to ensure that females were producing eggs. Plants were observed daily until eggs could be detected.

6.2.2 Oviposition

Oviposition was investigated at 5 different temperatures. The lowest temperature that could be investigated with the equipment available was 10 ^{o}C. So the temperatures used ranged from 10-23 ^{o}C. The experimental set up was similar to that described in section 5.2.2 and figure 5.1 (Chapter 5). Each of the humidity chambers contained 12 cages each with a male/female pair. One of these

chambers was kept at each of 10, 15, 18, 20, and 23 ^{o}C in Contherm Scientific constant temperature cabinets. The photoperiod in each cabinet was 10 hours light and 14 hours dark, to ensure that insects got a similar photophase as in autumn. Eggs were counted daily and fresh leaves were provided and filter paper linings were changed every other day. Any changes in leaves, filter paper linings, and inside walls of cages were noted.

6.3 RESULTS AND DISCUSSION

Table 6.1, Figures 6.1, 6.2, 6.3, and 6.4 present the oviposition rate, mean number of eggs produced by a female, mean oviposition period and mean number of eggs laid by females during 10 day time intervals respectively.

Table 6.1 Mean oviposition rate, Mean number of eggs produced by females, and the oviposition period of L. jacobaeae at five different temperatures.

Temperature (^{o}C)	Mean oviposition rate*(eggs/day)	Mean no. of eggs laid*	Oviposition period*(days)
10	1.52 (0.85)	70.91 (38.88)	45.00 (0)
15	4.11 (1.17)	222.08 (99.59)	53.94 (18.29)
18	4.59 (1.14)	279.33 (111.45)	61.25 (19.74)
20	5.43 (1.44)	369.91 (111.26)	67.75 (8.48)
23	5.71 (1.38)	295.91 (114.92)	51.50 (13.25)

* mean of twelve females, standard errors are given in parentheses.

Figure 6.1 Mean oviposition rate of L. jacobaeae at five different temperatures.

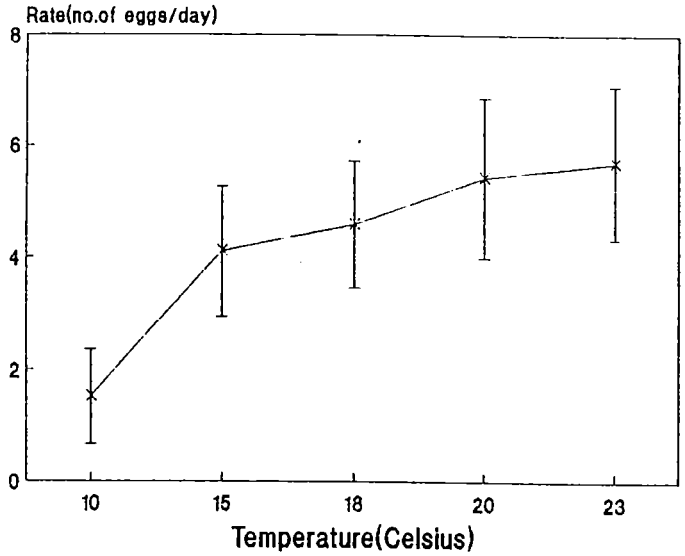


Figure 6.2 Mean total life production of eggs by L. jacobaeae females at, five different temperatures.

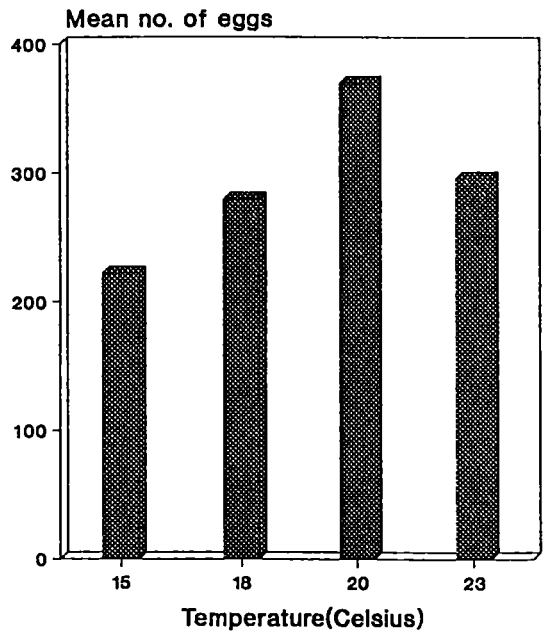


Figure 6.3 Mean oviposition period of L. jacobaeae at four different temperatures

