Interaction of population processes in ragwort (*Senecio jacobaea* L.) and ragwort flea beetle (*Longitarsus jacobaeae* Waterhouse)

A Thesis presented in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Ecology at Massey University.

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Declaration of Originality

This thesis represents the original work of the author, except where otherwise acknowledged. It has not been submitted previously for a degree at any University.

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Abstract

The primary goal of this study was to improve understanding of biological control of weeds by investigating how population processes in ragwort and herbivorous insect interact.

Specific aims were to measure the consumption rates of the three larval instars of ragwort flea beetle (*Longitarsus jacobaeae*), to investigate how the process of herbivory by ragwort flea beetle affects the population density of ragwort, and to investigate how soil moisture influences the population densities of ragwort flea beetle and ragwort.

An extraction apparatus was constructed to obtain *L. jacobaeae* larvae from ragwort roots and root crowns. This apparatus was 84% efficient.

A preliminary survey of ragwort flea beetle numbers included ragwort plants from Ballantrae, Turakina, and Pahiatua (Southern North Island, New Zealand). The larval population was highest at Ballantrae but the adult population was highest at Turakina.

Data were collected from Ballantrae from 1996 to 1998 to develop the interaction model between *L. jacobaeae* and ragwort. The interaction depended on the effect that soil water content had on the populations of both *L. jacobaeae* and ragwort, the effect that larval density has on larval mortality, and the effect of ragwort density on the population of *L. jacobaeae* larvae. Soil water content was positively correlated with the increase in numbers of *L. jacobaeae*. *L. jacobaeae* larval mortality was dependent on larval density. High numbers of larvae per plant resulted in a reduction in the number of larvae over time (13.6 larvae/plant on November 1997 to 1.8 larvae/plant in December 1997). The average number of larvae extracted at Ballantrae was lower in October and November 1996 (4.4 and 4.6 larvae/plant) than in October and November 1997 (13.4 and 13.6 larvae/plant). However, the average numbers of rosettes was higher in October and November 1996 (7.6 and 5.78m $^{-2}$) than in October and November 1997 (2.8 and 2.7 m $^{-2}$). There was a significant inverse correlation between the numbers of *L. jacobaeae* larvae and ragwort rosettes (-0.4608). When

0.8983 in 15 day old larvae, 0.9261 in 30 day old larvae, and 0.9454 in 45 day old larvae. The lowest percentage survival (0.9067 in 15 day old larvae) was found at the highest larval density (40 larvae per plant). Finally, the same experiment was tested in a field and the data from this was used to construct an interaction model for *L. jacobaeae* and its food, ragwort. This model was based on the correlation between soil water and populations of *L. jacobaeae* and ragwort; the effect of larval density on the mortality of larvae and on the weight loss of ragwort; and on the effect that ragwort density has on the mortality of *L. jacobaeae* larvae. Mean soil water was 12 ± 0.29 to 76 ± 1.81 % over the first 15 days, then 36 ± 1.10 to 82 ± 0.99% up to 30 days, and 35 ± 0.76 to 65 ± 1.78% up to 45 days of larval life. These were the soil water contents that occurred during the field experiment. The model showed that the highest larval survival again occurred when few larvae were introduced to ragwort plants (17.5% survival from 0-15 days, 14.33% from 16-30 days, and 18.5% from 31-45 days). High larval densities also produced the lowest survival (8.4% survival over 0-15 days, 5.87% over 16–30 days, and 6.7% over 31-45 days).

The effect of plant density on larval survival was also tested in the field. The highest larval survival (10.76%) occurred when there were on 16 plants m^{-2}, and the larvae were 0 to 15 –days old. The lowest larval survival (6.61%) occurred with 16-30 day old larvae on plants at a density of 4 plantsm^{-2}. A cohort life-table was constructed for predicting population fluctuations of *L. jacobaeae*. Values from this life table were used to model populations of *L. jacobaeae*, ragwort and the interactions between these species using "STELLA" software. Data for the ragwort model was obtained from published papers. Additional data from the experimental determination of feeding rates of *L. jacobaeae* larvae were used when both the *L. jacobaeae* and ragwort models were combined to examine the interactions between these species. This latter model was used to estimate population fluctuations of *L. jacobaeae* and its food over two years. It indicated that *L. jacobaeae* is a very effective control agent for ragwort, and that it can cause ragwort populations to decline to extinction within two years.

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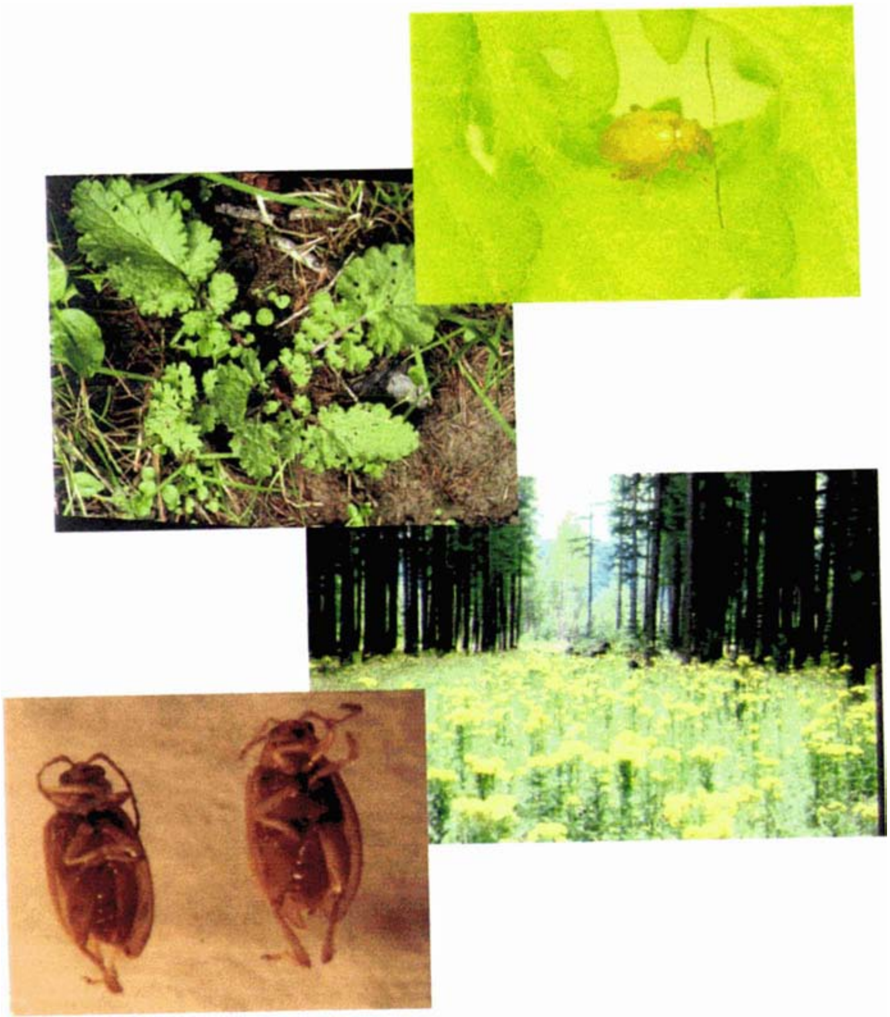
SECTION I

INTRODUCTION AND METHODS

Chapter one: Introduction



INTRODUCTION

1.1 Introduction

Ragwort, (*Senecio jacobaea* L.), is a common poisonous weed of grassland throughout most of New Zealand. It is particularly common in steep hill country where control by chemical or mechanical methods is expensive, and often uneconomic (Syrett, 1983). The value of losses due to ragwort in New Zealand has not been estimated. However, $0.6 million per year was spent on ragwort control from a budget of $9 million from public funds in 1979-80 under the Noxious Plant Control Scheme (Syrett, 1983). Ireson (1995) pointed out that losses caused by ragwort plants to the Tasmanian dairy industry alone, due to reductions in pasture production, are estimated to exceed A $ 1 million. Ragwort was one of the first weeds to be the subject of a biological control programme in New Zealand with the introduction of cinnabar moth (*Tyria jacobaeae* L.) from 1926-39 and two seed-flies (*Pegohylemyia* spp.) from 1928-39. All were introduced from Europe (Syrett, Scheele, & Philip, 1984; Dymock, 1985) but these insects became established in few regions (Syrett, 1983). A third biological control agent, the ragwort flea beetle (*Longitarsus jacobaeae* Waterhouse), was subsequently introduced into New Zealand in 1981 to try to improve the success of the biological control programme.

1.2 *Senecio jacobaea* L., Ragwort (Asteraceae)

Biology and Ecology of Ragwort

Ragwort is predominantly a biennial or perennial herb. It usually dies after flowering, but it frequently behaves as a perennial particularly if damaged (Harper & Wood, 1957).

Vegetative and Reproductive Stages

The following three vegetative stages of ragwort are generally recognized: seedlings with five or fewer expanded leaves; single rosettes that are usually up to one year old, (but single rosettes may be large and older than one year); and multiple rosettes which are older than one year and have more than one root crown (Forbes, 1977). These are largely arbitrary categories that are used for convenience. Wesselingh and

Klinkhamer (1996) found that flowering in ragwort depend on plant size at both the time of vernalization and the time of photo-induction in spring. Forbes (1977) reported that 57% of all plants died as seedlings, 35% died as vegetative rosettes, and the 8% that flowered died immediately afterwards. Thompson (1985), by contrast, found that about 20% of plants flowered.

Schmidl (1972) found that 2% of ragwort plants in Australia were annuals, 45% were biennials and 39% were perennials. In England, Forbes (1977) reported that 8% were annuals, 39% were biennials, and 53% were perennials. No plants were annuals in New Zealand according to Thompson (1985), but between 5% and 20% were biennials, 4% to 20% were triennials, and 1% to 5% survive as non- flowering third year rosettes. A rosette of about 12.5 - 15.0 mm in diameter forms in the first year depending on soil fertility and competition (Harper & Wood, 1957). Poole and Cairns (1940) found that first year rosettes may grow up to 30mm in diameter in New Zealand. Rosettes usually produce flowering stalks in the second year under favourable conditions. However, a minimum rosette size must be reached before the plant will flower, especially in a disturbed environment (van der Meijden & van der Waals- Kooi, 1979). The percentage of plants that regenerate after flowering varies from 1% to 44% (Poole & Cairns 1940; Schmidl 1972; Forbes, 1977; Thompson, 1985). Wardle (1987) concluded that the horizontally growing rosette inhibits surrounding vegetation and that young ragwort plants colonize the space created by the death of the adult plant. The potential for regenerating after damage also contributes to the plant's survival for more than one generation, and promotes the persistence of ragwort populations (Wardle, 1987).

Seeds

In New Zealand, ragwort sheds ripe seeds from the end of December until March or April. There are two types of seeds termed disc and ray. Both are about 2 mm long and 0.6 mm wide (Poole & Cairns, 1940). Disc seed is formed in the centre of the capitulum and bears a number of small hairs for dispersal by wind. Ray seed originates from the outside of the capitulum and is glabrous (hairless). Disc seeds usually have a shorter germination period and a higher germination rate than ray seeds (Poole & Cairns, 1940). Ray seeds are shed later than disc seed, they do not have dispersal structures and they have a thicker pericarp than that of disc seed (McEvoy, 1984).

Estimates of seed production vary between 1000 and 250,000 seeds per plant depending on geographic location and the number of capitula the plant produces (Cameron, 1935; Harper & Wood, 1957; Schmidl, 1972; van der Meijden, 1971; van der Meijden & van der Waals-Kooi, 1979). In New Zealand, Poole and Cairns (1940) reported that *S. jacobaea* at Ruakura produced 1,000 - 2,500 capitula per season and each capitulum contained 55 seeds. Large plants at Piopio produced 3,375 capitula with 60 seeds per capitulum and plants in Southland produced 6480 capitula per season. Plants in the United Kingdom produced between 68 and 2489 capitula and had an average of 70 seeds per capitulum (Cameron, 1935) whereas plants in Australia produced between 14 and 1936 capitula per season (Bornemissza, 1966).

Dispersal

Most ragwort seeds do not move far from the parent plant despite the large numbers produced. Those seeds that do move further are dispersed by wind, birds, farm animals and water. Wind dispersal generally results in the seeds remaining within a few metres from their source but they can be carried long distances by convection currents under suitable conditions (Poole & Cairns, 1940; McEvoy & Cox, 1987).

Farm animals may disperse the seeds but this has not been studied (Wardle, 1987). Poole and Cairns (1940) noted that dispersal by water occurred along the Waipa River and South Karori stream and resulted in seedlings often occurring on the lower streambeds. The possible colonization of new areas by seed transported over long distances by water was discussed by McEvoy and Cox (1987).

Germination and Dormancy

Seeds germinate in 4 to 8 days under optimal conditions (about 15°C) (Cameron, 1935). Baker-Kratz and Maguire (1984) found that maximum germination occurred 18 days after flowering in peripheral achenes, and after 21 days in central achenes. Seed germination also varies with climate (Schmidl, 1972), light, temperature and moisture (van der Meijden & van der Waals-kooi, 1979; Baker-Kratz & Maguire, 1984), and depth in the soil (Poole & Cairns, 1940; van der Meijden & van der Waals-

Kooi, 1979). Schmidl (1972) found 85% of summer-produced seeds germinated and 60% of late flowering plants germinated. Temperature and soil moisture content are predominant factors in seed germination. Ninety three percent of seeds germinated at 15^0C with a soil moisture content of 29% (van der Meidjen *et al.*, 1988). Crawley and Nanchapong (1985) reported that 78.8% of seeds germinated after plants were defoliated by cinnabar moth larvae. Within the flowers, percentage germination varies from 2 to 98% in disk achenes and from 2 to 82% in ray achenes (McEvoy, 1984). Seed germination also varies from 50% to 86% depending on the depth of soil covering them (Poole & Cairns, 1940). Beskow (1995) found that emergence of ragwort seedlings is higher on bare soil than under pasture canopy. Longer period survival of ragwort seedlings is depended on the sward height varied between 2 and approximately 7 cm when herbage mass was between 500 and 2,000 kg DM (dry matter) ha^{-1}.

Ragwort seeds can lie dormant for 6 to 8 years (Harper, 1958). Seeds remain viable in the top 20 mm of the soil for at least 4-5 years and some that are deeper than 40 mm are still viable after 10-16 years (Thompson & Makepeace, 1983; McEvoy, 1985; McEvoy *et al.,* 1991). Cameron (1935) and van der Meijden (1976) reported that covering seed with 1 cm or more of soil could prevent germination. Frost and drought may induce dormancy and thus delay germination in the field (van der Meijden & van der Waals-Kooi, 1979). However, Baker-Kratz and Maguire (1984) reported that innate dormancy in ragwort was "apparently non-existent". Beskow *et al.* (1994) and Beskow (1995) found that grazing and trampling by stock resulted in higher germination when compared with ungrazed control plots, although many of the seedlings (>58%) subsequently died over summer.

Establishment

Seedlings generally establish in the absence of both long grass and a closed sward (Cameron, 1935; van der Meijden & van der Waals-Kooi, 1979); dense vegetation greatly reduces their success at establishment (Crawley & Nanchapong, 1985). Establishment is certainly high in the clear area beneath mature ragwort plants in Oregon, U.S.A. (McEvoy, 1984).

Weed Status

Distribution

Ragwort is native to Europe and western Asia (Schmidl, 1972). It is a continental species that extends as far east as Siberia and as far south as Asia Minor (Harper & Wood, 1957). It is present in Rumania, Hungary, Bulgaria, northern Greece, and in North Africa (Harper & Wood, 1957). Other countries where ragwort has been introduced are mostly coastal areas of U.S.A and other areas of Canada, Argentina, British Columbia, New Zealand and Australia (Schmidl, 1972; Cox & McEvoy, 1983). It is widely distributed in west and southwest England, especially Pembrokeshire, Anglesey and in much of eastern Ireland (Harper & Wood, 1957).

Abundance

Ragwort was first recorded in New Zealand near Dunedin in 1874 (Poole & Cairns, 1940). It increased rapidly in Southland and in parts of Auckland, Wellington, and Taranaki after it was first introduced (Thomson, 1922). Ragwort spread quickly throughout New Zealand and most serious infestations occur in the light rhyolite and derived soils of the central plateau of North Island and adjacent districts (Poole & Cairns, 1940). Ragwort is now found in most areas that receive more than 800 mm of rain annually in New Zealand (Poole & Cairns, 1940). It was declared a noxious weed in the Second (optional) Schedule of the Biosecurity Act of 1993, and was placed in the First Schedule of the Act of 1908 (Radcliffe, 1969).

Ragwort was introduced into Tasmania and Western Australia in 1940 (Gardner & Royce, 1948). Schmidl (1972) reported that the infestation of ragwort in southern Victoria, Australia is about 40,000 ha of pasture, forest plantations and abandoned farmland. Amor, Lane, and Jackson (1983) found that in Australia the abundance of ragwort was generally highest on ungrazed sites and lowest on sites grazed by sheep except in summer when the density was greatest on some sites grazed by cattle. In England, Dempster and Lakhani (1979) found that 8-9 ragwort plants /m^2 occurred at Weeting Heath in 1968.

Changes in the population density of ragwort from one year to the next are thought to depend less upon the damage inflicted by cinnabar moth caterpillars and more upon the amount of rainfall at the time of seedling establishment (Cameron, 1935; Harper

& Wood, 1957; Harris *et al.,* 1978; Dempster & Lakhani, 1979; Myers, 1980). McEvoy (1984) reported that on the central coast of Oregon, USA, ragwort populations could be depressed by the introduction of two phytophagous insects, ragwort flea beetle (*Longitarsus jacobaeae* L.) and cinnabar moth (*T. jacobaeae*). He found that dormant seeds and vegetative buds declined to 32% of their former abundance and actively growing seedlings, rosettes, and flowering plants declined to 3% of their former abundance following increases in population densities of ragwort flea beetle and cinnabar moth. The abundance of ragwort decreased 110-fold in Oregon when a combination of ragwort flea beetle larvae and cinnabar moth larvae were present. The former attacked petioles, stems and roots in winter while cinnabar moth larvae attacked shoots in summer (McEvoy, 1984).

Toxicity of ragwort

Ragwort is toxic to cattle and horses and causes the disease known variously as "Pictou" cattle disease in Canada (Long, 1910), "Sirasykae" in Norway, and "Winton disease" in New Zealand (Gilruth, 1904; Leonard, 1950). Ragwort contains at least six pyrrolizidine alkaloids: jacobine, jacodine, jacoline, jaconine, senecionine, seneciphylline (Connor, 1977; Field & Daly, 1990). Ragwort can cause poisoning as a chronic hepatotoxicity in cattle resulting from recurrent sublethal doses of pyrrolizidine alkaloids (Connor, 1977). Pyrrolizidine alkaloids can also cause a cirrosis-like condition of the liver in cattle and horses (Johnson, 1978). Although cattle and horses are most often affected, human food contaminated with pyrrolizidine alkaloids from ragwort may be hazardous to human (Johnson, 1978). Toxicity is due to three nitric acid alkaloids: jacobine, jacodine, and jaconine (Barger & Blackie, 1937). The highest concentration of pyrrolizidine alkaloids is 0.3% which occurs in dried flowers (Aplin, Benn, & Rothshild, 1968; Buckmaster, Cheeke, & Shull, 1976; Dickinson *et al.,* 1976; Denzier *et al.,* 1977).

Bissect and Rees (1937) reported that death might not occur until 1 to 5 months after a lethal dose has been eaten and the symptoms of poisoning may not appear until a week before death. Johnson (1978) found in Tillamook, Oregon that the cattle died if a total of 2% of their body weight of the ragwort plants was eaten within a 20-day period. Poisoning symptoms by pyrrolizidine alkaloids on cattle are loss of condition, diarrhoea, hyperexcitability and coma. Horses may show unsteadiness, aimless wandering, ataxia and secrete dark urine (Mortimer & White, 1975). Sheep are occasionally poisoned by pyrrolizidine alkaloids and their growth is slowed when eating it (Aston & Bruce, 1933). Lambs may have poisoning symptoms similar to those of cattle and these symptoms are often confused with facial eczema (Mortimer & White, 1975). The pyrrolizidine alkaloids have cumulative effects (Mattocks, 1968). Animals convert these alkaloids to pyrroles that are partly excreted in urine (Mattock, 1968; Johnson, 1978). Some pyrroles, however remain strongly bound to tissue in the lung and liver (Mattock, 1968).

Ragwort is avoided by cattle and horses when sufficient alternative food is available. Two problems that are usually greater than the toxicity of ragwort are that pasture is partly vitilised because of ragwort (stock avoid the ragwort and thus do not eat pasture beside each ragwort plant) and also this species is usually designated as noxious (Kerry Harrington per. Comms). However, during periods of drought, when grass is in short supply or has dried out, they will eat ragwort. Aston and Bruce (1933) found that mature cattle might develop a lethal addiction to the plant. The toxic alkaloids remain in dried ragwort plants so that poisoning may follow ingestion of contaminated hay, dried grass and silage (Willmot, 1949; Donald *et al.*, 1956). Apparently ragwort becomes highly palatable when it is dead or dying after being sprayed with certain herbicides. It is important, therefore, to keep stock off sprayed pasture until the dead ragwort plants have disappeared.

1.3 Methods of Management

Mechanical Removal

Mechanical control measures for ragwort have proven to be largely ineffective because it can regenerate from small fragments of root (Poole & Cairns, 1940; Islam & Crawley, 1983). Chipping is generally unsuccessful because new meristem forms and the plant regrows from this (Poole & Cairns, 1940). Pulling plants out or cutting them down when they are flowering are impracticable control measures (Wardle, 1987). Crawley and Nanchapong (1985) suggested that some degree of control might be achieved by mowing the primary flowers at such a height that the sward does not open up and Thompson (1985) supported this mowing during a critical period of 1-2 weeks during late flowering can kill many ragwort plants because they have insufficient reserves to recover (Bornemissza, 1966). Flame-throwers can be used successfully to kill about 93% of seedlings while seeds remaining on burnt plants were not viable (Wardle, 1987).

Management by Herbicides

Various chemical herbicides have been used to control ragwort, but success varies according to the type of herbicide and time of application. Young plants can be killed more easily than older plants (Wardle, 1987). Sodium chloride was widely used as either a spot or blanket treatment for ragwort control (Harper, 1958; Radcliffe, 1969), but it is no longer available in New Zealand (Kerry Harrington per. Comms.). Black (1976) reported that 2,4-D can control younger plants, but it performs poorly on late rosettes, and budding or flowering plants. Thompson (1980) found that 2,4-D ester applied in May (autumn) gave less effective ragwort control than applications in October (spring) in New Zealand. Picloram is superior to 2,4-D at controlling ragwort (Coles, 1967; Thompson, 1974; 1977; Forbes, 1978) and MCPA can be applied to control ragwort, but it is less effective than 2,4-D (Thompson & Saunders 1984; Forbes, 1978). However, in New Zealand MCPA and 2,4-D are the most commonly used herbicides to apply for controlling thistles and ragwort (K. Harrington pers. Comm.). These two herbicides can cause suppression in white clover growth for a number of weeks after application. Generally they will not kill the clover, but the suppression in growth can have an impact on animal production (K. Harrington, pres. Comm.). Harrington (pers. Comm. 2000) explained that if ragwort populations are at low densities, farmers may decide to spot-treat the ragwort plants, rather than apply herbicides across all the paddock by ground-based or aerial boom spraying. In New Zealand, metsulfuron (Escort) is now popular for spot-spraying of weeds, but this damages clover as well as ryegrass component, increasing pasture damage further (K. Harrington, pers Comm.,2000). Application of 2,4,5-T can effectively control ragwort (Forbes, 1978) but 2,4,5-T has not been available in New Zealand for many years and glyphosate can suppress ragwort, but subterranean parts of the plants cannot be killed with these herbicides (Wardle, 1987). Mixtures of herbicides such as 2,4-D/picloram, 2,4-D/dicamba can also be used to control ragwort (Thompson, 1983).

Later stages of ragwort are more difficult to control by using weedicides. The "Matthews Technique" for controlling ragwort was developed in New Zealand (Wardle, 1987). The mature plants are allowed to complete flowering and seeding in an ungrazed situation so that the majority of root crowns and roots die (Wardle, 1987). Subsequent establishment of seedlings can be controlled by applying low dosages of 2,4-D. Herbicides, however, have some deleterious side effects. Generally the most harmful is that they can seriously damage clovers (Wardle, 1987; Honore, Rahman, & Dyson, 1980; Forbes, 1982; Thompson & Saunders, 1984).

Management by Grazing

Sheep can be used to control ragwort because they are relatively resistant to pyrrolizidine alkaloids poisoning. Swick, White, and Cheeke (1982) found that sheep apparently have a lower capacity to metabolize pyrrolizidine into pyrroles, which is harmful for ruminant livestock. Sheep grazing reduces the vigour of the plants so that only insignificant rosettes and no flowering shoots are formed (Poole & Cairns, 1940; Harper & Wood, 1957). Ragwort density declined from 9.2 plants/m^2 to 0.2 plants/m^2 in New Zealand after one year when subjected to 3.0 stock units in mob stock (Betteridge *et al.,* 1994).

Management by Microbial agents

Harper (1958) recorded four fungal diseases that attack ragwort in the UK: *Puccinia expansa* Link., *P. diaicae* Mayn., *Sphaerotheca humuli* Burr., and *Bremia factulae* Regel. These fungi cause rust diseases on ragwort but their effectiveness as microbiological control agents are unknown. In New Zealand, beet western yellow virus disease on ragwort plants, *S. jacobaea* (Pennycook, 1989 vol 1 & 3) and fungal diseases caused by *Pythium* sp. (Leterothalic forms) (Robertson, 1973 *in* Pennycook, 1989 vol. 2) and *Pythium* sp. (Spaerosporangiate forms) (Robertson, 1980 *in* Pennycook, 1989 vol. 2) on *Senecio* spp. are recorded.

Management by Tetranychid mite

A tetranychid mite *Halotideus destructor* Tuck., is known to attack the seedling and rosette stages of ragwort, and causes severe damage in South Africa (Schmidl, 1972). Seedlings can be killed and the growth of more advanced rosettes can be so retarded that flowering in the same season may be prevented (Schmidl, 1972). The mite is most active on ragwort in the spring but it also damages useful pasture crops (Schmidl, 1972).

Management by Insects

A number of insects attack and cause damage to ragwort in New Zealand. Syrett (1983) recorded six insects that are commonly associated with ragwort: the magpie moth, *Nyctemera annulata* Boisduval, (Lepidoptera: Arctiidae); the pyralid stem borer, *Homoeosoma farinaria* Turne, (Lepidoptera: Pyralidae); a cutworm larva, *Ariathisa comma* Walker, (Lepidoptera: Noctuidae); the agromyzid stem borer, *Melanagromyza senecionella* Spencer, (Diptera: Agromyzidae); the leaf miner, *Phytomyza syngenesiae* Hardy, (syn. *Chromatomyia syngenesiae* Hardy) (Diptera: Agromyzidae); and an aphid, *Brachycaudus helichrysi* Kaltenbach, (Hemiptera: Aphididae).

The magpie moth is the most conspicuous insect that attacks ragwort in New Zealand and it can cause considerable damage (Miller, 1970). The larvae feed on the leaves and seeds and cause extensive defoliation when their population density is high (Quail, 1901; McLaughlin, 1967). However, their effectiveness is decreased because they are parasitised by two braconids (*Microplitis* sp., *Apanteles* sp.) (Syrett, 1983; Valentine 1967), and two tachinids (*Pales nyctemeriana* Hudson and *P. casta* Hudson) (Miller, 1970). Syrett (1983) also recorded that the pupae of magpie moths are commonly parasitized by the ichneumonid *Echthromorpha intricatoria* F.

The pyralid stem borer is well established throughout New Zealand but long term control of ragwort by this insect is also unsuccessful because of parasitism (Miller, 1970). Cutworm larvae often damage ragwort, but they are not suitable biological control agents because they also damage crop plants. The agromyzid, *M. senecionella,* is common in New Zealand but has little effect on ragwort (Miller, 1970). The leaf miner, *P. syngenesiae* can damage ragwort leaves, but cannot be widely used as biological control agent because it is a problem in glasshouses and because it is parasitized by *Dacnusa aerolaris* Nees (Hymenoptera: Braconidae: Dacnusinae (Alysinae)) and by *Chrysocharis pubicornis* (Hymenoptera: Eulophidae) (Kelsey, 1937; Syrett, 1983). The aphid, *Brachycaudus helichrysi,* occurs on ragwort flowers and may cause them to mat together with honey-dew, but it has little effect and so is not suitable as a biological control agent (Miller, 1970).

Cinnabar moth Tyria jacobaeae (*Lepidoptera: Arctiidae*)

The cinnabar moth is the most conspicuous insect amongst the introduced biological control agents. It was introduced into New Zealand between 1929 and 1932 (Syrett, 1983) and is univoltine throughout its range, with an overwintering pupal stage (Beban, 1991). Adults emerge as early as August, but are common from November in New Zealand (Miller, 1970). The yellowish eggs are laid in clusters on the underside of leaves, and take about two weeks to hatch (Beban, 1991). Even small larvae usually damage the leaves soon after eclosion. They occur in the field from September to December in New Zealand (Miller, 1970). The young larvae are yellowish with a black head, but older larvae have the characteristic black and yellow-banded body. The larval period is about one month (Beban, 1991).

Cinnabar moth has had limited success as a biocontrol agent of ragwort. Some factors affecting this moth's mortality reduce its success as a biocontrol agent in some places. These factors are parasitism (Miller, 1970; Harris *et al.,* 1975; Dempster, 1975; Bornemissza, 1966; van der Meijden, 1980), predation (Wilkinson, 1965; Bornemissza, 1966; Myers & Campbell, 1976), viral disease (Bornemissza, 1966), microsporidian disease (Bucher & Harris, 1961; Harris *et al.,* 1975), fungal disease (Bornemissza, 1966), and unfavourable climatic conditions (Harris *et al.,* 1975). In addition, the moth's limited powers of dispersal (Wilkinson *et al.,* 1970) have led to local extinction of both ragwort and cinnabar moth in the Netherlands (van der Meijden, 1976). In England, Dempster (1982) found that ragwort and cinnabar moth populations experience high amplitude fluctuation and there is no control by cinnabar moth at low plant densities but if plant densities are low, then control is unnecessary. Ragwort has been maintained at low levels by cinnabar moth over a five-year period in England and USA (Oregon) (Stimac & Isaacson, 1978) but, the moth populations are stabilizing at levels below that needed for complete defoliation of ragwort in British Columbia and Canada (Harris, Thompson, Wilkinson, & Nearly, 1976).

Ragwort Seedfly Hylemyia *spp.* (*Diptera: Anthomyiidae*)

Two species of ragwort seed fly (*Hylemyia (=Pegohylemyia) seneciella* Meade and *H. jacobaeae* Hardy) larvae reduced seed production by feeding in the flower heads (Miller, 1970; Dymock, 1985). Both *P. seneciella* and *P. jacobaeae* were present in the consignments into New Zealand from England between 1928 and 1939 (Miller, 1970; Holloway, 1983). Their life histories have been described by Cameron (1935), Frick & Andres (1967), Miller (1970), Syrett (1983), and Dymock (1985). The pupae overwinter in the soil and imagines emerge in spring or summer. Female flies lay eggs in both closed and open capitula between the bases of the florets or along the green bracts. The eggs hatch in 3 to 4 days. The three larval instars feed on the immature seed and part of the receptacle (Cameron, 1935). When fully grown, the larvae fall to the ground and pupate in the soil.

When larvae are present in the inflorescence, they produce a brown spot in the disc florets (Cameron, 1935) which become matted together by larval secretions and covered with a dark grey mould. The pappus may also be extruded. After larvae leave to pupate, the inflorescences are black and sticky and the pappus is unshed (Miller, 1970). The exit hole can easily be seen at the base of the receptacle (Syrett, 1983).

Dymock (1985) concluded that seedfly had little effect on ragwort populations because of high pupal mortality (pupae do not damage plants, so presumably it is not high pupal mortality that is the reason for the low impact, but the subsequent low adult and then larval population) and because they only damage the early flowers. Competition for early flowers is intense but overall a large proportion of ragwort seed escapes damage. Mortality of larvae and pupae was caused by four species of hymenopteran parasites (Cameron, 1935), by nematodes in the genus *Rhabditis* and by fungi (Hoy, 1958).

The seed fly was introduced into Australia from New Zealand in the 1930s and 1950s but failed to establish (Waterhouse, 1967). Seedfly also failed to establish in British Columbia and Prince Edward Island when it was introduced there in 1968 (Harris, Wilkinson, Neary, & Thompson, 1971) but it did establish in California and has established in Oregon and Washington (Frick, 1968).

In New Zealand, seedfly infests up to 77% of the flower heads during the peak flowering period (Miller, 1970). Kelsey (1955) found that 91% of the early flowers and 42% of the main crop were attacked but no late flowers were damaged. Because ragwort populations are seldom limited by seed production (Crawley, 1990) it is not surprising that ragwort seed fly is not an effective biological control agent

Ragwort Flea Beetle Longitarsus jacobaeae *Waterhouse* (*Coleoptera: Chrysomelidae*)

Background

Ragwort flea beetle, *L. jacobaeae*, occurs naturally in Britain and Scandinavia and through Central and Eastern Europe to Siberia, Tibet and Turkestan (Shute, 1975). The Italian strain was introduced into New Zealand (Syrett, 1983). It was the third biological control agent introduced to control ragwort in New Zealand (Syrett, 1983). This was largely because cinnabar moth, *T. jacobaeae,* frequently defoliates ragwort but it is only partially successful as a control agent in many areas (Julien, 1987). *L. jacobaeae* was imported into quarantine at Lincoln (New Zealand) in November 1981 from Salem, Oregon, U.S.A. (Syrett, 1986). Releases were subsequently made at Inchbonnie (Westland) and Canvastown (Marlborough) in 1983; and at Kaiparoro Road, Ngatira (Tokoroa), and Cobb reservoir in March 1984 (Syrett, 1986). Little is known about the population ecology of this insect and no research has been published on its effectiveness at controlling its food plant despite a variety of studies of topics related to this in New Zealand by Syrett (1983; 1985; 1986), Syrett, Scheele, and Philip (1984), Philip and Syrett (1988), Harmans and Syrett (1989), and Thompson (1980, 1985). Syrett (1983) mentioned that insects recorded from ragwort plants in New Zealand and discussed the potential for introduced biological control agents such as the cinnabar moth, *T. jacobaeae*, ragwort seedfly, *Pegohylemyia* spp., and the flea beetle, *L. jacobaeae.* Syrett (1985) tested adult feeding, oviposition, and host specificity of *L. jacobaeae* against representative species of native *Senecio* and two common pasture species. Syrett (1986) also studied the seasonal distribution of life stages of *L. jacobaeae* at Lincoln (New Zealand) between 1983 and 1985.

Longitarsus. jacobaeae has also been released in California (Hawkes, 1968; Hawkes & Johnson, 1978), Australia (Cullen & Moore, 1980), and Canada (Harris *et al.,* 1984), and the beetle continues to be redistributed to new locations within New Zealand (P. McGregor pers. Comm.).

Description and Life History

The life history strategies of *L. jacobaeae* were studied in most detail by Syrett (1986) in New Zealand, by Cullen and Moore (1980) in England, and by Ireson, Friend, Holloway, and Paterson (1991) in Australia.

Syrett (1986) reported that adults of the Italian strain occur year round in New Zealand but peak in February. In Australia, peak population densities of adult beetles occur in January (Ireson *et al* 1991) that has an adult aestivation period during summer (Frick & Johnson, 1973). The beetles are orange and between 2.5-3.0 mm long. Females are usually bigger than males (Newton, 1933), and their last abdominal segment is convex in contrast to that of males which is concave (Frick, 1971). *L. jacobaeae* is considered univoltine (Newton, 1933; Frick, 1971; Frick & Johnson, 1972; 1973; Cullen & Moore, 1980; Syrett, 1986; Windig, 1991), but evidence is accumulation that in some parts of New Zealand a proportion of the population may have two generations per year (P.McGregor pers. Comm.). The imagines mostly feed on the underside of ragwort leaves. Frick and Johnson (1973) studied feeding and oviposition rates of adult beetles of the Italian strain and found that each male or female ate less on average in 1966 (0.3 Feeding Unit (FU)/day) than in 1967 (0.9 FU/day)(where one FU= 1.6 mm^2 of leaf area). It is because temperatures were warmer in 1967. The beetles can disperse rapidly and have been found 5-6 km from the closest known release point within three years (Hawkes & Johnson, 1978), but establishing populations may spread much slower than this (P.McGregor pers. Comm.).

Zhang and McEvoy (1995) found that ragwort flea beetles could orient upwind to ragwort plants and that there is a seasonal variation in beetle response to host odour. In a subsequent study they investigated the developmental state and starvation time of adult beetles, as well as local density of the plant population and distance to the nearest plant, discovering that all these factors could affect the upward response of these beetles to ragwort plants (Zhang & McEvoy, 1996).

Adult beetles make holes in the leaves when they feed on the foliage, but this damage is not sufficient to kill ragwort plants beyond the seedling stage (Frick, 1970b; Harris, Wilkinson, & Myers, 1984). However, McEvoy (pers. Comm.) suggests that feeding by adults may effect significant mortality on very small seedling plants. Hawkes and Johnson (1978) found that although adult feeding in the spring is not significant, feeding may be heavy in autumn when they come out of aestivation.

Mating has not been investigated in detail but the female emits a sex pheromone that attracts the male beetle (Zhang & McEvoy, 1994). Cullen & Moore (1980) reported that adult females oviposit soon after emergence but Frick (1970 b; 1971), found that this occurred two to four weeks after emerging. Female beetles lay eggs on ragwort root crowns or up to 40 mm deep in the soil, both near the ragwort or far away from it (Frick, 1970 b; 1971;Cullen & Moore, 1980). Each female can lay 217 to 1106 eggs during the 53 to 262 days of her adult life (Frick & Johnson, 1973). Page (2000) found that rosette ragwort plants are more attractive to *L. jacobaeae* for oviposition than flowering ragwort plants where both plants were grown in the glasshouse sheep grazed pasture. This appears to be due to the higher humidity near ground level, associated with rosette plants. In dairy pasture, where some ragwort was clipped regularly to simulate sheep grazing, Betteridge *et al.* (unpublished data) found more ragwort flea beetle larvae in the larger than in the smaller rosettes. However, there was no clear pattern when these data were expressed on a per gram DM (dry matter) basis. McEvoy (pers comm.) found that as ragwort density in the field increased, the number of beetle/plant decreased, possibly because at low plant density, ragwort biomass was greater than at high density. However, McEvoy questioned whether any relationship exists between adult *L. jacobaeae* number and ragwort mortality rate.

The eggs are elongate or oval with rounded ends. They are about 0.66 mm long and are rather less than half as broad (Newton, 1933). Eggs are yellow or orange and become darker just before hatching. The surface consists of a network of polygonal pits (Newton, 1933). Eggs are delicate and can be easily destroyed by drying (Frick, 1970 b). They usually hatch in two to four weeks, and then the newly hatched larvae enter the roots or root crowns of ragwort to feed (Frick, 1970 b).

Newly hatched larvae are about 1.5 mm long and 0.25 mm wide (Newton, 1933). The head, prothorax shield and anal plate are dark grayish brown with the head being darkest (Newton, 1933). The legs and segmental plates are light brown. Larvae feed inside the root crowns from late autumn to spring. The larval stage lasts 3-4 months in the laboratory but it is longer in the field (Frick, 1970 b). Larvae usually prefer the tissue near the epidermis in root crowns but they also feed externally on the lateral roots (Frick, 1970 b). The damage they cause by feeding shows as brown scarred grooves on the surface of the lateral roots (Frick, 1970 b). When the root crowns are heavily attacked, the larvae generally bore up into the petioles of the lower leaves, which subsequently wilt and die (Frick, 1970 b). There is no information on feeding rates or how larval feeding on roots or root crowns affects ragwort, nor is anything known about the relationship between larval densities and their effects on the plants. Full-grown larvae are about 6 mm long and just over 1 mm wide (Newton, 1933). The body is white and the head, anal plate and prothoracic shield are, respectively, dark brown, brown and light brown (Newton, 1933). Once mature the larvae leave the plant and pupate in the soil around the root crowns. Pupation takes about three weeks (Frick, 1970 b).

Host Plant Specificity

It is important to know what the hosts are of exotic phytophagous insects before any are introduced as biological control agents of a weed (Harris and Zwolfer, 1968). McEvoy (1996) pointed out that host specificity of biological control agents is one of the basic criteria used to evaluate the risks that biological control agents pose for non-target organisms. It is therefore essential to test host specificity before releasing introduced biological control organisms.

Ragwort is the only recorded food plant of ragwort flea beetles (Newton, 1933; Frick, 1970a). Newton (1933) found that all ragwort flea beetles survived in the laboratory when fed on ragwort, whereas 80% survived on *Senecio aquaticus*, 50% survived on cultivated sunflower (*Helianthemum* sp.), and none survived feeding on aster (*Callistephus* sp.), Michaelmas daisy (*Aster* sp.), marguerite (*C. leucanthemum*) or golden rod (*Solidago virgaurea*). Frick (1970 a) tested host specificity of ragwort flea beetles on 65 species of host plants. He found that the adults fed from 54% to 96% as much on *Senecio serra* Hook, *S. triangularis* Hook, *S. adonidifolius* Lois, *S.*

paludosus L., *S. cruentus* DC, *Cacalia suaveolens* L., and *Erechtiles arguta* DC. as they did on ragwort (Frick, 1970 a). Frick (1970 a) found that females laid eggs up to 60% less often on these species than they did on ragwort, and some had either a shorter life span or a greatly lengthened period of preoviposition. The larvae had narrow host specificity (Frick, 1970 a; Syrett, 1985).

Syrett (1985) determined the host specificity of ragwort flea beetles on seven closely related species of *Senecio* and two important, common pasture species (*Trifolium repens* L. and *Lolium perenne* L.). She found that adult beetles fed 100% of the time on *S. jacobaea*, 51% on *S. wairauensis*, and 24% on *S. glaucophyllus*, but they did not feed on *S. monroi*, *S. lagopus*, *L. perenne* L., or *T. repens* L. Female ragwort flea beetle oviposited neither *S. monroi*, woody shrub, nor *S. lagopus*, a thick leaved perennial sp. (Syrett 1985). Female beetle oviposited 79% on *S. jacobaea*, 16% on *S. wairauensis*, 5% on *S. quadridentatus*, and 2% on *S.glaucophyllus* (Syrett 1985). Thus, the ragwort flea beetle was considered to be highly host specific to ragwort and unlikely to damage New Zealand's native *Senecio* species or important pastoral crops (Syrett, 1985).

Effectiveness as a Biological Control Agent

Ragwort is difficult to control by defoliation because it regenerates rapidly. This was proven to be the case in western North America (Meijden *et al.,* 1988). Harris *et al.,* (1978) found that cinnabar moths did not control ragwort in British Columbia in spite of its biological success in achieving widespread annual defoliation. The introduction of both ragwort flea beetles and cinnabar moths, however, on the central coast of Oregon caused over 95% decrease in the actively growing ragwort (i.e., seedlings, rosettes, and flowering plants) and a 32% decrease in the dormant stages (i.e., seed and vegetative buds) following an increase in populations of these herbivores (McEvoy, 1984). Ragwort flea beetles reduced ragwort density from 71-rosette/ m^2 to 0.6-rosette/ m^2 over a period of 4 years in United State (Hawkes & Johnson, 1978) and in combination with cinnabar moths have successfully controlled ragwort at sites in Oregon (Hawkes, 1981; McEvoy, 1985), Washington and California (Mastrogiuseppes *et al.,* 1983; Pemberton & Turner, 1990), and to a lesser degree in Canada (Julien, 1987). However, Hawkes (1981) pointed out that in Oregon cinnabar moth established poorly in wetter coastal areas, and here the flea beetle alone did not provide adequate control.

According to McEvoy *et al.* (1989) root feeding by flea beetles is more successful for controlling ragwort than defoliation by the cinnabar moth. Ragwort flea beetle alone can reduce vegetative ragwort densities by 95% and flower production by 39%. Damage by ragwort flea beetles can also reduce the ability of flowering plants to compensate for defoliation and defloration to the extent that capitulum production may be reduced by 98% with no viable achenes produced (James, McEvoy, & Cox, 1992).

Factors affecting the survival of L. jacobaeae

There has been little research on the factors that affect the survival of different stages of *L. jacobaeae.* Hawkes and Johnson (1978) found that summer moisture stress could strongly affect the population density of *L. jacobaeae.* Cullen and Moore (1980) found that some eggs collected from the surface of the soil in the field during summer showed signs of partial dehydration but this does not seem to affect hatching rates significantly. However, temperature does plays an important role in egg hatching because Windig (1991) found that eggs do not hatch in the laboratory below 5°C.

Ireson and Terauds (1982) recorded that carabid, staphylinid, arachnid, and acarine species were the most common predators of *L. jacobaeae* in summer and early autumn during oviposition and egg hatching. Predation experiments in the laboratory showed that eggs and neonate larvae of ragwort flea beetles were eaten by adult carabid beetles (*Mecyclothorax* sp.) and staphylinid beetles (Aleocharinae) (Ireson & Terauds, 1982). The eggs of ragwort flea beetles were also parasitised by a mymarid wasp (*Anaphes euryale* Debauche.) (Windig, 1991). Two common ant species (*Formica polyctena*, and *Lasius alienius* L.) may also feed on the eggs of ragwort flea beetle in Netherlands (Windig, 1991). Light intensity and the two ant species are positively correlated with ragwort flea beetle abundance (Windig, 1993). Light intensity does not affect the abundance of ragwort plants, but it is seemed to be key factor for *L. jacobaeae* because Windig (1993) found that *L. jacobaeae* is rarely present in the shaded sub-populations of ragwort. He also found that light intensity is important for the distribution of *L. alienius* (open areas) and for *F. polyctena* (shaded areas).

1.4 Aims of this project

High numbers of ragwort flea beetle (*L. jacobaeae*) larvae kill ragwort plants and they can reduce plant densities to low levels. Therefore any larvae that can not pupate successfully by the time the plant dies must either move to another plant or die. Thus the rate of mortality of larvae on individual ragwort plants can be expected to change as larval density per plant changes.

The relationship between larval crowding and the probability that the plant will die is likely to be complex. In particular, larvae do not all arrive on a plant simultaneously; instead, some larvae will have progressed well through their development by the time other larvae are only just hatching. Plant death is likely to result from the cumulative effect of larval feeding, so the pattern of egg-laying and larval development throughout the season will affect the probability of plant death.

Larvae may be able to move from a dying plant to a nearby plant that still offers an adequate food supply. The probability of survival for such a larva therefore depends on the spacing between plants, or in other words, on plant crowding. However, if larvae do transfer successfully between plants after one plant dies, then the rate of mortality of plants will increase; thus when the insect is present we expect a relationship between plant mortality and plant density.

If *L. jacobaeae* larvae can transfer from plant to plant, but only over relatively short distances (i.e. there is some distance beyond which transfers are never successful), and if *L. jacobaeae* mortality depends on larval density when there are no nearby plants to which the larvae can transfer, then rates of mortality of both the plant and the insect are affected by densities of both the plant and the insect. The investigation of this interdependent relationship between the plant and the insect forms the central theme of this thesis.

The specific aims of this research were

1. To measure the consumption rates of the three larval instars.
2. To understand how plant consumption by ragwort flea beetle affects the population density of ragwort.
3. To understand how plant density (crowding) affects the survival of ragwort flea beetle larvae.
4. To measure the effect of soil moisture on the population density of ragwort.