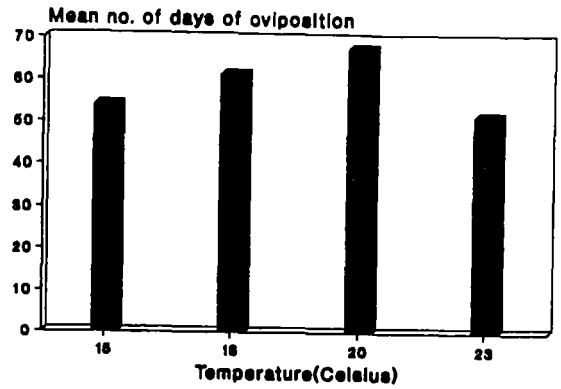


Figure 6.4 Mean number of eggs produced by a L. jacobaeae female during 10 days time intervals at five different temperatures.

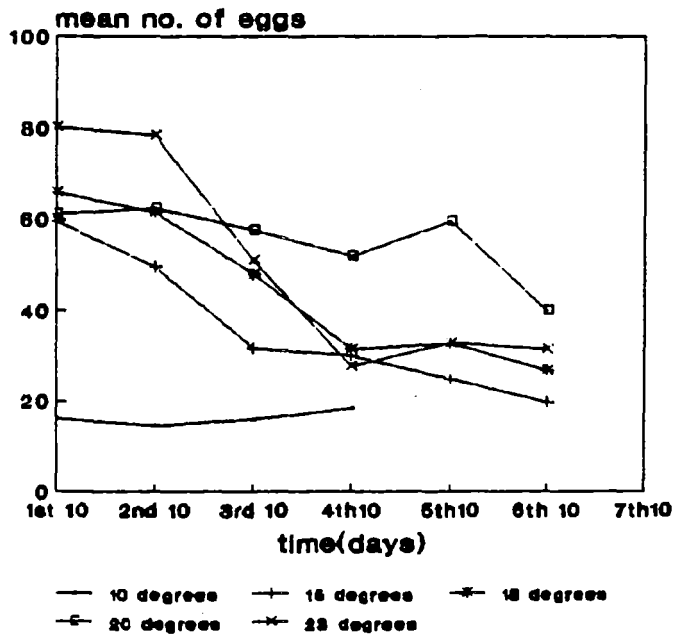
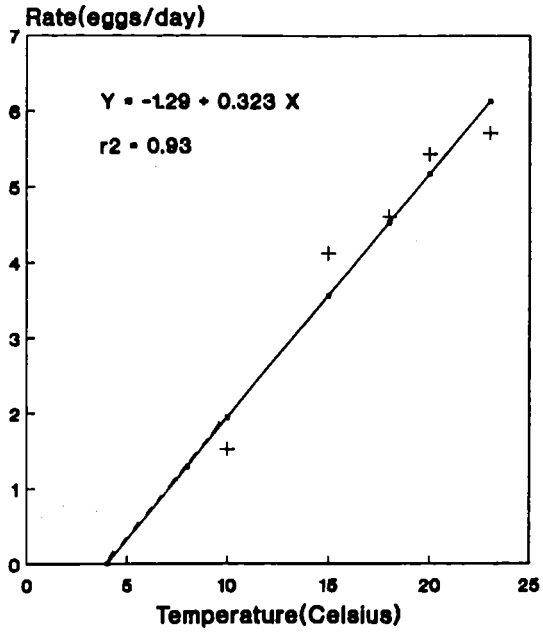


Figure 6.5 Mean oviposition rate of L jacobaeae at different constant temperatures showing lower temperature threshold.