This information will help us understand how *L. jacobaeae* populations can persist when they can apparently kill all the aboveground stages of the plant in a local population. It will also help us to predict the likely long-term consequences of *L. jacobaeae* infestation for ragwort populations.

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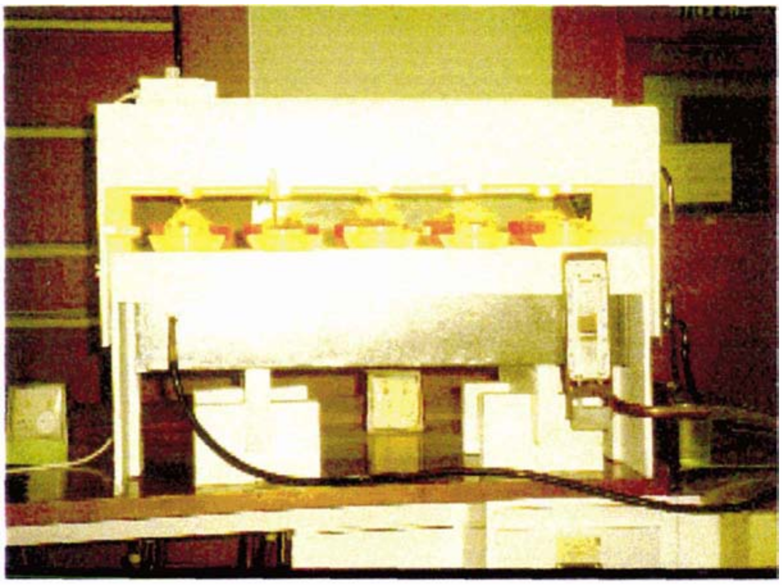
Chapter two: Materials and General Methods

(Methods for specific aspects of the study are described in the appropriate chapters)



A. An apparatus for extracting ragwort flea beetle larvae and other organisms from soil.

2.1 Abstract

The extracting apparatus consisted of a heating box, collecting funnels, and a cooling tank. Ragwort flea beetle *Longitarsus jacobaeae* larvae were extracted from soil/ragwort samples. Most *L. jacobaeae* larvae (42%) were extracted between 12 and 15 hours after the start. Efficiency of the apparatus was 84%.

2.2 Introduction

A major problem in examining the population ecology of an insect with a soil dwelling stage is ensuring that quantitative samples are taken so that consistent and quantitative data are gathered. Techniques for extracting soil animals from have generally been based on providing a gradient of temperature and light; this gradient drives organisms from the soil to be collected in a container.

Southwood (1978) reviewed different techniques for extracting insects and other soil arthropods and MacFadyen (1962) described an improved funnel type extractor for soil arthropods. Ragwort flea beetle larvae, *Longitarsus jacobaeae* Waterhouse, have been extracted from plant material with Tullgren funnels (McEvoy, *et al.*, 1991; Ireson, *et al.*, 1991; McEvoy, *et al.*, 1993), and they have been collected by dissection and hand searching of the petioles and root crowns (Hawkes & Johnson, 1978; Ireson & Terauds, 1982; Syrett, 1986; Windig, 1991, 1993).

The aim of my study was to develop an extracting apparatus that quantitatively samples soil dwelling *L. jacobaeae* larvae. It was also desirable to develop an extraction apparatus that extracted *L. jacobaeae* larvae and other soil living organisms quicker than can be done using a Tullgren funnel.

2.3 Materials and Methods

The extraction apparatus was based on that described by MacFadyen (1961). It consisted of three components: (1) a heating box, (2) the collecting funnels, and (3) a cooling tank (Figure 2.1).

Heating box

The heating box was 1150 mm long x 460 mm wide x 410 mm high. Ten spotlight bulbs (Philips, Reflector R 63, 60 watt) were arranged in two rows in a wooden box (140 mm in height) situated above the apparatus. The heating box could be moved vertically from 410 mm to a minimum of 200 mm above the funnel deck using adjustable screws that slid within double slots. All spotlight bulbs were controlled by a commercial simmerstat so that the temperature on the surface of the soil samples would reach about 50°C after 24 hours. The gap between the spot light bulbs and the funnels was kept to about 60 mm to avoid lateral temperature gradients.

Collecting Funnels

The length, breadth, and height of each funnel deck was 1050 x 460 x 410 mm. Ten funnels, arranged in two rows, were placed in holes (120 mm in diameter) in the plywood funnel deck. Each funnel was 150 mm in diameter and had a lower opening of 12 mm in. A rubber tube was attached to the lower end. This could be closed with a clip. The funnels were filled with water to the level of the top of the funnel desk before the soil samples were added in their extraction containers. Each extraction container consisted of two separate vertical containers made of polyvinyl chloride (PVC) pipe (80 mm in diameter) that fitted one above the other by means of a short sleeve. A wire sieve of 0.05 mm mesh (hole size) was glued underneath of the upper container and the soil sample was placed in this. Another sieve of 0.01 mm mesh was attached beneath of the lower container. The upper container was 50 mm high and the lower one was 15 mm high. Only the lower container was submerged in the water in the funnel. Each spot light bulb was positioned 20 mm above of the soil sample during extraction.

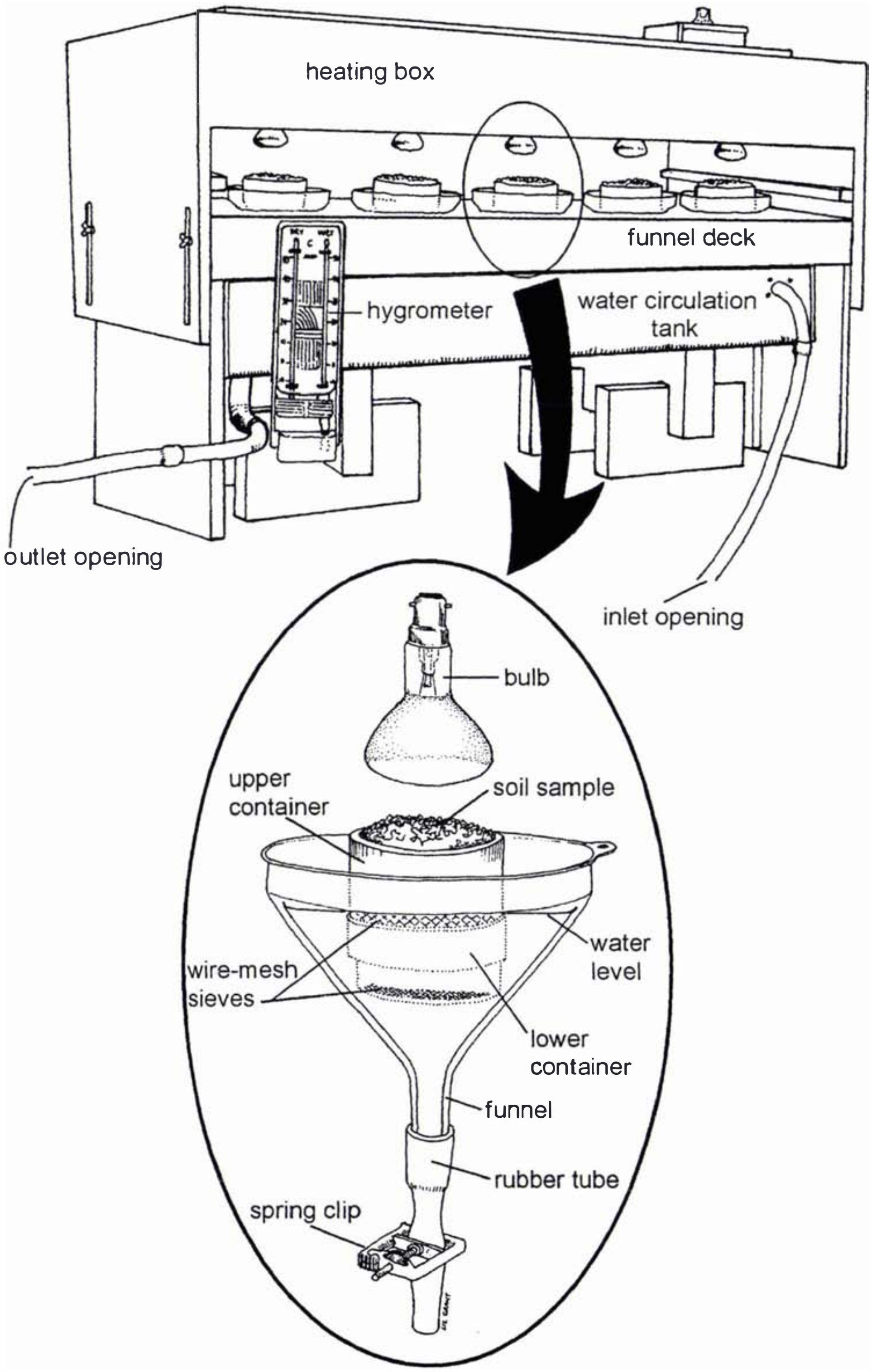


Figure 2.1 Extracting apparatus for *Longitarsus jacobaeae*.

Cooling

A cooling water tank (1000 x 350 x 80 mm) constructed from aluminium sheet was placed under the wooden frame of the funnel desk. Cold tap water was introduced into the tank through a 10 mm inlet and drained into a sink through a 20 mm diameter outlet. The lower parts of the funnels were cooled by immersion in this water.

Extraction

Ten core samples of soil containing ragwort roots and crowns were collected from the AgResearch hill country research station (Ballantrae). Each sample (diameter 80 mm, depth 80 mm) was sub-divided horizontally into two sub-samples 40 mm deep. Each sub-sample was then placed undisturbed, upper surface downwards, on the sieve of an upper sample container. The lower container was then fitted and both containers were placed in the water filled funnels so that only the second container was submerged. The spotlight bulbs were switched on at position "2" on the simmerstat and the heat applied to the samples was then increased gradually by increasing the simmerstat setting. The simmerstat settings were adjusted to keep the temperature below 49.4°C. Water was circulated in the cooling tank to cool the lower parts of the funnels. The temperature on the top and bottom of the samples was measured with a microthermometer inserted into the soil sample. The water temperature, room temperature, and humidity were also recorded. The extraction was terminated when the surface of the sample reached a temperature of about 50°C after 24 hours.

After completing the extraction, each sample container was removed from the funnel and the fine mesh together with larvae of *L. jacobaeae* and other small soil organisms that had collected on it was removed from the lower container. These organisms were then counted under a binocular microscope. Finally any organisms that had passed into the water through this sieve were recovered and counted by opening the clip on the rubber tube attached to the funnel and flushing the water through a 0.05 mm mesh sieve.

Rearing first instar larvae of L. jacobaeae

Adult beetles were collected from Ballantrae in May 1996 by using a portable suction machine. Male and female were sexed according to Frick (1971). Fifteen pairs of beetles were reared in a plastic rearing box (21 x 15 x 11 cm). Six rearing boxes were set up to collect enough eggs for the efficiency test (see below), laborarory and glasshouse experiments (Chapter 5). A plastic cup (4 cm in height and 9 cm in diameter) with five ragwort leaves was placed in each plastic rearing box. Five fresh ragwort leaves were placed into five circular holes (0.5 cm in diameter) in the lid of the plastic cup, two thirds filled with water which covered the bases of the petioles. Wilted leaves were removed and replaced with fresh leaves daily. Five filter papers were also placed on the bottom of each rearing box to collect eggs in the method modified from Paul Peterson (Landcare Research) and Delpachitra (1991). Eggs on the leaves, ribs, filter papers were removed by a fine brush (000 Haydn finest Sabel brush).

Collected eggs were carefully placed on a filter paper on capillary matting in a petri dish. Filter paper and capillary matting in the petri dish were soaked with 10 cc of water. Eggs were collected every day. 250 eggs were placed in each petri dish and placed in a humidity controlled chamber. The humidity controlled chamber was kept at 4 °C to stop develop until enough eggs were accumulated for the efficiency test. When over seven thousand eggs were collected, four petri dishes containing 1000 eggs (250 x 4) were moved into a 20°C temperature controlled chamber. After 12 days, petri dishes with eggs in 20°C temperature controlled chamber were checked every day for newly hatched larvae. The newly hatched larvae were carefully transferred into each experiment.

Efficiency test

The efficiency of the extraction apparatus was estimated by running 50 sterilized soil samples each with 10 newly hatched *L. jacobaeae* larvae through the apparatus. Each soil sample occupied an upper container to a depth of 15 mm. They were sterilized by heating in an oven at 105^{0}C for 24 hours, then soaked in water and stored individually in plastic bags at 4^0C for 7 days. The newly hatched *L. jacobaeae* larvae were introduced into each sample 24 hours before extraction and left at room temperature.

2.4 Results

After 24 hours of extraction the apparatus produced a temperature differential of approximately 20°C between the top and the bottom of the soil sample and a gradient of 5°C/cm through the soil (Table 2.1). The *L. jacobaeae* larvae moved downward through the sample and emerged at the bottom where they were collected on the sieve in the second container. After 6 hours extraction 2.6% of the larvae had left the soil and a maximum (42%) left between 12 and 15 hours after the start of the extraction process (Table 2.1 and Figure 2.2). The greatest number of larvae left the soil during an interval 12 -15 hours after extraction started even though the temperature gradient was near maximum after 12 hours (Figure 2.2). Fewer larvae left the soil after 15 hours and 97% of all larvae extracted had left the sample 18 hours after the start (Table 2.1). The last larvae had left the samples by 21 hours.

Table 2.1 Changes in the soil temperature and the number of *L. jacobaeae* leaving the soil over time during the extraction process.

Time from start of heating (hr)	Soil Temp: °C at Top	Soil Temp: °C at Bottom	Gradient °C / cm	No. of larvae extracted	% of Total
0	14.2	14.2	0	0	0
3	22.4	16.4	1.5	0	0
6	29.8	18.6	2.8	1	2.63
9	36.5	22.1	3.6	5	13.16
12	40.1	23.3	4.2	12	31.58
15	41.0	24.2	4.2	16	42.11
18	45.0	26.6	4.6	3	7.89
21	46.8	27.6	4.8	1	2.63
24	49.4	29.0	5.1	0	0

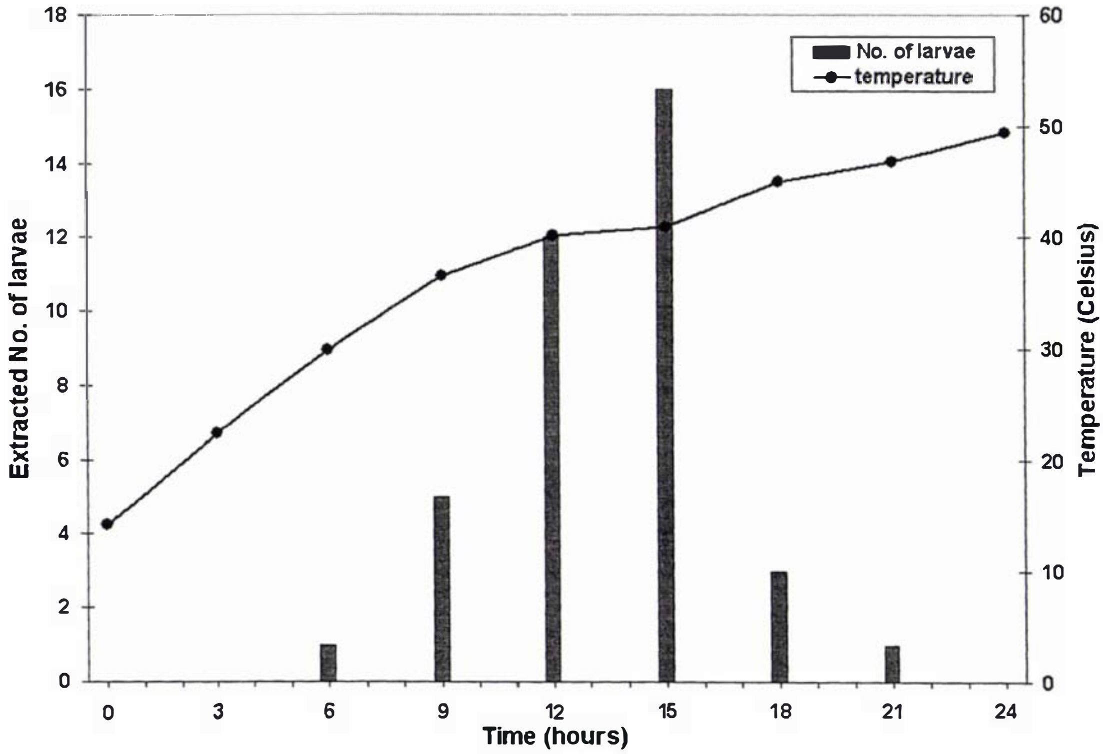


Figure 2.2 Relationship between extraction time (hours), temperature, and the number of *L. jacobaeae* larvae recovered.

The efficiency of the extracting apparatus was estimated by comparing the number of *L. jacobaeae* larvae that were extracted with the number that were introduced into the test samples (Table 2.2). Efficiency varied from 75% to 89% with a mean of 84 ± 2.65%.

Table 2.2 Extraction efficiency using 1st instar *L. jacobaeae* larvae.

Replicates	No. of larvae introduced	No. of larvae extracted	% recovered
1	100	85	85
2	100	86	86
3	100	75	75
4	100	85	85
5	100	89	89

2.5 Discussion

This extracting apparatus can extract RFB larvae and other soil living organisms such as mites, nematodes, earthworms, and insects.

Gabbutt (1959) quoted 59-88% efficiency for his Tullgren extractor for extracting soil living Coleoptera, Araneae and one species of pseudoscorpion *Chthonius ischnocheles*. Hermann. Kempson, *et al.*, (1963) found that the efficiency of their extractor was 70-95%, while Block (1966) obtained variable extraction efficiencies of 55-85%. My extracting apparatus was on average 84% efficient for extraction of *L. jacobaeae* larvae from soil with ragwort plants, but I did not test the efficiency of extraction for other soil organisms. I also did not investigate why those larvae that were not extracted remained in the apparatus. It is likely that at least some of these died during the 24 h period before extraction, possibly as a result of being transferred from cultures to the extraction machine. Such mortality would result in an under-estimate of the efficiency of the extraction apparatus. The wide range of extraction efficiencies recorded in the literature is probably caused by differences in the physical texture of the soils, volumes of the samples, and the different responses of the test organisms. Thus the ability of organisms of different stages and species to move through soil will affect the efficiency of the extracting apparatus.

Usher and Booth (1984) extracted soil micro-arthropods from soil at room temperature to 35^0C (for the longer extraction periods) or to 40^0C (for the shorter extraction periods). My apparatus extracted *L. jacobaeae* larvae from soil and plant material from 14.2^0C to 49.4^0C during 24 hours. The highest temperature gradient during this period was 5.1°C. Pande and Berthet (1973) showed that gradient extractors are reasonably efficient for small arthropods, and this is confirmed by my results, which show that this apparatus efficiently and quickly extracts root feeding *L. jacobaeae* larvae.

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B. Description of statistical analysis

Introduction

Canonical correlation analysis is the multivariate analyse used in Chapter 4 for sample surveys and experiments with multiple variables. Canonical correlation analysis was used for studying the relationship between two sets. A 3x 4 factorial design (univariate method) was used in Chapter 5 and Chapter 6 for investigating the effectiveness of three larval durations and 4 larval densities on the weight of ragwort rosette plants. A split plot design (univariate method) was used to examine movement of *L. jacobaeae* 1st instar larvae in nine compartments in Chapter 7. A 2 x 3 x 4 factorial design was applied to investigate interplant movement of *L. jacobaeae* 1st larval instar in two distances. A 4 x 4 factorial design was used in Chapter 8 for examining the effect of four larval densities and four plant densities on the mortality of *L. jacobaeae* larvae and ragwort. Multivariate analyses (Canonical correlation analysis, Multiple correlation analysis) were based on Greenacre (1984) and Manly (1994). Factorial designs and split plot design and analysis of variance were based on Cochran and Cox, (1957), and Ostle and Malone (1988).

Statistical Analysis

Chapter Three: Preliminary studies of ragwort flea beetle and the different stages of ragwort plant in three sites.

The establishment of ragwort flea beetle adults and larvae on different stages of the ragwort plant at three different study sites was examined using mean and standard error of the ragwort flea beetle adult, larvae, seedlings, rosettes, and flowering plants of ragwort at three sites (Pahiatua, Ballantrae, and Turakina).

Chapter Four: Ballantrae studies: L. jacobaeae *and its food plant, ragwort: Further analysis of a simple interaction model.*

Multiple correlation analysis and canonical correlation analysis with SAS programmes were used in this study. To examine the interrelationships the variables were divided into two sets such as: percent soil water for one set and *L. jacobaeae* larvae, adults, ragwort seedlings, rosettes, and flowering plants for another set; or *Longitarsus jacobaeae* larvae and adults for one set and ragwort seedlings, rosettes, and flowering plants for another set; or percent soil water and weather records (rain, air temperature, and soil temperature) for one set, and *L. jacobaeae* larvae and adults and ragwort seedlings, rosettes, and flowering plants for another set for the canonical correlation analysis.

Chapter Five: Laboratory studies to measure the consumption rates of ragwort by the L. jacobaeae *larvae*

Experimental Design

A 3 X 4 factorial design with 5 replicates was used for the experiment (Appendix 5.1). This experiment was conducted on ragwort rosettes to investigate the effectiveness of three larval durations (15 days, 30 days, and 45 days) after "infestation" and four densities of larvae per plant (0, 10, 20, and 40 per plant). The experimental unit of 60 plants was divided into 5 blocks each of 12 plants. Twelve experimental treatments were then allocated randomly into each of the blocks (Appendix 5.1). The data were analysed using **SAS/STAT** version 6.12 volume II (Appendix 5.2).

Aims

The major aims of this experiment were to examine

(a) how three larval feeding durations affect the weight of rosette plants;

(b) how larval densities affect the weight of rosette plants; and

(c) how larval densities affect the three durations.

Hypotheses

In this experiment I tested the following hypotheses:

(a) that the feeding durations of *L. jacobaeae* larval consumption do not affect rosette plant weight;

(b) that the four larval densities of *L. jacobaeae* on rosettes do not affect rosette plant weight; and

(c) that the interaction between larval feeding durations and larval densities does not affect rosette plant weight.

Glasshouse studies to measure the consumption rates of ragwort by the L. jacobaeae *larvae*

Experimental Design

The statistical model for this experiment was a factorial design with two factors (three feeding durations and four larval densities similar to laboratory experiment) and four blocks (Appendix 5.3, SAS programme in Appendix 5.4).

Aims

The major aims of this experiment were the same as those of the laboratory experiment.

Hypotheses

In this experiment the hypotheses were the same as those of the laboratory experiment.

Chapter six: Statistical Procedures for movement

The Soil main plot treatments were split into nine subplots (the nine tray compartments) (Middle centre, Middle cotton-wool, Middle ragwort roots, Lower centre, Lower cotton-wool, Lower ragwort roots, Upper centre, Upper cotton-wool, and Upper ragwort roots.)(Figure 6.1 and Appendix 6.1).

All statistical analyses used the General Linear Model (GLM) procedure of SAS (SAS 1987, version 6.0) (Appendix 6.1). ANOVA was used to test for any effects of soil on the movements of 1st instar larvae, any relationship between the nine tray compartments on the movement of *L. jacobaeae* 1st instar larvae, and any interaction between the soils and the nine tray compartments on the movement of *L. jacobaeae* 1st instar larvae. Some compartments had no *L. jacobaeae* 1st instar larvae in them (Appendix 6.1).

Chapter Seven: Experimental determination of the effect of interplant distance on the ability of larvae to transfer between plants.

The experimental design was a 2 x 4 x 3 factorial design. Two distances (plants grown 50 mm or 100 mm away from center plant), four larval densities (0,10, 20, or 40 larvae per plant), three larval durations (15 days old larvae, 30 days old larvae, or 45 days old larvae), and four replicates (total=96) were used as treatments and blocks in this experiment.

All statistical analysis was done with SAS/STAT version 6.0 (Appendix 7.1). ANOVA (analysis of variance) was used to test the ability of *L. jacobaeae* larvae to move two distances (50 mm and 100 mm) in relation to 12 treatments (the effects of three larval durations and the effects of four larval densities). The "General Linear Model" (GLM) was used to perform ANOVA.

Chapter Eight: Field studies to determine larvae survival in relation to larval density and plant density.

The study area was partitioned into three 64 m^2 plots to investigate the effectiveness of three larval feeding durations (15 days, 30 days, and 45 days). Each plot had 4 blocks (4 replications) and 16 treatment combinations. The treatments were allocated at random to 0.5 m x0.5 m small plots within a 3.25 m x 3.25 m area. The experiment was carried out as a 4 x 4 factorial design with 4 replicates: four blocks x four densities of ragwort rosette plant (1, 2, 4, and 8 plants) x four densities of larvae per plant (0, 10, 20, and 40 per plant) = 64 small plots (Appendix 8.6). Analysis of variance (ANOVA) was used to examine the effects of different levels of crowding of *L. jacobaeae* larvae and ragwort on the survival of larval *L. jacobaeae*, the effects of larval crowding and ragwort density on the growth of ragwort plants, and the effects of larval feeding durations on the weight gain of ragwort rosettes. These ANOVAs were carried out using the General Linear Model (GLM) procedure of SAS/STAT version 6.0 (Appendix 8.6). Plots of residuals against predicted values demonstrated that error variances were similar among treatments, plots of expected normal order scores against residuals indicated that the errors were normally distributed, so no transformations were applied.

In this experiment I tested the following hypotheses: (1) that different levels of crowding of *L. jacobaeae* larvae and ragwort do not effect the survival of larval *L.*

jacobaeae, (2) larval crowding and ragwort density do no effect the growth of ragwort plants, and (3) larval feeding durations do not effect the weight gain of ragwort rosettes.

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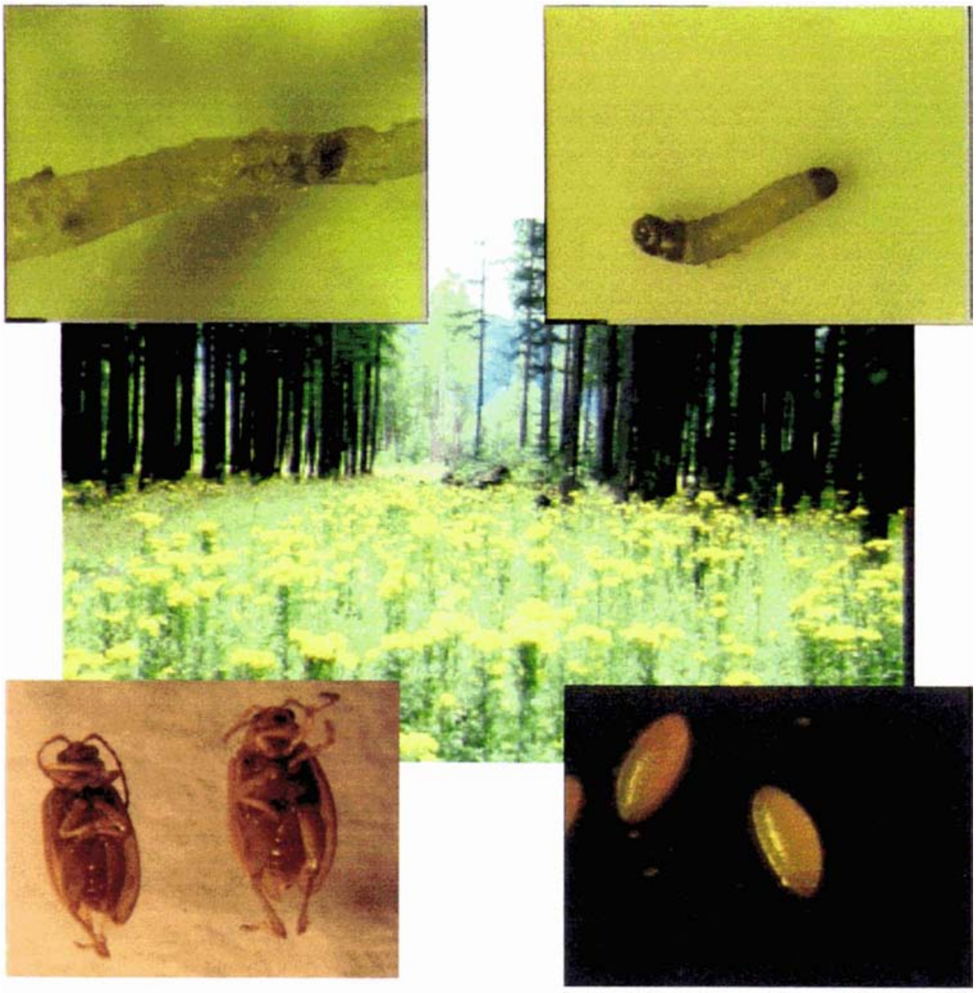
SECTION II

FIELD STUDIES

Chapter Three: Preliminary Studies

Chapter Four: Interaction between *L. jacobaeae* and ragwort

Chapter Three: Preliminary Studies



Preliminary studies of *Longitarsus jacobaeae* and the different stages of ragwort plant in three sites

3.1 Abstract

A preliminary population study investigated the numbers of *Longitarsus jacobaeae* larvae, adults and different stages of ragwort plants at Ballantrae, Turakina, and Pahiatua. The *L. jacobaeae* larval population was higher at Ballantrae than at Turakina and Pahiatua, but the adult population was highest at Turakina. Overall, both larval and adult populations were highest on the rosette stage of ragwort. The relationship between *L. jacobaeae* and ragwort plants varied between the study sites.

3.2 Introduction

Ragwort, *Senecio jacobaeae* L. (Compositae), is an important weed through out New Zealand (Harman and Syrett, 1989). The ragwort flea beetle, *Longitarsus jacobaeae* Waterhouse (Coleoptera: Chrysomelidae) was introduced into New Zealand in 1981 and first released for biological control of ragwort in the field in 1983 (Syrett, 1986). The hope was that a more effective control of ragwort would result when *L. jacobaeae* was present together with the cinnabar moth, *Tyria jacobaeae* L. (Lepidoptera: Arctiidae), and the ragwort seed fly, *Botanophila jacobaeae* (Hardy) (Diptera: Anthomyiidae). The latter two had been previously introduced as biological control agents for this weed (Syrett *et al.,* 1984). *Longitarsus jacobaeae* is now well established throughout most areas where it was released (Syrett *et al.,* 1991, Hayes, 1994, Harman *et al.,* 1996) but as yet there is no published information about how effective this suite of biological control agents has been with ragwort in New Zealand. Ragwort has been successfully controlled in western Oregon after the introduction of the same three insects (*L. jacobaeae*, *T. jacobaeae*, and *B. jacobaeae*) (McEvoy *et al.,* 1991). In one study site in western Oregon where *T. jacobaeae* was established, ragwort declined to less than 1% of its former abundance after the introduction of *L. jacobaeae* (McEvoy *et al.,* 1991). *L. jacobaeae* is also well established in Australia (Cullen and Moore, 1980) and Netherlands (Windig, 1991).

It is difficult to disentangle the relative effects of biological control agents when more than one is present, and so it is difficult to do this for ragwort in New Zealand where *L. jacobaeae* and *T. jacobaeae* often occur together. However, as a first step, I present preliminary observations on how *L. jacobaeae* and ragwort populations fluctuated in three study sites in the Manawatu-Wanganui region between April and August 1996. *T. jacobaeae* certainly defoliates the plant and apparently reduces its numbers at least locally in the Manawatu-Wanganui region. Sampling *T. jacobaeae* was beyond the scope of my study but information was acquired on the relationship between *L. jacobaeae* and ragwort populations.

3.3 Study Areas

Three sites were chosen: Ballantrae, Turakina, and Pahiatua. The Ballantrae site was the Ballantrae Hill Country Research Station, located 35 km east of Palmerston North on the eastern side of the Ruahine Range. This farm covers 484 ha of moderate to steep hill country that is moist in summer (Figure 3.1). The Turakina site is located between Bulls and Wanganui, about 60 km west of Palmerston North. The Pahiatua site is located in the northern Wairarapa, 65-km northeast of Palmerston North and 16 km south of Woodville (Figure 3.1).

3.4 Materials and Methods

Sampling Technique

All sites were sampled twice per month between 14 April 1996 and 15 September 1996. At each site one plot 30 by 30 m square was measured out and subdivided into 10 sub-plots of 6 m x 15 m. The plot was situated near the original release point of *L. jacobaeae* at each site. One 1 m^2 quadrat was then taken from a random position within each subplot. One soil core sample containing a ragwort rosette was collected from the centre or near to the centre of each 1 m^2 quadrat. Soil samples were taken to a depth of 80 mm using an iron cylinder 140 mm long by 80 mm in diameter with the lower edge sharpened to form a cutting edge.

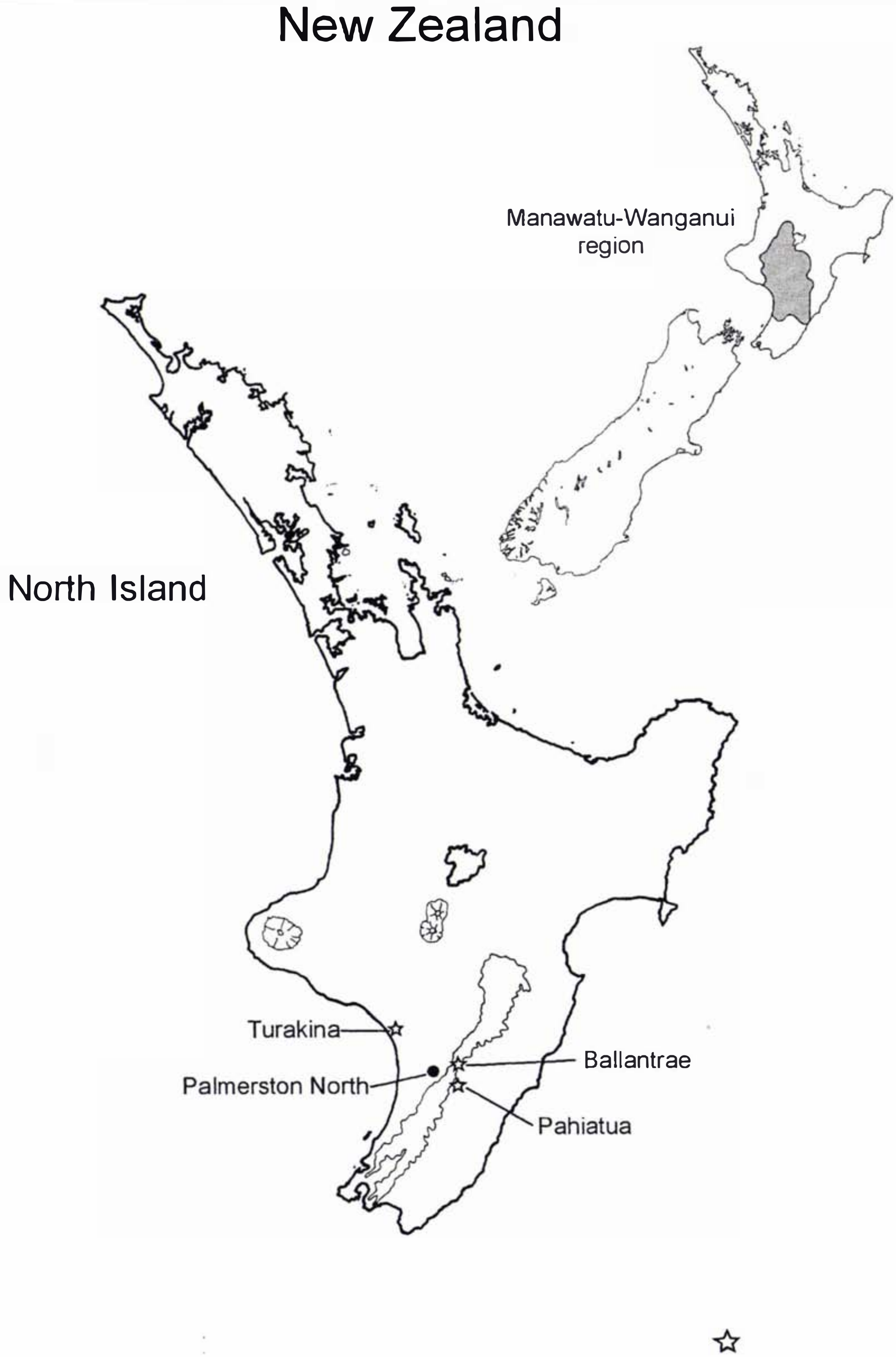


Figure 3.1 Locations of the three study sites at Ballantrae, Pahiatua and Turakina.

This was attached to a 1 m long handle. *Longitarsus jacobaeae* larvae were extracted the using apparatus described in Chapter 2 (A). All extracted larvae were recorded (Appendix 3.1).

In addition, ten samples of adult beetles were collected from the same ten 1 m^2 quadrats using a portable suction machine. Three ragwort plants from each quadrat were vacuumed, and spent two minutes for each plant. The catch for each sample from the portable suction machine was emptied into a tray and the beetles counted then released (Appendix 3.1). The numbers of different stages of ragwort plants (seedlings, rosettes, and flowering plants) were also recorded from the same ten 1 m^2 quadrats (Appendix 3.1).

Statistical Method

The relationships between the numbers of *L. jacobaeae* adults and larvae and the different stages of ragwort at the three sites was examined by their mean and standard error (Table 3.1) and correspondence analysis (Appendix 3.2).

3.5 Results

Results from the first 10 samples indicated that *L. jacobaeae* larvae occurred mainly within the top 40 mm of the soil sample, and few were extracted between depths of 40 mm to 80 mm so subsequent samples were restricted to the top 40 mm of the soil.

The mean numbers of *L. jacobaeae* and ragwort plants differed between the three sites (Pahiatua, Ballantrae, and Turakina) (Table 3.1).

Table 3.1 Mean (± standard error) numbers of *Longitarsus jacobaeae* and ragwort found at three study sites. (n =100).

Study sites	Adults /plant	Larvae /plant	Seedlings / m^2	Rosettes / m^2	Flowering plants/m^2
Pahiatua	4.75 ± 1.64	8.26 ± 2.48	1.36 ± 0.51	2.97 ± 0.54	0.34 ± 0.25
Ballantrae	7.25 ± 1.71	21.92 ± 2.22	0.81 ± 0.43	4.18 ± 1.29	0.59 ± 0.34
Turakina	14.32 ± 4.13	6.99 ± 1.47	0.86 ± 1.18	1.82 ± 1.03	0.83 ± 2.66

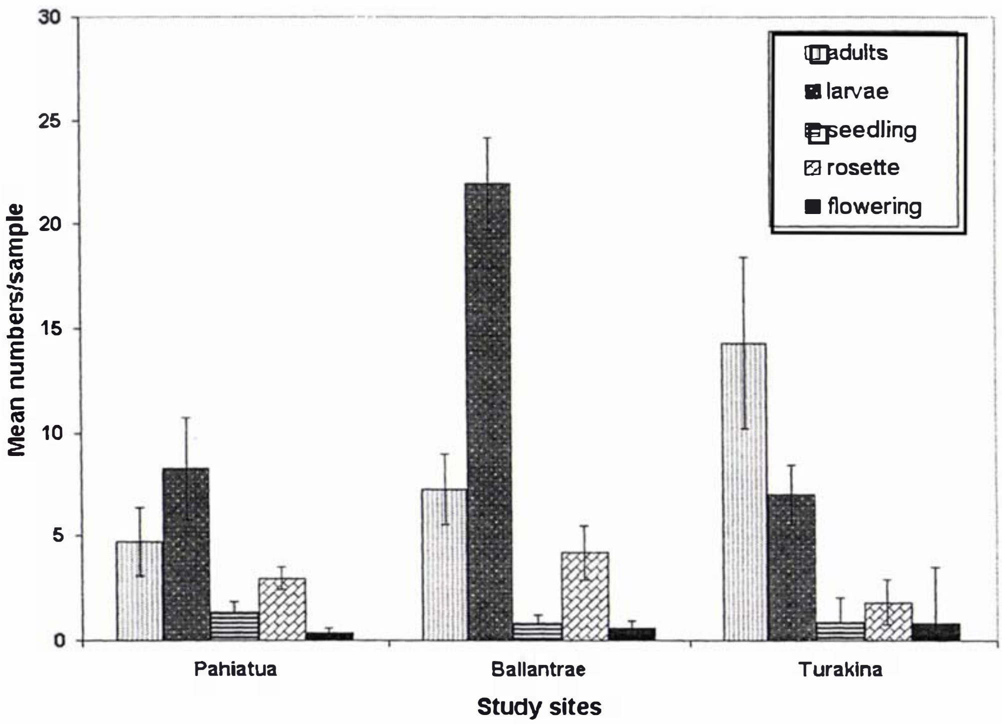


Figure 3.2 Mean numbers of *L. jacobaeae* and stages of ragwort plants found at the three study sites. Bars indicate ± 1 standard error.

Adult flea beetles were most numerous at Turakina, whereas more larvae were found at Ballantrae than at either Pahiatua or Turakina (Table 3.1, Figure 3.2). Rosettes were more common than either seedlings or flowering plants at all three sites (Table 3.1, Figure 3.2), with the highest mean number of rosettes being at Ballantrae. The mean number of flowering plants per sample was higher at Turakina than at the other two study sites. Slightly higher mean numbers of seedling occurred at Pahiatua than at Ballantrae and Turakina, but there was little difference in seedlings at the latter two sites.

These results were trends only, and my sample sizes were too small to disclose statistically significant differences (in most cases), but for the purposes of this preliminary study these data provided useful information about the variability of *L. jacobaeae* and ragwort populations. The coefficients of variation for the populations were 34.5% at Pahiatua, 23.6% at Ballantrae, and 28.8% at Turakina.

Correspondence analysis was carried out to examine populations of *L. jacobaeae* adults, larvae, ragwort seedlings, rosettes, and flowering plants in three different sites. Correspondence analysis was used to explain, in the context of the ordination of three sites (Pahiatua, Ballantrae, and Turakina), the basis of the abundance of *L. jacobaeae* adults, larvae, ragwort seedlings, rosettes, and flowering plants, although it can be used equally well on data that can be presented as a two-way table of measures of abundance with rows corresponding to one type of classification and the columns to a second type of classification. The contingency table, SAS programme, and the out-put were summarized in Appendix 3.2. Correspondence analysis confirmed that the majority of adults were found at Turakina, but larger numbers of larvae were found at Ballantrae and Pahiatua than Turakina.

The number of *L. jacobaeae* larvae over the entire study was highest at Ballantrae. Correspondence analysis shows that *L. jacobaeae* larvae are most associated with Ballantrae suggesting that larvae are favoured at Ballantrae in comparison with Turakina and Pahiatua. Similarly, ragwort rosettes are most associated with Pahiatua and ragwort flowering plants are most associated with Turakina (Appendix 3.2).

3.6 Discussion

These results indicate that the numbers of ragwort flea beetle and hence their effectiveness as a biological control agent may be affected by the number and different stages of ragwort plant (Table 3.1) as well as by the environmental factors that occur at different sites. For example, the high numbers of adult *L. jacobaeae* at Turakina suggest that something may be causing a higher mortality of the final instar larvae, pupae, and adults at Ballantrae and Pahiatua or that adults laid fewer eggs.
Cullen and Moore (1980) found in England that *L. jacobaeae* adults appearing in the field from early summer showed normal egg production and develop despite hot, dry conditions. They were concerned that two year old ragwort plants with *L. jacobaeae*

larvae on them could produce seeds, and die before the larvae become fullgrown. This may also apply to New Zealand, because Harman and Syrett (1989) reported that peak numbers of adults and larvae of *L. jacobaeae* appeared when ragwort flowering plants are senescing. It is clearly necessary to study the various ecological factors and biology of the ragwort flea beetle to better understand their biological effectiveness in relation to controlling ragwort.

When the three different sites in my study are considered, Turakina is best for survival of ragwort flea beetle adults but Ballantrae appears to be the most suitable for the larvae. Adult populations probably do not differ significantly among sites. Further research is required to find why *L jacobaeae* adult populations are much higher at Turakina than at the other two sites and why larval populations are much higher at Ballantrae than at Turakina and Pahiatua.

A very much larger study is now required if the effectiveness of *L jacobaeae* as a biological control agent of ragwort is to be understood. In order to do this, data needs to be collected on density dependent factors (such as feeding, food availability, and natural enemies) and density independent factors (especially the weather) that affect both *L jacobaeae* and ragwort.

3.7 References

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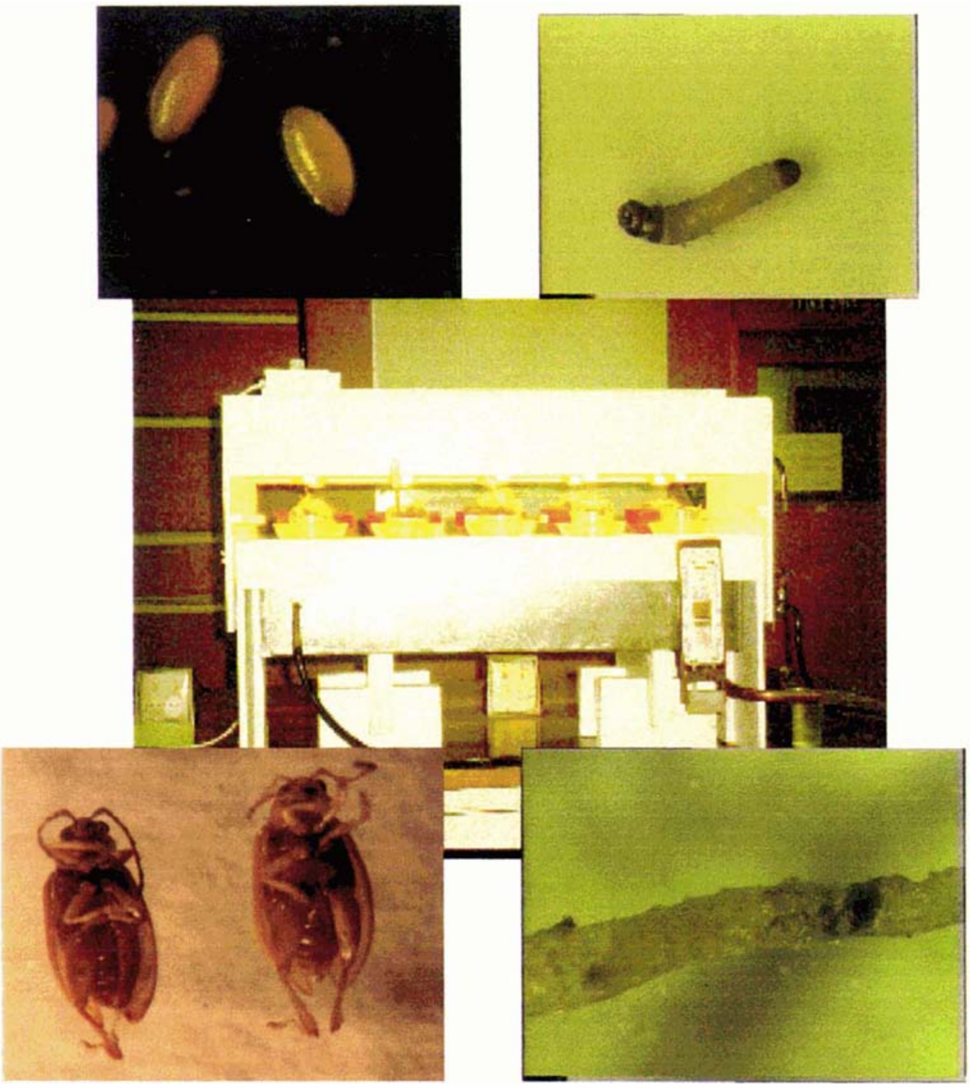
Chapter Four: Interaction between L. jacobaeae and ragwort



Analysis of a simple interaction model of *Longitarsus jacobaeae* and its food plant, ragwort.