6.3.1 Oviposition Rate

Oviposition rate at different temperatures was significantly different ($P<0.05$). A least significant difference test was done to compare treatment means, and there was no significant difference among treatment means at 15, 18, 20, and 23 degrees but at 10 ^{o}C oviposition rate was significantly lower. The mean number of eggs laid during 10 day time intervals showed that although there is no significant different there is a drastic decline in egg laying at 23 ^{o}C after about 20 days. At the other 3 temperatures the decline of the number of eggs laid with the time is low and is almost the same (fig 6.4). Among these three temperatures 20 ^{o}C can be considered as the optimum since insects maintain egg laying at a high level at least until the 50th day with a low decline by the 60th day. Among oviposing females at the five different temperatures the maximum oviposition rate was observed at 20 ^{o}C, it was 9.6 eggs per day. The minimum oviposition rate of 1 egg per day was observed at 10 ^{o}C. A linear regression analysis was done to get an idea about the lower threshold for oviposition and it was estimated as 4 ^{o}C (Figure 6.5).

6.3.2 Total Number of Eggs and Oviposition Period

I should mention here that 2 females at 10 ^{o}C never laid eggs during the experiment period. Frick (1973) has reported that in artificial laboratery conditions, some L jacobaeae females went into a prolonged dormancy which lasted until the death or for an extended period. Therefore, it could be assumed that those females were still undergoing reproductive diapause period. On the other hand it could be suggested that 10 ^{o}C is relatively close to the lower threshold temperature (4 ^{o}C) for oviposition and that coould be the reason why these two insects did not oviposit throughout that period. Other important thing is at 10 ^{o}C the experiment was only conducted for 45 days due to a failure in the temperature cabinet. Therefore, it is impossible to come to a straight conclusion. Among the other temperatures tested the maximum oviposition period of 91 days occurred at 18 ^{o}C with a total of 428 eggs (ovi. rate = 4.7). The minimum oviposition period observed was 24 days with total of 132 eggs (ovi.rate = 5.5) at 15 ^{o}C. The maximum number of eggs laid observed was 683 within 71 days (ovi.rate = 9.6) at 20 ^{o}C. The minimum number of eggs laid was 62 within 51 days (ovi.rate = 1.2) at 15 ^{o}C.

6.3.3 Female Mortality During Oviposition

At 15 ^{o}C only one female died after 23 days, and at 23 ^{o}C, 2 females died at 55 and 56 days respectively. At other temperatures no females died while ovipositing.

6.3.4 Feeding Behaviour During Oviposition

No exact measurements were taken with relevance to feeding. Informal notes were recorded regarding feeding and excretion during the experiment. These notes show that insects at 10 ^{o}C feed very little or, on some days did not feed at all. Cages and filter paper linings in this treatment were clean all the time showing that there was very little or no excretion at this temperature. Compared to this, insects that were at higher temperatures (15, 18, 20 and 23 ^{o}C) feed intensively showing numerous feeding holes in leaves. The cages and filter paper linings were observed to be dirty all the time due to intensive excretion.

6.4 CONCLUSION

The oviposition results presented here confirm that L. jacobaeae responds favourably to temperatures higher than 10 ^{o}C. A suitable range would be between 15 and 23 ^{o}C, because at 23 ^{o}C there was a drop off of number of eggs produced and the oviposition period. Frick (1973) has

done some work related to temperature and oviposition, but there wasn't any information at temperatures lower than 24 ^{o}C. Therefore, comparision of results is impossible. Feeding and excretion results suggest that L. jacobaeae adults hardly feed at temperatures lower than 10 ^{o}C. Considering both oviposition and feeding results, it could be suggested that the effective lower threshold temperature for oviposition might be nearer to 10 ^{o}C than the 4 ^{o}C postulated by the Campbell et al. 1974) extrapolation method. However, there is a similarity of lower threshold temperature for the egg development (4.3 ^{o}C) (see Chapter 3) and for the oviposition (4 oC) when use the Campbell et al. (1974) method.

Comparision of mean monthly temperatures during autumn with the favourable temperature range for oviposition of L. jacobaeae suggest that, at the time insects start egg laying temperatures are more favourable but, the temperature decreases with time during autumn, and the environment becomes less suitable for oviposition, until by May, only two sites in the nothern North Island (i.e. Gammons road and Kaihu) remain above 10 ^{o}C. However, presumably when the temperature rises above 10 ^{o}C at other sites the insects will be able to resume egg laying but the overall rate of oviposition will be much reduced.