4.1 Abstract

The experiment was carried out at Ballantrae. Soil samples with ragwort plants were collected for extraction. Percent soil water was measured. Soil water was positively correlated with *L. jacobaeae* larval population and negatively correlated with *L. jacobaeae* adult population, ragwort seedlings, rosettes and flowering plants. Populations of *L. jacobaeae* larvae and adults were negatively correlated. Populations of ragwort seedlings and flowering plants were positively correlated and higher than rosette population. Percent soil water was positively correlated with rainfall and negatively correlated with air temperature and soil temperature. Mean number of rosettes was greater in October and November 1996 (7.6 ± 1.68 and 5.8 ± 0.52) than in October and November 1997 (2.8 ± 0.29 and 2.7 ± 0.26 m^{-2}). The numbers of *L. jacobaeae* were negatively correlated with the numbers of ragwort seedlings. The strongest correlation between *L. jacobaeae* and ragwort plants occurred between the larvae and rosettes and was negative (-0.4608).

4.2 Introduction

In all classical biological control programmes, exotic natural enemies such as phytophagous insects, nematodes, fungi, and bacteria are introduced to control weeds by reducing and maintaining their populations below an economic threshold level. The introduction of exotic natural enemies is a long-established practice that has met with some success (Huffaker & Messenger, 1976, Goeden, 1988). The success rate for biological control of weeds is estimated to be from 17% (Crawley, 1989) to 39% (Julien *et al.*, 1984). Ragwort was recently controlled successfully in northern California (Pemberton & Turner 1990) and Oregon (McEvoy *et al.*, 1991) by herbivorous insects, which were introduced from Europe. In North America, three introduced phytophagous insects, the cinnabar moth *Tyria jacobaeae* L. (Lepidoptera: Arctiidae), a ragwort seedfly *Botanophila seneciella* (Meade) (Diptera: Anthomyiidae), and a ragwort flea beetle *Longitarsus jacobaeae* Waterhouse (Coleoptera: Chrysomelidae), caused ragwort populations to decline to low levels (<1-3% of its former abundance) (McEvoy *et al.,* 1991). All three of these insects have also been introduced to New Zealand, Australia, England and the Netherlands in an attempt to control ragwort. They have been successful in New Zealand (Harman *et al.,* 1996). Despite the success of the biological control programme in New Zealand, there has been no published quantitative evidence to evaluate the effectiveness of cinnabar moth or ragwort flea beetle on ragwort in New Zealand (Syrett, 1989) but ragwort seed fly was shown to have a negligible effect (Dymock, 1987). The aim of this thesis is to elucidate the relationship between ragwort flea beetle *L. jacobaeae* and ragwort and this chapter addresses

1. How soil water content affects populations of *L. jacobaeae* larvae and ragwort plants;
2. How larval density affects larval mortality; and
3. How ragwort density affects larval and adult *L. jacobaeae* numbers.

4.3 Methods

Study area: Ballantrae

This research was carried out at Ballantrae Hill Country Research Station, 35 km east of Palmerston North on the eastern side of the Ruahine Ranges. Ballantrae was chosen as an intensive study site after preliminary studies showed that *L. jacobaeae* larvae were more numerous there than in other places close to Palmerston North.

Sampling Technique

A preliminary investigation was made using samples taken at Ballantrae, on one occasion (14 May 1996) to check the methodology and to check the number of samples required. This is detailed in Appendix 4.1.

Ten samples of ragwort plants together with the surrounding soil were taken once per month between May 1996 to August 1998. The sampling technique is described in Chapter 3. A total of 280 samples were taken.

Adult beetles were collected from 10 randomly selected quadrats using a portable suction machine (Chapter 3). The beetles from each quadrat were counted by emptying the replaceable plastic collection container onto a plastic tray. Population densities of ragwort seedlings, rosettes, and flowering plants were estimated by counting their numbers within a 1 m^2 aluminium frame placed in the centre of a further 10 randomly selected quadrats (Appendix 4.1). Temperature, humidity, rainfall, and soil temperature were recorded in a weather station at Ballantrae (Appendix 4.2).

Soil water content

Ten samples from the Ballantrae study site were taken at the same time and places as the ragwort samples above. These were randomly collected using a soil corer (85mm in diameter and 140 mm in depth). The soil samples were wrapped in aluminium foil immediately after they were collected. Each soil sample was divided vertically into 4 samples in the laboratory, individually wrapped in aluminium foil, then weighed (W2). They were then dried in an oven at 105°C for about 24 hours. After drying they were removed from the oven and allowed to cool before being weighed again (W3). The dry soil was then removed and the aluminium foil was cleaned dried and weighed (W1). The percentage of soil water was subsequently calculated as follow:

Percent soil water = (W2 - W3) / (W3 - W1) X 100 (Appendix 4.3).

Method of Extraction

Larvae of *L. jacobaeae* were extracted from the soil samples using the method described in Chapter 2.

Statistical Analysis

Canonical correlation analysis was used to investigate the relationship between:

(a) *L. jacobaeae* (larvae and adults) and ragwort (seedlings, rosettes, and flowering plants)

(b) The soil water content and the weather (rain, air temperature, and soil temperature) and *L. jacobaeae* (larvae and adults) and ragwort (seedlings, rosettes, and flowering plants).

Two measures of *L. jacobaeae* (numbers of larvae, and of adults) and three measures of ragwort (numbers of ragwort seedlings, ragwort rosettes, and flowering plants) were used for the analysis. In this experiment, I defined a small ragwort plant with 7 true leaves as seedling stage and more than 7 or fewer true leaves as rosette stage. Both canonical correlation analysis and analysis of variance (ANOVA) were performed using SAS/STAT. The programmes are given in Appendix 4.4 & 4.5.

4.4 Results

(1) Effect of soil water content on populations of L. jacobaeae *larvae and ragwort*

The correlation between soil water content and a range of variables is shown in Table 4.1.

Table 4.1 Correlation coefficients between percent soil water, *L. jacobaeae* (RBF) larvae, *L. jacobaeae* adults, ragwort seedlings, rosettes, and flowering plants. Significance levels, given in brackets, test the null hypothesis that the corresponding correlation was not different from zero).

	% soil water	RFB larvae	RFB adult	seedlings	rosettes	flowering plants
%soil water	1.0000 (0.0)					
RFB larvae	0.5911 (0.0009)***	1.0000 (0.0)				
RFB adults	-0.1507 (0.4439)ns	-0.0480 (0.8084) ns	1.0000 (0.0)			
seedlings	-0.3717 (0.0515) ns	-0.0736 (0.7098) ns	-0.4338 (0.0211)*	1.0000 (0.0)		
rosettes	-0.2507 (0.1983) ns	-0.4608 (0.0136)	0.0176 (0.9292) ns	0.2110 (0.2812) ns	1.0000 (0.0)	
flowering plants	-0.4421 (0.0185)*	-0.3627 (0.0578) ns	0.1147 (0.5610) ns	0.5524 (0.0023)**	0.4781 (0.0101)*	1.0000 (0.0)

'***" = $p<$ **0.001**, '**' = $P <$ **0.001**, "*' = p <**0.05**, $ns \geq p$ **0.05**

Soil water content showed a positive correlation with the number of *L. jacobaeae* larvae (0.5911) (Table 4.1). The highest soil water content occurred at Ballantrae during August 1997 and the largest population of *L. jacobaeae* larvae was found in October – November 1997 (Figure 4.1). The lowest soil water level was measured during April 1997, and March 1998 when there was a low population of larvae (Figure 4.1). The percentage of soil water was low during July 1996, April 1997, and February – March 1998 (Figure 4.1) when there were also low population densities of ragwort seedlings and flowering plants (Figure 4.2). Ragwort does not flower at Ballantrae during the wetter periods of the year (July –October, Figures 4.2), but ragwort survives as rosette then. Thus the large numbers of larvae that occur during this period survive in the roots of ragwort rosettes.

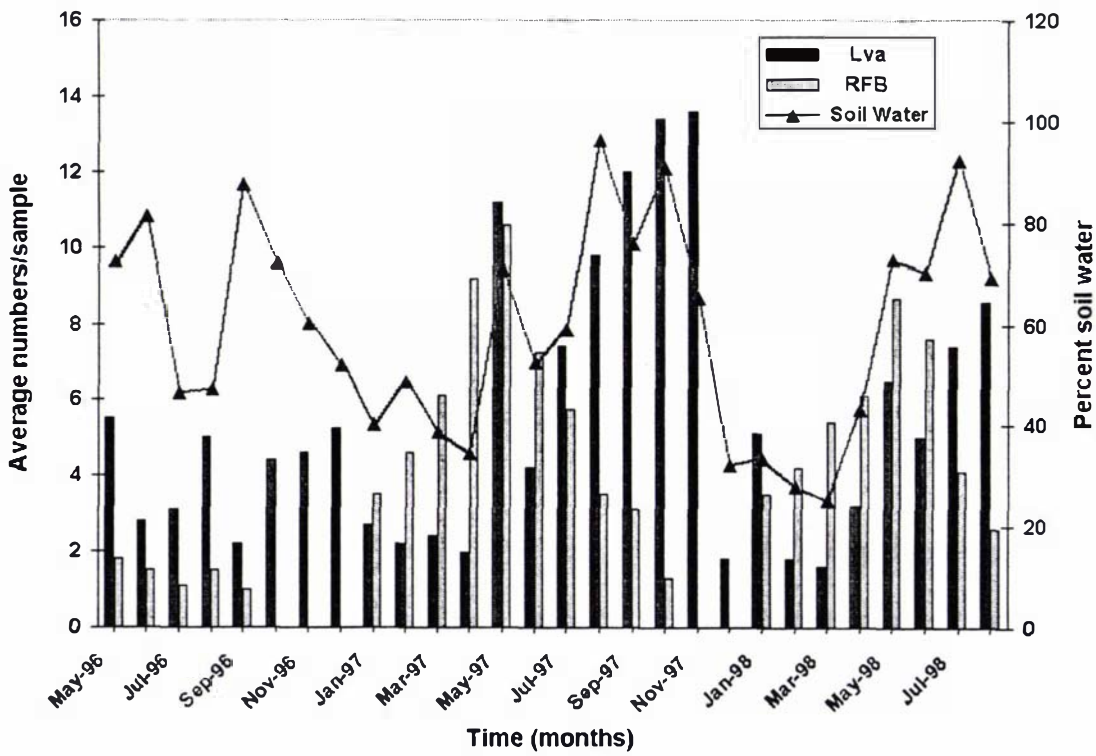


Figure 4.1 The relationship between percent soil water and the mean population density of *L.jacobaeae* larvae and adults at Ballantrae between May 1996 to August 1998.

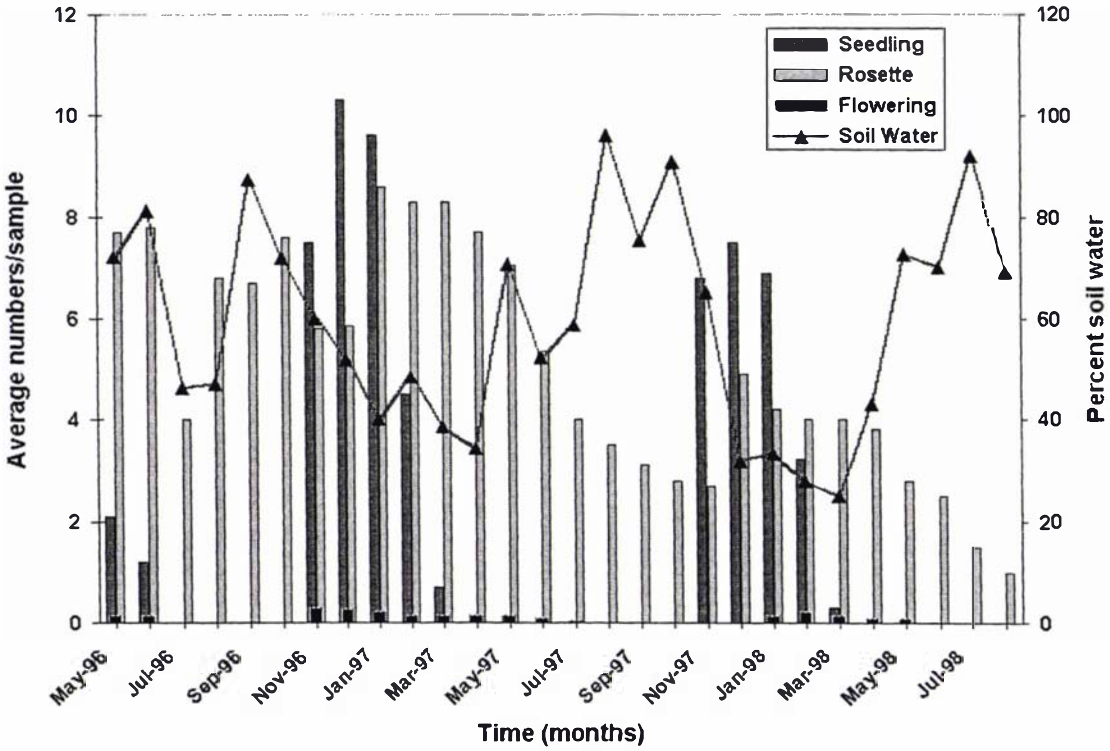


Figure 4.2 The relationship between percent soil water and the mean population densities of ragwort seedlings, rosettes and flowering plants.

Table 4.2 Correlations between the numbers of *Longitarsus jacobaeae* and *Senecio jacobaeae*, and soil water and weather records from Ballantrae.

Weather	Larvae	Adults	Seedlings	Rosettes	Flowering plants
Soil water	0.5861	-0.1450	-0.3643	-0.2331	-0.4339
Rain	-0.1957	-0.2765	-0.0347	-0.1092	-0.1662
Air temperature	-0.2506	-0.0313	0.5196	0.2137	0.3571
Soil temperature	-0.3219	-0.1783	0.6678	0.3245	0.6075

Soil water is positively correlated with *L. jacobaeae* larvae and negatively correlated with other variables (numbers of *L. jacobaeae* adults, ragwort seedlings, rosettes, and flowering plants; Table 4.2). Soil water is also positively correlated with rainfall whereas air and soil temperatures are positively correlated with all stages of ragwort plants as well as with each other (Figure 4.3; Table 4.2).

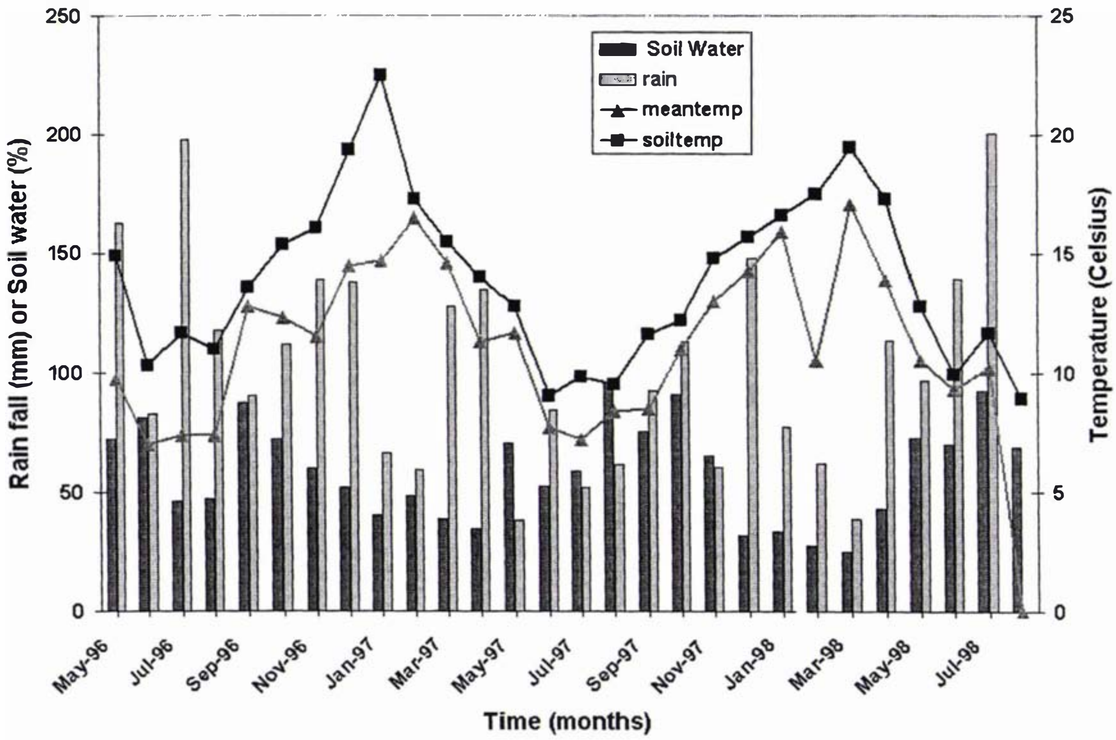


Figure 4.3 Average monthly weather records (air temperature, soil temperature, and rainfall) and percent soil water for Ballantrae between May 1996 and August 1998.

Table 4.3 ANOVA of the relationship between the percentage of soil water and the numbers of *L. jacobaeae* larvae and adults (Model) at Ballantrae.

Source	DF	SS	MS	F	P
Model	2	4244.28	2122.14	7.17	0.0035
Error	25	7401.66	296.07		
Total	27	11645.94			

There is a highly significant interaction (p=0.0035) between percent soil water and the numbers of *L. jacobaeae* larvae and *L. jacobaeae* adults (Table 4.3). However, the correlation between soil water and the larval population was highly significant (p = 0.0012) while that between percent soil water and the adult population was not significant (p = 0.4495) (Table 4.4). In contrast, the percentage of soil water content bears no relationship to the numbers of ragwort seedlings, rosettes, and flowering plants (Table 4.5).

Table 4.4 Results of multiple canonical correlation analysis between the percentage of soil water and the numbers of *L. jacobaeae* larvae and adults: parameter estimates.

Variable	DF	parameter estimate	standard error	T for H0:	P
Intercep	1	43.096	7.469	5.770	0.0001
RFBlarvae	1	3.387	0.923	3.666	0.0012
RFB adults	1	-0.831	1.081	-0.768	0.4495

Table 4.5 ANOVA of the relationship between the percentage of soil water and the numbers of ragwort seedlings, rosettes, and flowering plants at Ballantrae.

Source	DF	SS	MS	F	P
Model	3	2584.81	861.60	2.282	0.1048
Error	24	9061.13	377.55		
Total	27	11645.94			

(2) Relationship between larvae density and larval and plant survival

Ragwort seedlings reached a population maximum in January in both 1997 and 1998 but they were not found from April to October 1997 (the wetter period of the year). Overall the numbers of all three stages of ragwort (seedlings, rosettes, and flowering plants) declined from 1996 to 1998 at Ballantrae (Table 4.6, Figure 4.5).

The numbers of *L. jacobaeae* larvae were negatively correlated with the numbers of ragwort seedlings (Table 4.6 and Figure 4.5). No seedlings were found between April to October because they had either developed into rosettes or had been killed by *L. jacobaeae* larvae. *L. jacobaeae* larvae could therefore survive on rosettes from April to October.

Table 4.6 Mean and standard error of population densities of *L. jacobaeae* larvae and adults, and ragwort seedlings, rosettes and flowering plants, and the percentage of soil water content at Ballantrae.

Month	Larvae	Adults	Seedlings	Rosettes	Flowering plants	% soil water
May-96	5.50 ± 0.90	1.80 ± 0.138	2.1 ± 0.28	7.70 ± 0.29	1.5 ± 0.19	72.19 ± 3.15
Jun-96	2.80 ± 0.39	1.50 ± 0.14	1.2 ± 0.09	7.80 ± 0.40	1.5 ± 0.18	81.25 ± 5.09
Jul-96	3.10 ± 0.69	1.10 ± 0.143	0	4.00 ± 0.97	0	46.24 ± 2.64
Aug-96	5.00 ± 0.80	1.50 ± 0.29	0	6.80 ± 1.24	0	47.14 ± 1.73
Sep-96	2.20 ± 0.50	1.00 ± 0	0	6.70 ± 0.21	0	87.38 ± 4.30
Oct-96	4.40 ± 1.22	0	0	7.60 ± 1.68	0	71.96 ± 3.15
Nov-96	4.60 ± 0.83	0	7.5 ± 1.51	5.80 ± 0.52	3.1 ± 0.40	60.04 ± 3.25
Dec-96	5.25 ± 0.91	0	10.3 ± 1.54	5.86 ± 1.73	2.7 ± 0.39	51.84 ± 3.27
Jan-97	2.70 ± 0.57	3.50 ± 0.53	9.6 ± 0.76	8.60 ± 1.39	2.2 ± 0.25	40.16 ± 1.79
Feb-97	2.20 ± 0.53	4.60 ± 0.69	4.5 ± 0.42	8.30 ± 0.93	1.4 ± 0.23	48.56 ± 5.01
Mar-97	2.40 ± 0.48	6.10 ± 0.93	0.7 ± 0.09	8.30 ± 0.97	1.4 ± 0.25	38.67 ± 3.34
Apr-97	1.98 ± 0.34	9.20 ± 0.87	0	7.70 ± 0.53	1.4 ± 3.24	34.43 ± 2.14
May-97	11.20 ± 0.76	10.60 ± 0.63	0	7.05 ± 0.41	1.0 ± 0	70.69 ± 5.99
Jun-97	4.20 ± 0.51	7.25 ± 0.80	0	5.35 ± 0.58	0.5 ± 0	52.40 ± 4.60
Jul-97	7.40 ± 0.59	5.75 ± 0.65	0	4.00 ± 0.44	0	58.78 ± 3.05
Aug-97	9.80 ± 0.59	3.50 ± 0.48	0	3.50 ± 0.34	0	96.19 ± 8.44
Sep-97	12.00 ± 0.79	3.10 ± 0.42	0	3.10 ± 0.42	0	75.54 ± 8.15
Oct-97	13.40 ±0.79	1.30 ± 0.18	0	2.80 ± 0.29	0	90.76 ± 6.48
Nov-97	13.60 ± 0.73	0	6.8 ± 0.24	2.70 ± 0.26	0	65.11 ± 4.11
Dec-97	1.80 ± 0.36	0	7.5 ± 0.43	4.90 ± 0.28	1.4 ± 0.40	31.98 ± 2.48
Jan-98	5.10 ± 0.37	3.50 ± 0.27	6.9 ± 0.62	4.20 ± 0.33	2.3 ± 0.33	33.41 ± 3.24
Feb-98	1.80 ± 0.25	4.20 ± 0.72	3.2 ± 0.34	4.00 ± 0.45	1.4 ± 0.18	27.82 ± 5.99
Mar-98	1.60 ± 0.22	5.40 ± 0.40	0.3 ± 0	4.00 ± 0.42	1.0 ± 0	24.94 ± 3.55
Apr-98	3.20 ± 0.33	6.10 ± 0.43	0	3.80 ± 0.25	1.0 ± 0	42.97 ± 4.04
May-98	6.50 ± 0.50	8.70 ± 0.79	0	2.80 ± 0.29	0	72.59 ± 9.78
Jun-98	5.00 ± 0.30	7.60 ± 0.54	0	2.50 ± 0.43	0	70.13 ± 3.91
Jul-98	7.40 ± 0.67	4.10 ± 0.41	0	1.50 ± 0.22	0	92.14 ± 8.87
Aug-98	8.60 ± 0.42	2.60 ± 0.24	0	1.00 ± 0.20	0	69.15 ± 5.03

The mean number of rosettes was greater in January and February 1997 (8.6 ± 1.39 and 8.3 ± 0.93 m $^{-2}$) than in January and February 1997 (4.2 ± 0.33 and 4.0 ± 0.45 m $^{-2}$) (Table 4.6). However, numbers of both ragwort rosettes and flowering plants were high in February, March, and April 1977, December 1997, and February - March 1998 when the population density of *L. jacobaeae* larvae was low. The populations of ragwort rosettes and flowering plants were low when numbers of *L. jacobaeae* larvae were slowly increasing from June 1997 to November 1997 (Table 4.6).

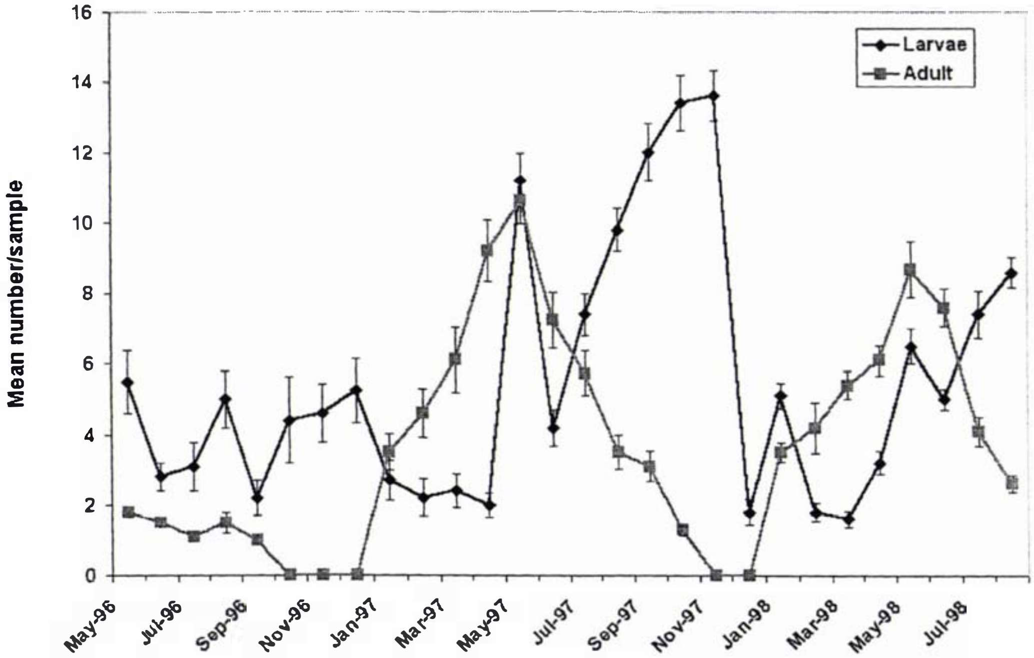


Figure 4.4 Changes in the numbers of *L. jacobaeae* larvae and adults from May 1996 to August 1998 at Ballantrae. Bars indicate ± 1 standard error.

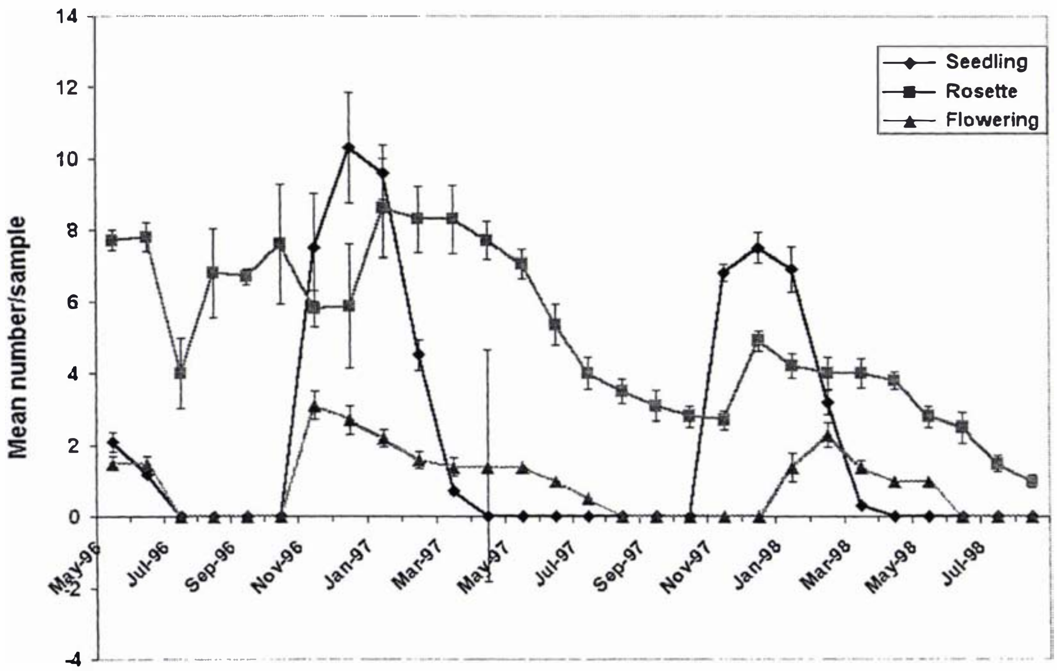


Figure 4.5 Changes in the numbers of ragwort seedlings, rosettes, and flowering plants from May 1996 to August 1998 at Ballantrae. Bars indicate ± 1 standard error.

The highest mean numbers of *L. jacobaeae* larvae and adult per sample were recorded in 1997, but lower mean numbers per sample were recorded in 1996 and 1998. The highest mean numbers of ragwort seedlings, rosettes, and flowering plants were recorded in 1996 and then all reduced in 1997 and in 1998 (Table 4.7).

Table 4.7 Mean numbers of *L. jacobaeae* larvae, adult, ragwort seedlings, rosettes and flowering plants per sample and the water content of the soil at Ballantrae between 1996 and 1998.

Names	Mean sample (1996)	Standard Error	Mean sample (1997)	Standard Error	Mean sample (1998)	Standard Error
Larvae	4.11	0.62	7.44	2.34	4.90	1.28
Adults	0.86	0.38	4.58	1.70	5.28	1.05
Seedlings	2.64	2.05	2.43	1.81	1.30	1.26
Rosettes	6.53	0.64	5.53	1.17	2.98	0.62
Flowering plants	1.10	0.65	0.78	0.39	0.71	0.43
% Soil water	64.76	7.87	58.61	10.76	54.14	12.49

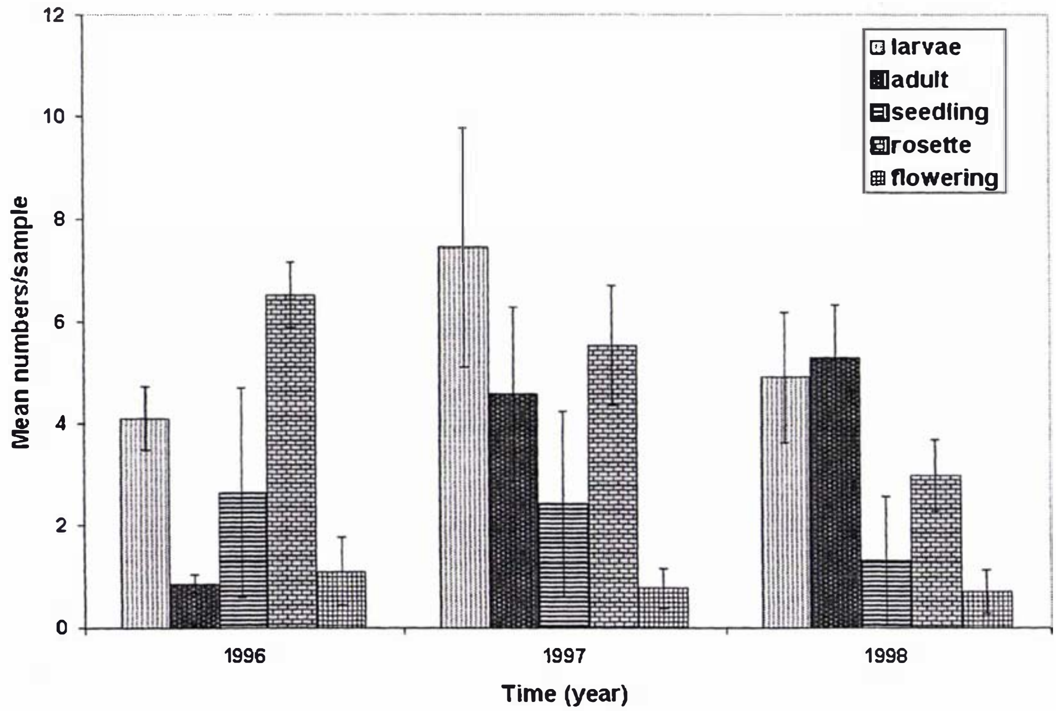


Figure 4.6 Correlations between the numbers of *L. jacobaeae* larvae and adults, and ragwort seedlings, rosettes, and flowering plants found at Ballantrae between 1996 and 1998 (Bars indicate ± 1 standard error).

3. The relationship between L. jacobaeae *(larvae and adults) and ragwort (seedlings, rosettes, and flowering plants)*

The largest correlation amongst the measures of ragwort plants was 0.5524 (Table 4.8.), and this was between seedlings and flowering plants. When both seedling and flowering plant populations were high, the rosette population was lower.

Table 4.8 Canonical correlation matrix for *L. jacobaeae* and ragwort variables.

	larvae	adults	seedlings	rosettes	flowering plants
larvae	1.0000				
adults	-0.0480	1.0000			
seedlings	-0.0736	-0.4338	1.0000		
rosettes	-0.4608	0.0176	0.2110	1.0000	
flowering plants	-0.3627	0.1147	0.5524	0.4781	1.0000

The strongest correlation between *L. jacobaeae* and ragwort plants occurred between the larvae and rosettes and was negative (-0.4608) (Table 4.8). The second largest correlation was between *L. jacobaeae* adult and ragwort seedlings (-0.4338, Table 4.8). Thus *L. jacobaeae* adult numbers increased while the population of seedlings was decreasing. Wilks' lambda test provide highly significant correlation between larvae and other variables (adults, seedlings, rosettes, and flowering plants (p = 0.0051).

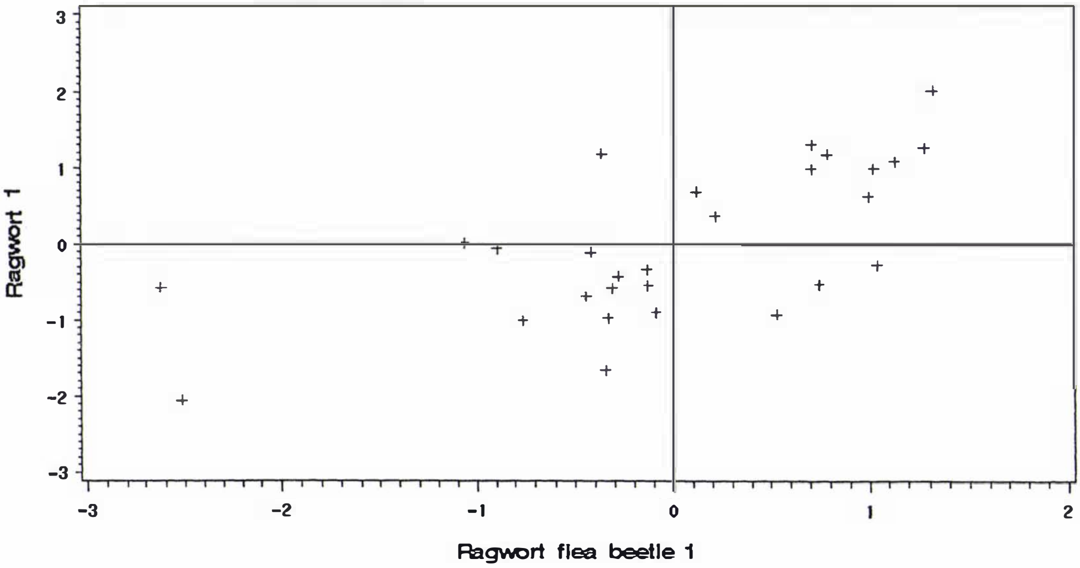


Figure 4.7 Plot of the first canonical scores for the mean values for *L. jacobaeae* larvae and for ragwort rosette plants of each sample.

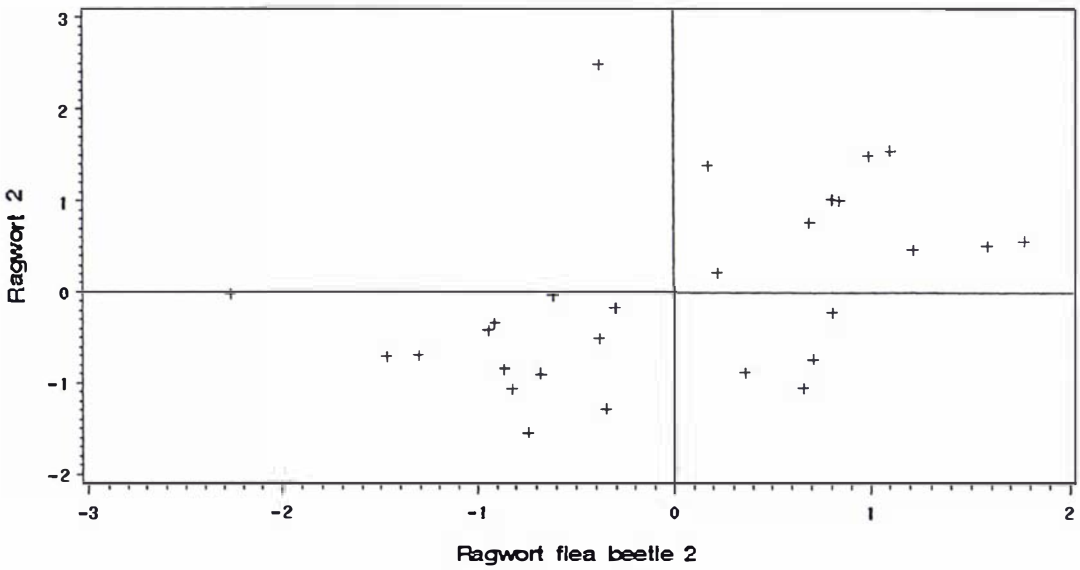


Figure 4.8 Plot of the second canonical scores for the mean values for *L. jacobaeae* adults and for ragwort seedlings and flowering plants of each sample.

Plots of the scores for the first and second canonical variates for *L. jacobaeae* and ragwort plants indicated that the relationship was not linear (Figure 4.7 and Figure 4.8).

(4) Relationships between soil water content and rainfall, air temperature, and soil temperature, and L. jacobaeae *(larvae and adults) and ragwort (seedlings, rosettes, and flowering plants) at Ballantrae*

The strongest correlation with soil water content was with the numbers of *L. jacobaeae* larvae (canonical coefficient = 0.5861; Table 4.9). A high soil water content was also positively correlated with rainfall (canonical coefficient = 0.0976) and negatively correlated with air temperature and soil temperature (Table 4.9). Soil water was also negatively correlated with *L. jacobaeae* adults, ragwort seedlings, rosettes, and flowering plants.

Table 4.9 Canonical correlations between weather records, and numbers of *L. jacobaeae* and Ragwort at Ballantrae.

Variables	%soil water	rain	air temp.	soil Temp.	larvae	adults	seed-lings	rosett-es	flowering plants
%soil water	1.0000								
Rain	0.0976	1.0000							
Air temp.	-0.4678	-0.2132	1.0000						
Soil Temp.	-0.5810	-0.1383	0.8319	1.0000					
larvae	0.5861	-0.1957	-0.2506	-0.3219	1.0000				
adults	-0.1450	-0.2765	-0.0313	-0.1783	-0.0373	1.0000			
Seedlings	-0.3643	-0.0347	0.5196	0.6678	-0.0545	-0.4473	1.0000		
Rosettes	-0.2331	-0.1092	0.2137	0.3245	-0.4372	-0.0089	0.1791	1.0000	
Flowering plants	-0.4339	-0.1662	0.3571	0.6075	-0.3428	0.1034	0.5428	0.4478	1.0000

Largest negative correlation was between rain and the numbers of *L. jacobaeae* adults (canonical coefficient = -0.2765) but rainfall was also negatively correlated with ragwort seedlings, rosettes, and flowering plants between April 1996 and August 1998 (respectively, -0.0347, -0.1092, and --0.1662; Table 4.9). There was a high correlation (0.8319) between air temperature (environmental temperature) and soil temperature as could be expected. However, the air mean monthly air temperature was correlated with the number of ragwort seedlings, rosettes, and flowering plants (canonical correlation coefficients of 0.5195, 0.2137, and 0.3571 respectively) between May 1996 and August 1998 as also was the mean monthly soil temperature (canonical correlation coefficients of 0.6678, 0.3245, and 0.6075 respectively (Table 4.9). There was also a significant correlation between soil water and other variables (larvae, adults, seedlings, rosettes, and flowering plants ($p = 0.0211$; Wilks' Lambda)).

4.5 Discussion

Few studies have examined the relationships between environmental factors and either ragwort flea beetle or ragwort. None have addressed the effects of soil water content except for three studies on seed germination. Van der Meijden and van der Waals-Kooi (1979) found that 92.5% of ragwort seed germinated at 15°C when the soil moisture was 29%, and that a germination rate of over 80% occurred between 10°C and 25°C. They also found that germination was best if the temperature varied diurnally over a 10°C range. Baker-Kratz and Maguire (1984) found that maximum and fastest germination occurred when the temperature alternated between 20°C and 30°C. McEvoy (1984) found that disc achenes germinated faster than ray achenes when they were alternately wet and dried for one hour at 20°C.

Poole and Cairns (1940) found that ragwort can grow well in New Zealand wherever rainfall exceeds 870 mm per year. Dempster and Lakhani (1979) reported that summer rainfall plays an important role in increasing the numbers of ragwort plants at Weeting Heath (England) and Crawley and Gillman (1989) found that seed production, rainfall and the production of regrowth shoots affect population fluctuation of ragwort plants.

My data from Ballantrae showed that the correlation between rainfall and numbers of *L. jacobaeae* adults was small and, like the other variables (air temperature, soil temperature), was negatively correlated (Table 4.9). The negative correlations between rainfall and ragwort seedlings (Table 4.9) were insufficiently high to decide if a lack of rainfall could have a negative effect on the ragwort population. More information is required from different sites to better understand the relationship between rainfall and ragwort population dynamics.

I found that the number of *L. jacobaeae* larvae appeared to be largely related to soil water content at Ballantrae except in extreme conditions such as being flooded for a long time. In this study, there was a weak correlation between soil water content and rainfall. Beskow (1995) also found that rainfall and soil mositure content were very weakly correlated. However, increased soil water content could cause a reduction in the numbers of *L. jacobaeae* adults, ragwort seedlings and flowering plants. In my study, soil water content was measured according to the different seasons between 1996 to 1998. The results cannot exactly answer whether soil water content effects the survival of *L. jacobaeae*. More research such as relationship between artificial different soil moisture contents (e.g. 40%, 50%, 60%, 70%, etc.) and larval survival and ragwort plant growth is still required to understand how soil water affects the populations of different stages of *L. jacobaeae* and ragwort plants.

Harper (1958) pointed out that large numbers of ragwort rosettes could compete effectively with grass and clover, but the factors that affect competition between ragwort plants are not known although plant density does directly affect the fluctuations of ragwort populations.

In Nova Scotia, Harris (1973) reported that defoliation by cinnabar moth larvae can lead to reduced numbers of ragwort plants when the plants have insufficient recovery time before the winter. However, feeding by cinnabar moth larvae has little effect on the reduction of numbers of ragwort plant in western North America (van der Meijden, 1971), in Canada (Harris, 1973), in the USA (Negal & Isaacson, 1974; Hawkes, 1981), and in England (Dempster & Lakhani, 1979; Crawley & Gillman, 1989). When both *L. jacobaeae* and the cinnabar moths are present together they have been reported to cause a decline in ragwort density in Oregon (Hawkes, 1981; McEvoy, 1985), Washington and Califonia (Mastrogiuseppes *et al.* 1983; Pemberton & Turner, 1990), but they have little effect on plant density in Canada (Julien, 1987). Hawkes and Johnson (1978) reported that *L. jacobaeae* reduced the density of ragwort from 71 rosette m^{-2} to 0.6 rosette m^{-2} during 4 years in Oregon but they do not mention whether the cinnabar moth or any other insect that feeds on ragwort was present as well. McEvoy *et al.* (1989) found that root feeding by *L. jacobaeae* larvae is more effective in reducing plant density than defoliation by cinnabar moth larvae. They also found that *L. jacobaeae* could reduce seedling and rosette densities by 95% and flowering plants by 39% as previously mentioned in Chapter 1.

Betteridge *et al.* (unpublished data) found that large rosettes had more *L. jacobaeae* larvae in them than small rosettes. However, there was no clear pattern when their data were expressed on a per gram dry matter basis suggesting that there was a variable density of larvae in relation to the roots. McEvoy (pers. Comm. 1999) found that as ragwort density in the field increased, the number of *L. jacobaeae* per ragwort plant decreased, and he suggested that this could be because ragwort biomass was greater at low plant density, than at high density. However, he also questioned whether any relationship exists between the number of adult *L. jacobaeae* and the mortality rate of ragwort.

At Ballantrae, large numbers of larvae per plant were found when plant densities were high (sometimes 17 rosettes m^{-2}). When larval numbers were high, rosette numbers were diminishing, possibly because the feeding activities of *L. jacobaeae* larvae reduced the population of ragwort rosettes. Feeding by larvae probably caused a decrease in rosette numbers at Ballantrae, followed by a decrease in larval numbers because of decreasing numbers of host ragwort plants. Low larval density may result in less food consumption than when larval densities are high, and this in turn may result in an increase in the plant population followed by an increase in the larval population in the following year. A dense population of ragwort seedlings may also result in a high mortality of ragwort by interspecific and intraspecific competition and lead to a decline in the ragwort population. Beskow (1995) also found that mortality of seedlings was 84% on bare soil and the numbers were reduced from 442 to 60 from October 1993 to January 1994. He concluded that mortality of seedlings was mainly due to competition from pasture plants. In my study, the total mean numbers of ragwort of all stages declined from 1996 to 1998. Feeding by *L. jacobaeae* larvae can certainly cause a weight loss in ragwort in the laboratory (Chapter 5).

Grazing by sheep occurred at my study site at Ballantrae and this may have affected the results. Betteridge *et al.* (1994) reported that grazing by sheep gave very good ragwort control in New Zealand.

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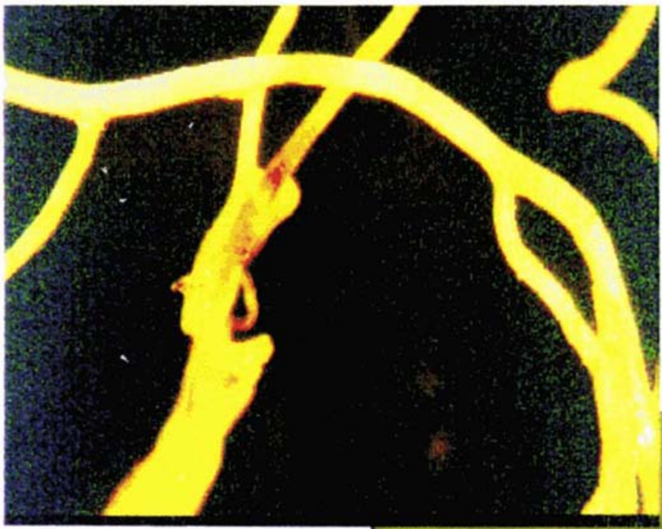
SECTION III

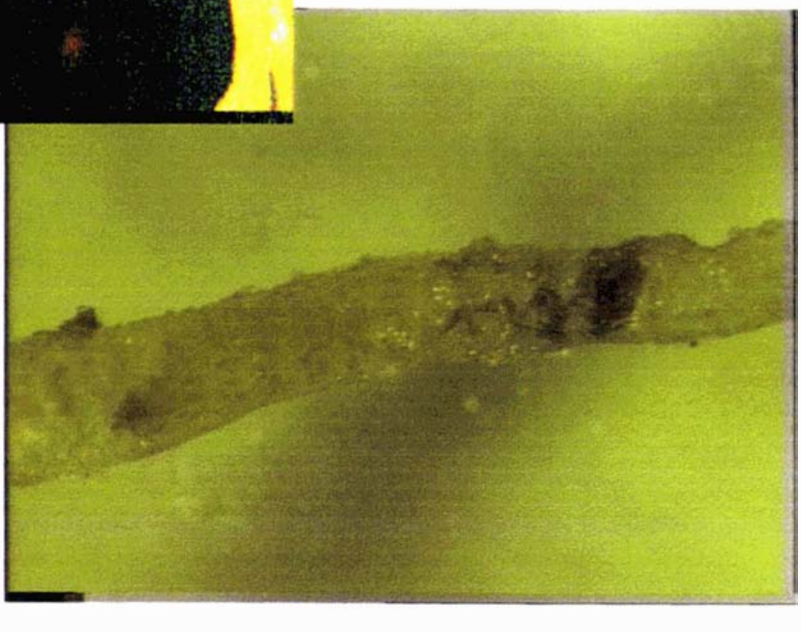
EXPERIMENTAL STUDIES

Chapter Five: Larval Consumption rates

(This paper was delivered to the 7th Australasian Conference on Grassland Invertebrate Ecology; 4-6 Oct. 1999, Perth.)