During the oviposition period of L. jacobaeae temperatures in the North Island are more favourable than in the South Island.

CHAPTER SEVEN

CONCLUDING SUMMARY

7.1 THE AIM OF THE STUDY

The aim of the study was to identify the impact of some environmental factors on the biology of the leaf feeding beetle L. jacobaeae in order to better understand its impact in New Zealand in a biological control programme against ragwort Senecio jacobaea.

7.2 THE OUTCOME OF THE STUDY

7.2.1 Egg Development

In natural environment conditions in New Zealand, the eggs of L. jacobaeae first appear in autumn (February or March). Experimental results showed that the mean incubation period at 15 and 18 ^{o}C was 25 days and 18 days respectively. In natural field conditions during autumn, when the mean temperature normally ranges from 19.1 - 12.3 ^{o}C (Appendix 1), eggs would probably take about 3 - 4 weeks to hatch, depending on the local temperature. These eggs would therefore hatch in April. Eggs which are laid late in the season, can withstand the cooler temperature and develop slowly during the winter, but still hatch and produce healthy larvae.

7.2.2 Larval Development

According to the egg development results, first instar larvae should appear in April in the field. Very low threshold temperature for larval development indicate that, although the developmental rate is low, larvae can tolerate the temperature during the winter, in the release areas of New Zealand (Appendix 1; 2). With the onset of higher temperatures in the spring larvae can develop at higher rates. The results reported here suggest two situations that might happen in the field:

(a) Eggs laid early in the autumn may hatch in April. The larvae face winter temperatures right from the first instar and may develop at a very low rate. They may complete the second instar by the end of the winter. From September second instar larvae face warmer spring temperatures, and they may develop at a higher rate to complete third instar and start pupation in November. At this time, higher summer temperatures may increase the development rate and adults may emerge during December.

(b) Eggs that did not hatch during Autumn, may overwinter in the field. With the onset of warm spring and summer temperatures, these eggs will hatch. During summer larvae will take only about 80 days to complete their development, so adult emergence could be expected to be occur in January.

7.2.3 Adult Reproductive Diapause

L. jacobaeae adults which emerge early in the summer, enter into reproductive diapause and continue until environmental conditions suitable for egglaying. Adults which emerge late in the summer or early in the autumn may start egg laying with out entering to diapause, because of environmental suitability at this time. They are able to start egg laying about three weeks after emergence. Therefore, it could be suggested that there will be no time lag of egg production between early emerging adults and late emerging adults. The only advantage to early emerging adults is that they might get higher quality food materials than late emerging adults, because ragwort plants senesce after flowering in late summer. A question can be raised is that if the food material they get is low in quality, how do they produce fertile eggs?. Flight muscle resorption may provied an answer for this, since there is a complete resorption of flight muscles during the egg development.

7.2.4 Oviposition

Adults start oviposition during the autumn in New Zealand, when the temperature conditions are most suitable. Temperatures of the insect release areas in the North and South Islands in New Zealand indicate that insects might have longer lasting oviposition periods in the North Island than in the South Island. The lowe threshold temperature for oviposition (4 ^{o}C) shows that insects can produce eggs, even at lower temperatures during the winter, but the rate of oviposition would be less than 1 egg/day, at temperatures below 10 ^{o}C.

7.2.5 Impact of Insect on the Plant

The most destructive stage of L. jacobaeae is the third instar larvae. Even though first and second instars feed on the root system of ragwort plant, they do not cause much damage. One larvae might complete up to second instar in a single root system of an about two month old plant, but a small single root system may not adequate for a third instar larva. This situation obviously varies with the age, size, and palatibility of the root system and the activity of the larvae.

One disadvantage for a biological control programme that could happen is the production of new shoots from damaged lateral roots. This was observed only twice during the experiment and it took long time to produce those shoots (shoot length was about 5 mm, even after two months). However, I should mention it because it could increase the plant population instead of control the plant.

7.2.6 When Should Adult Beetles be Released in the Field ?

Field observations show that L. jacobaeae adults released early in the autumn (newly emerged adults) have dispersed to other areas before producing their offspring, whereas adults released in late in the autumn (gravid females) produced their offsprings at the release site itself. These offspring then disperse to new areas and produce the second generation. Therefore, it could be recommended that gravid females should be better than newly emerged females for release in the field in that it may help to ensure a sufficient local population for establishment.

7.3 FUTURE RESEARCH

This thesis has gathered some basic biological information on L. jacobaeae on which the future establishment of insect can be based. However, there are still areas that need further research if the biological control programme is to be totally successful.

7.3.1 Effect of Larval Feeding on Plant

A more detailed investigation is necessary to measure the feeding rate of different larval instars in roots of different age groups. This type of study may help to establish a damage threshold value for the insect. At the same time it would be helpful to find out whether there is a compensatory effect of larval feeding on new root production from damage roots.

7.3.2 Flight Capability of Beetle

An understanding of flight muscle change with the age of the beetle, would provide better knowledge of the flight capability at different reproductive stages. This knowledge would help to a better understanding of dispersal ability, and it might help to decide the timing of insect releases.

7.3.3 Relationship Between the Size of the Metafemoral Spring, Pre Diapause, Diapause and Post Diapause Stages

Metafemoral spring is an important character to be studied, since it is considered to be critical in the locomotion of the beetle. Maulic (1929) beleived that any flea beetle that could not jump demonstrated degeneration of the metafemoral spring (cited by Furth, 1988). Therefore, further studies should be carried on to find out whether the condition of the metafemoral spring, and therefore the jumping ability, changes with the reproductive status of the adult beetle.

7.3.4 Economic Analysis

A basic economic hypothesis is that farmers will not adopt technology without an economic incentive, which means the new technology must carry some advantage over farmers existing technology. Therefore, if an individual farmer has to pay for their releases, returns with other control measures and with the biological control using L. jacobaeae should be calculated and compared.

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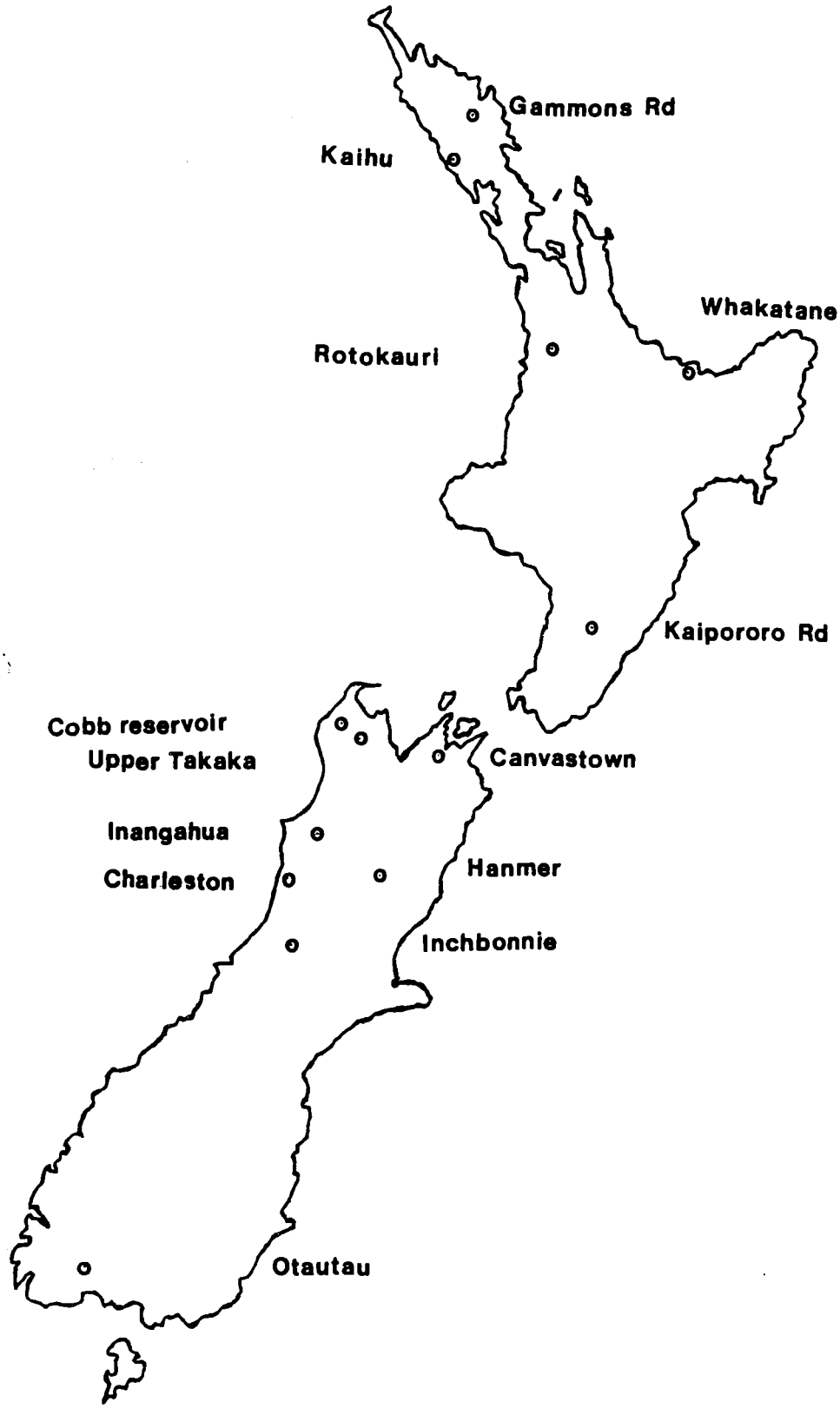
APPENDIX 1