("with minor amendments")





Measuring the consumption rates of ragwort by ragwort flea beetle *Longitarsus jacobaeae* (Coleoptera: Chrysomelidae)

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5.1 Abstract

Consumption rates of the three larval instars of ragwort flea beetle, *Longitarsus jacobaeae* Waterhouse, were measured in the laboratory and glasshouse to examine the relationship between numbers of larvae attacking the ragwort plant and the duration of larval feeding; and to estimate how many larvae are required to kill a host plant.

The average consumption rate in the laboratory and glasshouse was respectively 0.0064 g/day and 0.0043 g/day for first instar larvae; 0.0072 gm/day and 0.0051 gm/day for 2[nd] instar larvae; and 0.0076 g d^{-1} 0.0058 g d^{-1} for 3[rd] instar larvae. Ragwort plants increased by 0.0138 g/g d^{-1} when in the rosette stage in the laboratory and glasshouse. Survival of *L. jacobaeae* larvae depended on the density of larvae in ragwort roots. The highest daily survival rate occurred in the laboratory when larvae were left of ragwort for 45 dyas at densities of 10 per plant (probability of surviving one day = 0.9649) and in a glasshouse when larvae were left for 45 days at a density of 40 larvae (probability of surviving one day = 0.9587).

Assuming that ragwort will die if the rate of consumption of roots is greater than the production of roots then our results suggest that between 49-67 *L. jacobaeae* larvae are required to kill a single ragwort rosette growing at 0.0138g/g d^1. These estimates do not take into account the necrotic effects of root damage.

5.2 Introduction

Ragwort flea beetle, *Longitarsus jacobaeae* Waterhouse, is a specialist herbivore. The larvae live inside roots and root crowns of ragwort and it is this feeding that is considered to be the most damaging to ragwort (Delpachitra, 1991; Frick, 1970 b; McEvoy, and Rudd, 1993; Syrett, 1983). For this reason, it is important to understand the feeding behaviour of these larvae in relation to the biological control of ragwort. Although there are many foraging models for prey and predators (e.g. Holling, 1959; Krebs, 1973; Loiterton and Margrath, 1996; Rapport and Turner, 1975; etc.) few biologists have studied feeding by small insect herbivores (lepidopteran larvae) on host plants (Bergelson and Lawton, 1988; Bernays, 1997). We know of no comparable foraging models publications on the feeding behaviour of underground root feeders such as *L. jacobaeae* larvae, perhaps because of the difficulties of studying consumption rates underground. Our experiments were designed to answer the following questions:

- What are the consumption rates of each of the three larval instars of *L. jacobaeae*?
- What is the relationship between numbers of larvae in the plant and the duration of larval feeding?
- How many larvae are required to kill a host plant?

5.3 Methods

Laboratory experiment

Root-washed ragwort plants (grown from seed individually in soil in a glasshouse) were placed in petri dishes containing capillary matting soaked with 1% liquid fertilizer solution. The method was developed by P. Peterson (Landcare Research) and was a modification of the method of Delpachitra (1991). Sixty single rosette plants of the same age and approximately the same size were grown between one side of a petri dish and the capillary matting. The roots were spread out over the matting with the leaves extending outward through a V-shaped groove in the side wall of each petri dish. Black plastic was used to cover the plant roots to exclude light. The 60 petri dishes were supported so that each ragwort plant was vertical. When the plants were 30 days old, 0, 10, 20, and 40 one day old *L. jacobaeae* larvae were placed per plant between stem and root crown using a fine artist brush ('000' Haydn Sabel) and the ragwort plants were then left for 15, 30, or 45 days. Thus, the experimental design was a 3 x 4 factorial (duration x larval crowding) replicated 5 times. The experiment was conducted at 20°C in a controlled environment room. Most *L. jacobaeae* moult to the 2nd instar by 15 days and to the 3rd instar by 30 days. Fresh and dry weights of the plants were measured at the end of 15, 30 or 45 days as appropriate.

Glasshouse experiment

The laboratory experiment was repeated in a temperature controlled glasshouse at 20°C ± 1°C, using ragwort plants potted in soil. These plants were not fertilized but in all other respects the experimental protocol was identical to the laboratory experiment. When the plants were 30 days old, four densities of one day old *L. jacobaeae* larvae (0,10, 20, and 40 larvae/plant) were placed between stem and root crown of each ragwort plant above the soil surface using a fine brush.

Fresh weights of plants at 45 days, 60 days, and 75 days were obtained after washing the soil from the roots and root crown, and draining the plants for two hours. After larvae had been extracted the larvae were counted and the plants were dried in an oven at 60°C for 12 hours, then weighed.

L. jacobaeae larvae from laboratory and glasshouse experiments were extracted using the apparatus described in Chapter 2 (A).

5.4 Results

The time that larvae were on ragwort plants and the density of larvae per plant both had highly significant negative effects on ragwort growth in the laboratory and in the glasshouse (ANOVA p=0.0001 and p=0.0008 respectively for duration; p=0.0001 and p=0.0001 respectively for density, more detailed in Appendix 5.5). Time had a quadratic effect on the mean weight of single rosettes of ragwort for each larval density. However, there was a significant interaction between the time that larvae were on the plant and larval density on the weight loss of ragwort. When no larvae were present the mean plant weight increased gradually but decreased if the plant had

10, 20, or 40 larvae for between 15 days and 45 days. Thus different larval densities caused different weight reductions in single rosettes of ragwort depending on the time that larvae were present.

The average consumption rates of larvae in the laboratory and glasshouse are given in Table 5.1. The consumption rates can be based on Holling (1959) and calculated as follow:

$C\ R = \{C\ P\ W\ (gd^{-1}) - L\ I\ P\ W\ (gd^{-1})\} / F\ D\ (d)$

Where C R - consumption rates of larvae, C P W (gd^{-1}) - plant weight (0 larvae or controlled plant), L I P W (gd^{-1}) - plant weight (larvae feeding inside of the plant), and F D (d) - feeding duration (days) (more detailed are in Appendix 5.1 - 5.6).

Here most larvae are 1st instar at 15 days, most are 2nd instar at 30 days, and most are 3rd instar at 45 days. The mean feeding rates of *L jacobaeae* larvae were higher in the laboratory than in the glasshouse (Figure 5.1).

Table 5.1 Consumption rate of different stages of *L. jacobaeae* larvae in the laboratory and glasshouse.

Time	No. of alive larvae (lab.)	No. of alive larvae (glasshouse)	Feeding rate in lab. ($g\ d^{-1}$)	Standard Deviation	Feeding rate in glasshouse. ($g\ d^{-1}$)	Standard Deviation
15-days	29	16	0.0064	0.0022	0.0043	0.0013
30-days	17	17	0.0072	0.0030	0.0051	0.0022
45-days	15	18	0.0076	0.0043	0.0057	0.0029

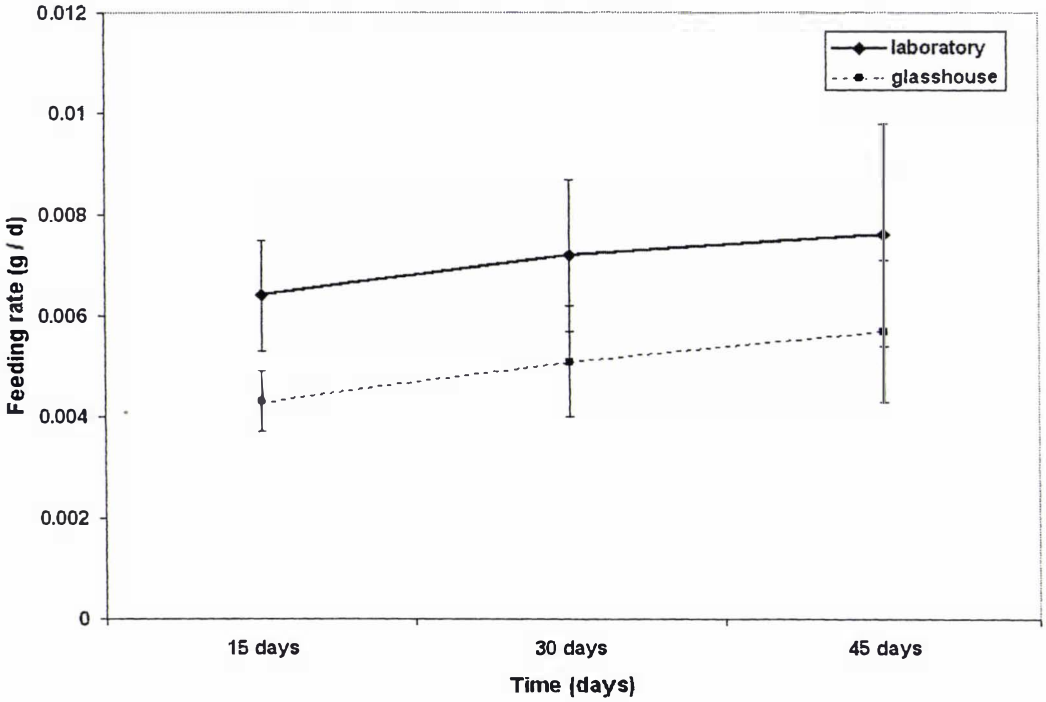

Figure 5.1 Feeding rate of *L. jacobaeae* larvae in the laboratory and glasshouse. Bars indicate ± standard error.

Ragwort plants without *L. jacobaeae* larvae increased in average weight by 0.6034 g d^{-1} (fresh weight) in the laboratory. The average observed growth rate is 0.0138 g / g d^{-1}. Growth of single rosette ragwort plants from 1 to 75 days in the laboratory and glasshouse is shown in Figure 5.2. The mean weight gain of single rosette ragwort plants in the glasshouse prior to 30 days was not measured because this would damage the plants. The weights of ragwort plants grown in the glasshouse were significantly higher than those grown in the laboratory.

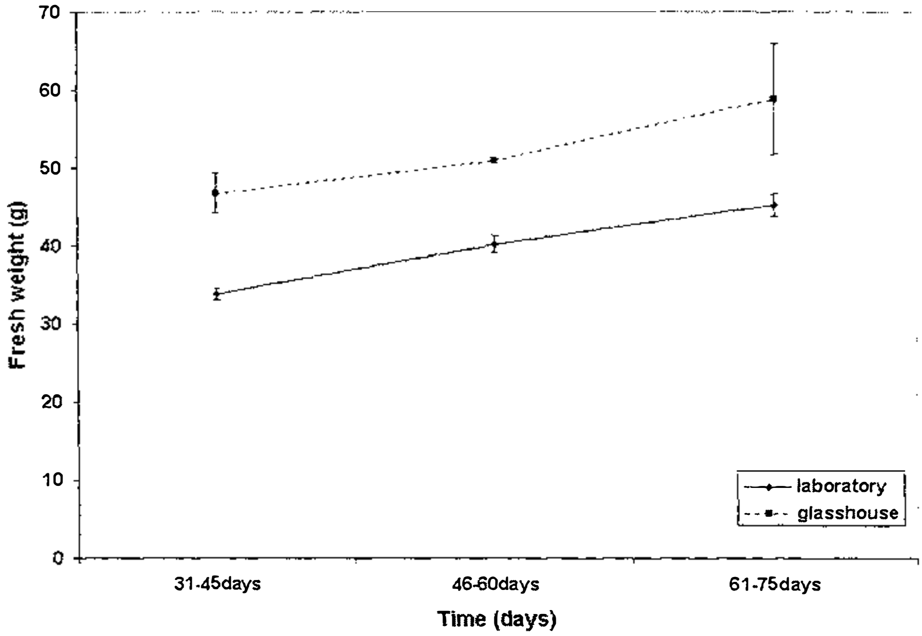

Figure 5.2 Average weight of single rosette ragwort plants in the laboratory and in the glasshouse. Bars indicate ±1 standard error.

The highest probability that *L. jacobaeae* larvae would survive for 1 day occurred when they were left on ragwort for45 days at a density 10 per plant in the laboratory and at 40 larvae /plant for the same duration in the glasshouse. Survival of larvae depended on the density of larvae in ragwort roots. Larvae at low density (10 larvae/plant) had a higher survival rate than those at high densities (40 larvae/plant) in the laboratory. The lowest probability of surviving 1 day occurred at 20 larvae/plant when the larvae were left for 15days in both the laboratory and the glasshouse (Table 5.2). The survival rate of older larvae was greater than that of younger larvae (Table 5.2).

Table 5.2 Relationship between survival of *L. jacobaeae* larvae in the laboratory and glasshouse and numbers of larvae per plant and lengths of time with ragwort. Survival is given as the probability of a larva surviving for one day.

No larvae/plant	Survival in the laboratory			Survival in a glasshouse		
	15-days	30-days	45-days	15-days	30-days	45-days
10	0.9306	0.9444	0.9649	0.8983	0.9261	0.9454
20	0.9092	0.9407	0.9540	0.6748	0.9291	0.9540
40	0.9067	0.9343	0.9521	0.8886	0.9343	0.9587

5.5 Discussion

Our results apply only to single rosette ragwort plants that are up to 75 days old and to *L. jacobaeae* larvae that are up to 45 days old. We found that *L. jacobaeae* larvae can be more effective at reducing the growth of ragwort plants when the plants are aged between 30 days and 75 days. Possibly plant weight gain may be high enough to resist the infestation of *L. jacobaeae* larvae when the plants are younger than 30 days old. When *L. jacobaeae* larvae damage ragwort roots, the plant may compensate in a variety of ways such as by growing new adventitious roots. However, no data were gathered on this. Feeding rates were negatively correlated with increasing densities of larvae and this indicates that there may be competition between the larvae. Such competition may be influenced by density dependent factors, but further experimentation is required to determine the exact relationships.

The different weight gains in the laboratory and glasshouse were undoubtedly due to the very different conditions under which they were grown. In addition, *L. jacobaeae* larvae grown in the laboratory were heavier than those of comparable age in the glasshouse but the reason for this is unknown. The roots of laboratory grown plants were appeared to be more easily damaged when they were examined than those in the glasshouse so they may have been softer and more easily penetrated by the larvae. Alternately, the lack of soil in the laboratory may have enabled the larvae to move between roots more easily. Thus their feeding and assimilation rates in the laboratory may have been higher.

Crowding of *L. jacobaeae* larvae clearly affects their feeding rates both in the laboratory and glasshouse but we do not know whether this is caused by intraspecific competition amongst *L. jacobaeae* larvae, or by other factors. Other factors such as softness of the roots or root crowns, the abilities of larvae to penetrate and move into or within roots or other physiological changes may also affect their feeding rates.

The numbers of *L. jacobaeae* larvae needed to kill a single rosette ragwort plant can be estimated by dividing the mean weight gain per day of ragwort plants (Figure 5.2) by the mean larval feeding rate (Table 5.2). When the age of the larvae is considered, then the estimates for killing a single rosette plant based on the laboratory experiment are 52 larvae that are15 days old, 49 larvae that are 30 days old, and 51 larvae that are 45 days old. In terms of the density of *L. jacobaeae* larvae alone, then theoretically 43 larvae should be required to kill single rosette ragwort plants at a density of 10 larvae/plant, or 58 larvae at 20 larvae/plant, and 69 larvae at 40 larvae/plant.

The estimated number of larvae required to kill single rosette ragwort plants in the glasshouse were 67 larvae up to 75 days old, and 81 larvae at 15 days of age, 66 larvae that are 30 days old, and 58 larvae that are 45 days old. When different densities of larvae were considered, then 45 larvae are required to kill a plant when there are 10 larvae/plant, and 74 larvae at 20 larvae/plant, and 108 larvae at 40 larvae/plant.

It is likely that our estimates of the infestation rates of *L. jacobaeae* larvae required to kill ragwort are low if applied to ragwort in the field. The infestation rate necessary to kill a ragwort plant probably depends on the feeding rates of the larvae and on their developmental stage. Thus larger larvae would have higher feeding rates than small larvae so fewer large larvae would be required to kill a ragwort plant. The plant may respond to the damage cause by larval feeding with compensatory growth, and the environmental conditions under which the ragwort is growing may also affect the ability of the plant to survive damage.

5.6 Acknowledgements

We would like to thank to Dr. Peter G. McGregor, Landcare Research for editing manuscript and his valuable suggestions, Mr. Hamish J. Mack, Ecology, Institute of Natural Resources, Massey University, and Mr. Paul Peterson, Landcare Research, Palmerston North for help to get RFB and ragwort plants. Aung Kyi was supported by New Zealand Ministry of Foreign Affairs and Trade under its Official Development Assistance Post Graduate Scholarship (NZODA PGS) scheme.

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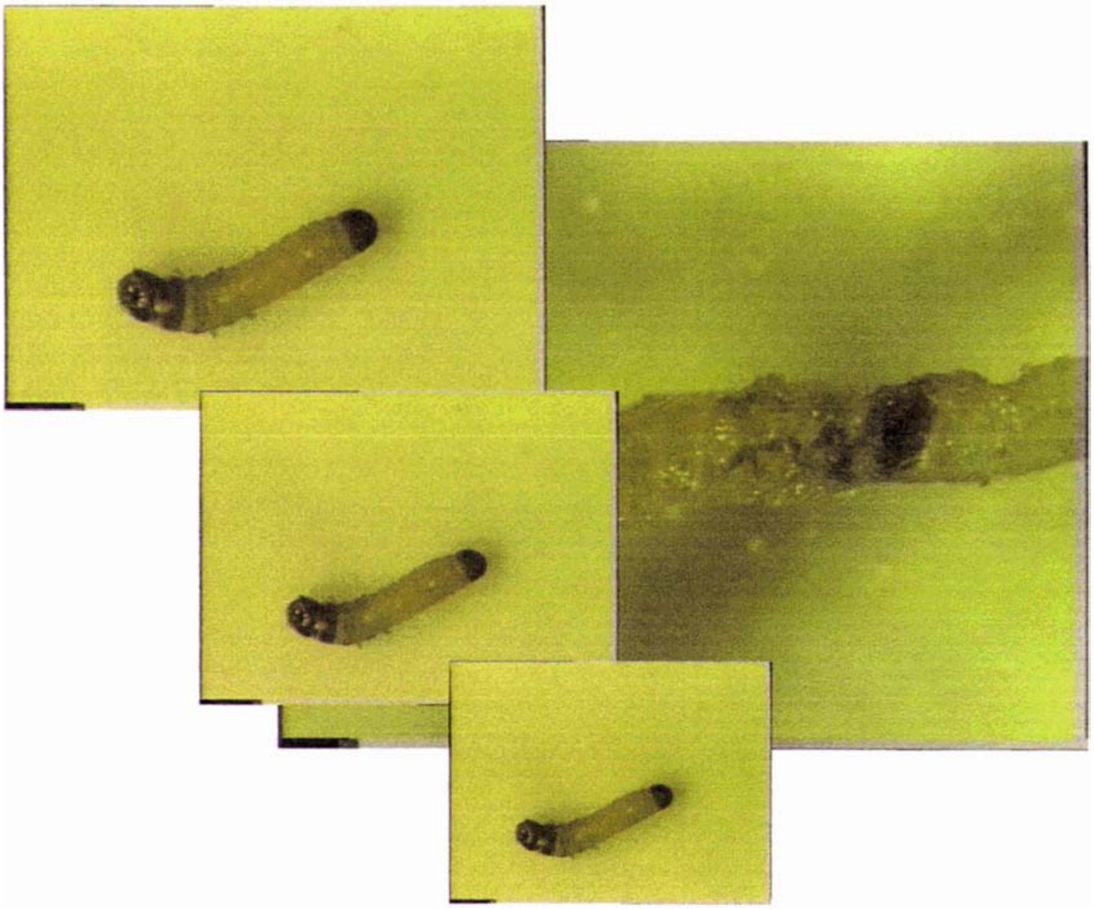
Chapter Six: Larval Movement



An experimental study of movement by first instar larvae of ragwort flea beetle (*Longitarsus jacobaeae* Waterhouse) through soil of different textures

6.1 Abstract

Movement of 1st instar larvae of ragwort flea beetle *Longitarsus jacobaeae* Waterhouse (Coleoptera: Chrysomelidae) was investigated in three different soils (sand, silt, and 50% sand + 50% silt) in the laboratory using a test chamber. Soil texture had no significant effect on movement. Horizontal movement was much greater than upward and downward vertical movement.

6.2 Introduction

Larvae of *Longitarsus jacobaeae* Waterhouse hatch in soil and then move to roots or root crowns to feed. Thus the ability of 1st instar larvae to move through soil is likely to play an important part in the inter-relationship between ragwort and ragwort flea beetle.

Nothing has been published on larval movement of *L. jacobaeae* but some information is available on movement through soil for larvae of another chrysomelid beetle, the western corn rootworm, *Diabrotica undecimpunctata howardi* Barber (Strnad & Bergman, 1987). Larval migration (Short & Luedtke, 1970; Suttle *et al.*, 1967) and survival (Turpin & Peters, 1971) of *D. u. howardi* has also been examined in relation to soil texture. Here I report on a laboratory experiment to determine whether 1st instar larvae of *L. jacobaeae* can detect ragwort roots in soil and move through soil towards them.

6.3 Materials and Methods

Movement of newly hatched (<one day old) *L. jacobaeae* larvae was examined in a test chamber designed so they could move between adjacent compartments filled with

different soils. The test chamber was a glass box (100 mm high x 150 mm wide x 300 mm long) with a tightly fitting glass lid (Figure 6.1). Three glass sided trays (33 mm high x 147 mm wide x 297 mm long) fitted one above the other inside the test chamber. The bottom of each tray was made from stainless steel wire mesh with holes 2 mm across. These holes allowed *L. jacobaeae* larvae to pass through. Each tray was divided into three equal (100 mm) compartments by two stainless steel wire mesh partitions (2 mm holes). Each 100 mm compartment was more convenient to divide two 50 mm wide sections and much easy to chose the centre point for releasing. When assembled in the glass test chamber, the trays created nine equal sized rectangular spaces, which were partitioned off by wire mesh (Figure 6.1).

Each compartment in each tray was filled with the same soil for a test. Three soil types were tested: sand (90% sand, 8% silt, 2% clay), silt loam (71% silt, 20% clay, 9% sand) or 50% sand + 50% silt loam. The soil classification followed Gibbs (1980), Strnad and Bergman (1987), and McLaren and Cameron (1993). The moisture content of all three soils was adjusted to 20 % (wt/wt).

Movement was tested on three separate occasions using a different soil each time. Soil was screened directly into the trays so that the resulting soil densities were 0.92, 0.84, and 0.76 g/cm^3 for the sand, 50% sand and 50% silt loam, and silt loam, respectively. The soil was then lightly pressed down. A 2 cm diameter plug of soil was then removed from the central compartment of the middle tray and 100 *L. jacobaeae* 1st instar larvae (< 24 h old) were introduced into the resulting cavity at the start of each test. Soil was loosely added into the hole above these larvae until it formed a level surface. Larvae were transferred with a very fine brush ("000" Haydn Sebel brush). One 3 cm diameter soil plug was also removed from near the outer edge of each end compartment. Each hole at the same end of each tray was filled with 100 g of freshly harvested ragwort roots and the holes at the opposite end were filled with 15 g of cotton wool. Ragwort roots were added 12 hours before the *L. jacobaeae* larvae were released into the test chamber. The three trays were then stacked on each other in the test chamber with the soil in contact between all trays. The glass lid was closed and the test chamber was placed in a dark temperature controlled room (20^{o}C) for 12h during the trial.