MEAN MONTHLY TEMPERATURE OF RELEVANT AREAS OF NEW ZEALAND

AREA	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	START	STOP	MET STATION
SOUTH ISLAND															
Canvastown	16.8	16.6	15.3	12.5	9.5	6.7	6.3	7.7	9.8	11.8	13.4	15.4	1959	1980	Rai Valley(G251)
Charleston	16.1	16.2	15.4	13.2	10.8	8.6	8.0	9.0	10.4	11.9	13.1	14.9	1947	1980	Greymouth(F422)
Cob Reservoir	13.3	13.8	12.3	9.4	6.3	4.0	3.1	4.0	5.9	7.8	9.5	11.7	1965	1980	Cob Dam(F162)
Hanmer	15.6	15.6	13.9	11.0	7.3	4.5	3.9	5.4	8.1	10.6	12.3	14.4	1906	1980	Hanmer Forest(G581)
Inangahua	15.8	16.0	15.3	13.1	10.9	8.8	8.2	8.9	10.2	11.5	12.9	14.6	1937	1980	Westport Airport(E835)
Inchchbonie	14.8	15.1	13.7	11.2	7.6	5.0	4.6	5.7	7.5	9.7	11.1	13.3	1973	1980	Otira substation(F851)
Otautau	14.0	13.9	12.7	10.4	7.4	5.4	4.7	6.0	8.2	10.2	11.4	13.1	1949	1980	Otautau(I102)
NORTH ISLAND															
Gammons rd	18.9	19.1	18.2	15.9	13.5	11.7	10.7	11.1	11.9	13.4	15.2	16.8	1973	1980	Kaikohe(A482)
Kaihu	18.6	19	18.1	15.9	13.5	11.7	10.7	11.2	12.2	13.8	15.3	16.8	1943	1980	Dargaville(A982)
Kaipororo rd	16.8	16.9	15.6	13.0	10.1	7.8	7.1	8.2	10.0	11.8	13.6	15.5	1928	1980	Mangamutu,Pahiatua(D48
Roto Kauri	18.0	18.6	17.2	14.5	10.9	8.9	8.1	9.5	11.1	12.8	14.9	16.4	1970	1980	Hamilton Airport(C832)
Whakatane	19.1	15.5	18.0	15.2	12.3	9.9	9.3	10.3	12.0	13.9	15.7	17.7	1947	1980	Whakatane(B993)

APPENDIX 2

SOME LONGITARSUS JACOBAEAE RELEASED AREAS IN NEW ZEALAND



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