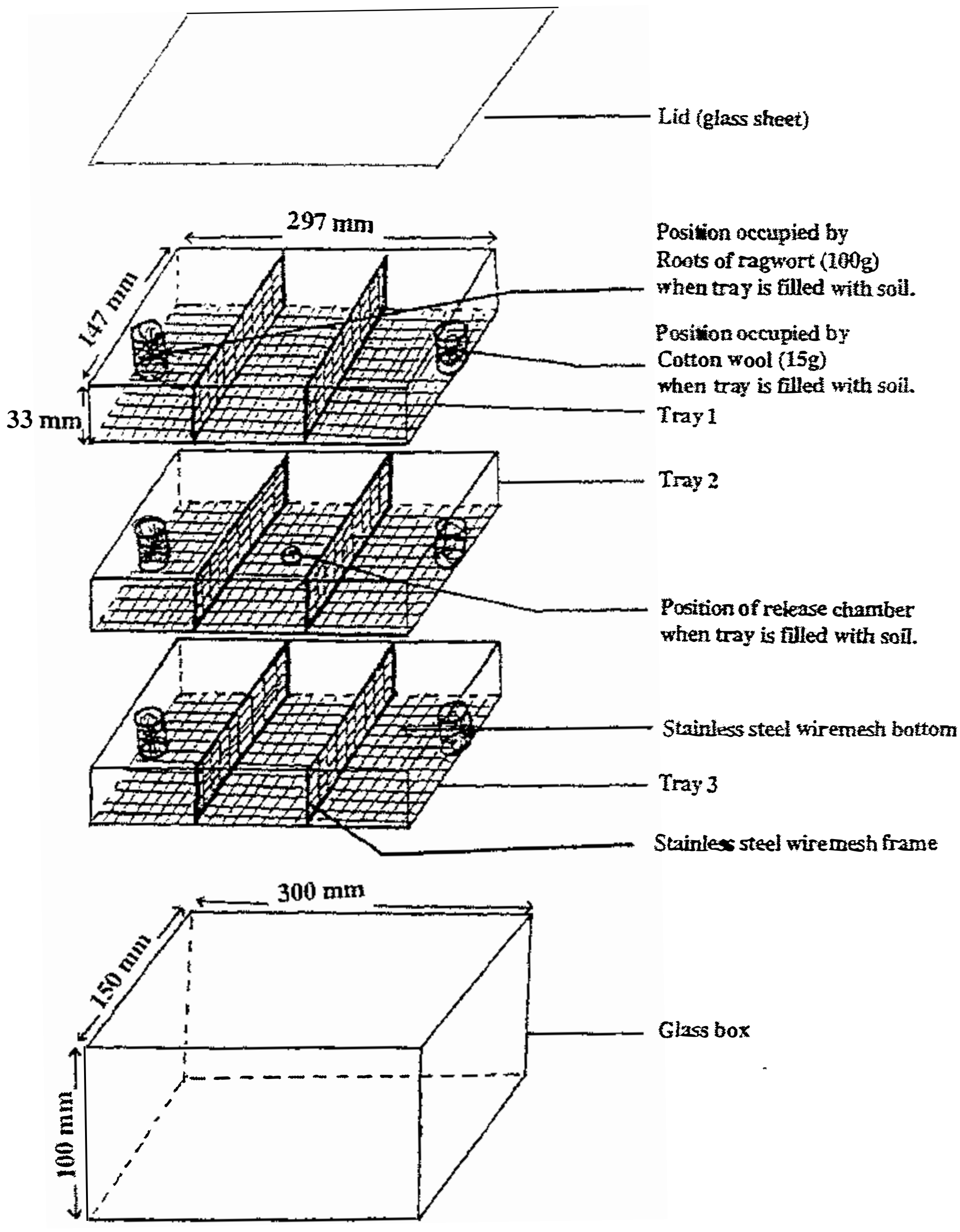


Figure 6.1 Exploded view of the test chamber used to investigate movement of first instar *L. jacobaeae* larvae through soil. The three glass-sided trays shown fit one above the other in the test chamber.

A preliminary experiment was also carried out to determine how far *L. jacobaeae* larvae move through uncompacted soil. Here, the soil was not pressed into each tray when *L. jacobaeae* larvae were introduced.

Twelve hours after the *L. jacobaeae* larvae were introduced, the soil in each tray was divided into six, 50 mm wide sections, and the larvae within each section were recovered by flotation in a magnesium sulphate solution of specific gravity 1.2 (Rohitha, 1992).

6.3.1 Statistical Analyses

Movement of *L. jacobaeae* larvae through soil was analysed as a split plot design with 4 replicated test chambers. Each tray compartment was considered to be a treatment. The main plot treatments (of the three types of soil) were split into nine subplots (the nine tray compartments) (Middle centre, Middle with cotton-wool, Middle with ragwort roots, Lower centre, Lower with cotton-wool, Lower with ragwort roots, Upper centre, Upper with cotton-wool, and Upper with ragwort roots)(Figure 6.1 and Appendix 6.1).

All statistical analyses were done using the General Linear Model (GLM) procedure of SAS (SAS 1987, version 6.0) (Appendix 6.1). ANOVA was used to test for any effects of soil on the movements of 1^{st} instar larvae, any relationship between the nine tray compartments on the movement of *L. jacobaeae* 1^{st} instar larvae, and any interaction between the soils and the nine tray compartments on the movement of *L. jacobaeae* 1^{st} instar larvae.

6.4 Results

First instar larval movement was not significantly affected by soil type, but it was significantly affected by the distance the larvae were from ragwort roots (Table 6.1).

Table 6.1 ANOVA of movement by *L. jacobaeae* 1st instar larvae in three different soils. The Type I MS was used for BLOCK*SOIL as an error term. The block term is the experimental replication.

Source	DF	Type I SS	Mean Square	F	P
Block	3	13.43	37.81	3.93	0.0723
Soil	2	23.22	11.61	1.21	0.3624
Sub-plots	8	14472.42	1809.05	223.42	0.0001

Table 6.2 Mean number and Standard Error of *L. jacobaeae* 1st instar larvae in different sectors (nine compartments) in three different soils during 12 hours. (Data from 4 replicates.)

Soil	Middle sectors			Lower sectors			Upper Sectors		
	centre	cotton	root	centre	cotton	root	centre	cotton	root
Sand	37± 2.7	1± 0.3	10± 0.7	25 ± 1.9	2± 0.3	6± 0.5	16± 2.4	1	2± 0.3
Silt	31± 1.2	1± 0.3	8± 1.1	33± 2.5	1	5± 0.8	18± 2.6	1	2± 0.5
Sa+Silt	32±0.6	2± 0.3	11± 1.0	35 ± 1.3	1	6± 0.4	11± 2.0	1± 0.3	1± 0.3

Sa+Silt* - 50% Sand + 50% Silt

Most larvae stayed close to where they were first placed, but a few did disperse (Table 6.2). Of those that did move, a significantly greater number moved vertically towards ragwort roots (Table 6.2 shows 25 + 16 =41, 33 + 18 = 51, and 35 + 11 = 46) than towards the cotton wool control within the middle layer (Table 6.2) and most of these were found between 50 and 150 mm from their release sites after 12 h in all three soil types (Table 6.2). Sand seemed to inhibit movement most as this contained the highest percentage of larvae that remained within 50 mm of the release site after 12 h. Silt appeared to favour the greatest horizontal movement followed closely by the sand/silt mixture.

Significantly more 1st instar larvae moved vertically than either upward or downward and of the larvae that moved vertically, more did so as they moved towards compartments containing ragwort roots than towards the cotton wool controls in all three soil types (Figures 6.2a, b, c, 6.3, and 6.4; Tables 6.2, 6.3).

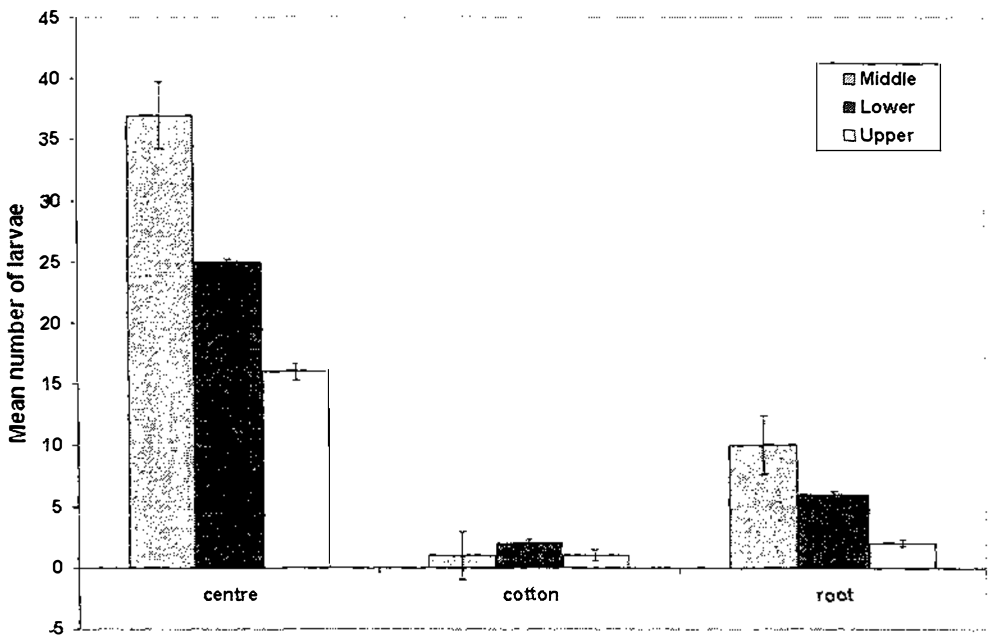


Figure 6.2 (a) Movement of *L. jacobaeae* 1st instar larvae in nine compartments (sectors) in sand. In figure 6.2 (a-c) "Middle, Lower, and Upper" sectors refer to the three trays shown in Fig 6.1. "Centre, Cotton wool, and Roots are three compartments in each tray. Bars indicate ± 1 standard error.

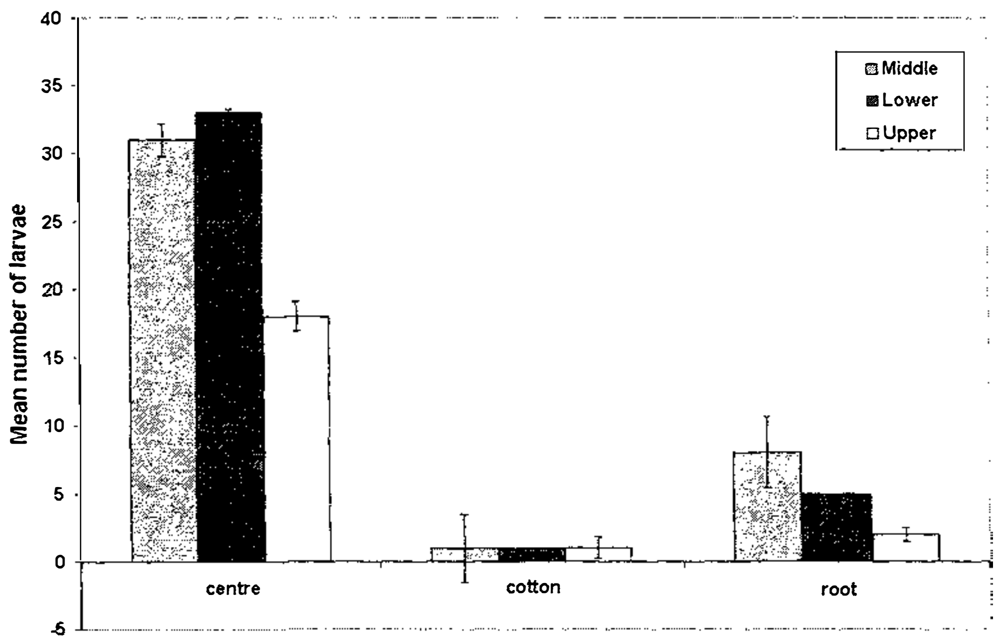


Figure 6.2 (b) Movement of *L. jacobaeae* 1st instar larvae in nine compartments (sectors) in silt. Bars indicate ± 1 standard error.

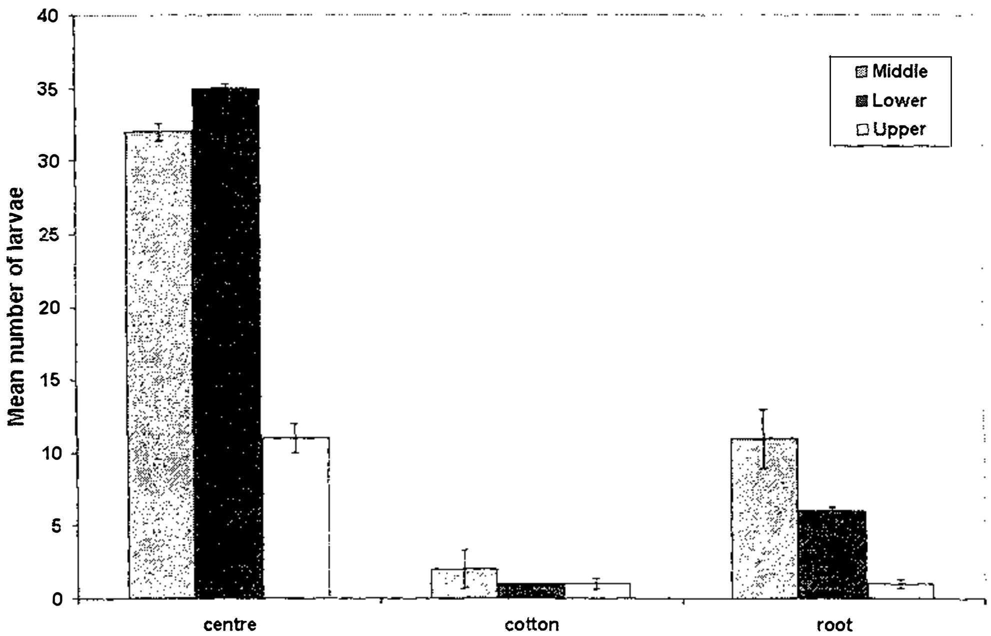


Figure 6.2 (c) Movement of *L. jacobaeae* 1st instar larvae in nine compartments (sectors) in 50% sand +50% silt. Bars indicate ± 1 standard error.

Table 6.3 Tukey's studentized range multiple comparison test for movement of *L. jacobaeae* 1st instar larvae in the test chamber. Means are for all three soil types combined; those with the same letter are not significantly different.

Sectors	Mean	N	Tukey Grouping	
A (Middle, centre)	33	12	A	
D (Lower, centre)	31	12	A	
G (Upper, centre)	15	12	B	
C (Middle, roots)	10	12	C	
F (Lower, roots)	5	12	D	
I (Upper, roots)	2	12	D	E
B (Middle, cotton wool)	2	12	D	E
E (Lower, cotton wool)	1	12		E
H (Upper, cotton wool)	1	12		E

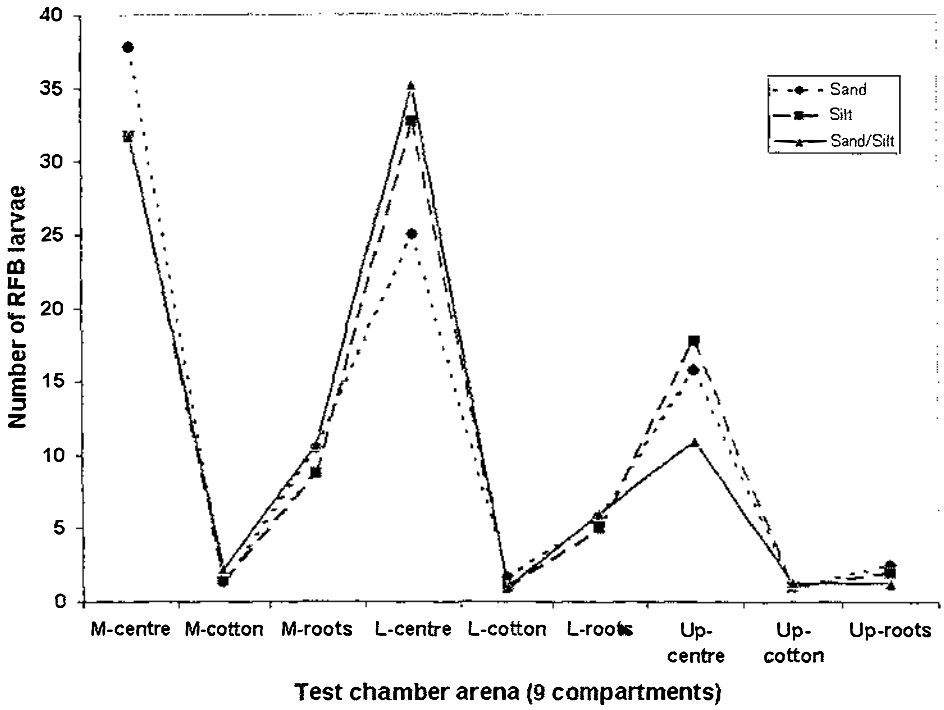


Figure 6.3 Means for movement experiment of *L. jacobaeae* 1st instar larvae in sand, silt, and sand silt mixture.

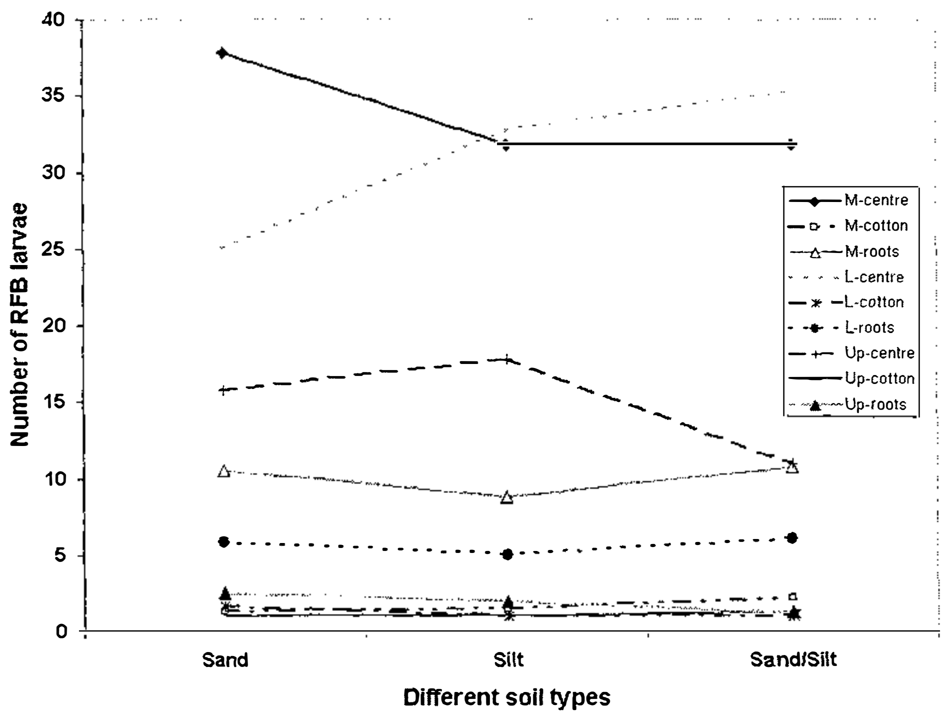


Figure 6.4 Means for movement experiment of *L. jacobaeae* 1st instar larvae at nine compartments in sand, silt, and sand silt mixture.

6.5 Discussion

Some 1st instar *L. jacobaeae* larvae moved both horizontally and vertically in all three-soil types but there was a highly significant interaction between the three different soils and nine compartments (Table 6.1) so their movement into the 9 compartments varied according to the soil they were in.

This experiment demonstrated that most 1st instar *L. jacobaeae* larvae remain near their release site (0-50 mm) in sand, silt and a sand/silt mixture (Table 6.3). Such behaviour may be related to the behaviour of the adult female, which usually oviposits on the root crowns of ragwort or in the soil near them (Frick, 1971). Thus newly hatched larvae normally only need to move a short distance to find roots or root crowns of ragwort in order to bore into them. Other soil dwelling insects that are known to remain close to their oviposition site, or that do not move far through soil include western corn root worm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) (Strnad & Bergman, 1987) and the bean leaf beetle, *Cerotoma trifurcata* Foster (Coleoptera: Chrysomelidae). For examples, 1st instar larvae of the western corn rootworm can travel up to 25 cm (Strnad & Bergman, 1987) and all larval instars of the bean leaf beetle can move as far as 30.5 cm (Marrone & Stinner, 1983) underground from their oviposition site to find food.

Soil texture is one of the most important factors that relates to survival and movement of root-feeding insects. For instance, survival of small larvae of the southern corn rootworm, *Diabrotica unidecimpunctata howardi* Barber, and the bean leaf beetle, *C. trifurcata*, (both Coleoptera: Chrysomelidae) is highest in fine-textured soils such as silt and loam but decreases with increasing particle size (e.g. sandy soils) (Lummus *et al.*, 1983; Marrone & Stinner, 1984). Strnad and Bergman (1987) found that density of soil affected movement of western corn rootworm and that larvae moved less than 5 cm in compacted sandy loam with density of 1.1g/cm^3, less than 20 cm at density of 1.3 g/ cm^3 and less than 5 cm at 1.5 g/cm^3 in sand. This reduction is due to a decrease in pore spaces in the soil of increased density. Newly hatched western corn root worm larvae have head capsules about 0.2 mm in diameter, and this is bigger than the average pore space when the bulk soil density is high so this may prevent movement through soil (Strnad & Bergman, 1987).

Other factors may that may affect movement of soil insects are soil moisture (Lummus *et al.*, 1983; Marrone & Stinner, 1984; Dennehy & Clark, 1987) and the presence or absence of light when larvae are near the surface. In addition, Forster (1975) noted that the distributions of root-feeding insects generally result from the heterogeneity of soil and the adaptations of these insects to this environment. For example, the sea aster root aphid *Pemphigus trehernei* Foster lives in saltmarshes and is restricted to pore spaces but these spaces are of limited occurrence here (Foster, 1975).

Strnad and Bergman (1987) found that the reduced pore space might prevent the passage of larvae of the western corn rootworm so it is likely that soil texture may affect movement. This may also affect movement of *L. jacobaeae* larvae, and hence their survival. However, more 1st instar larvae of *L. jacobaeae* moved further horizontally away from their release site in silt than in either sand or a sand/silt mixture (Table 6.4) so it appears that larger interstitial spaces rather than smaller ones seem to reduce movement in this species. Interestingly, the 1st instars of both *L. jacobaeae* and the western corn rootworm are about the same size but *L. jacobaeae* larvae only moved up 15 cm within 12 h whereas the larvae of western corn rootworm moved much further and faster (25 cm within 6 h) (Strnad & Bergman, 1987). However, Strnad and Bergman (1987) tested their larvae with a gas flow of 50% air: 50% CO_2 through the soil and this may have provided a much greater stimulus for movement than using roots in still air.

Root-feeding and underground dwelling insects principally use CO_2 emission from roots to locate a food source in the soil but different chemical compounds present in the roots can also act as attractants, deterrents or phagostimulants and thus help determine host-plant preferences (Brown & Gange, 1990). For example, third instar larvae of the grass grub *Costelytra zealandica* White (Coleoptera: Scarabaeidae) are attracted by root volatiles of pasture legume, *Lotus pedunculatus* (Sutherland & Hillier, 1974; Sutherland, Maron, & Hillier, 1975). Root-feeding and underground dwelling insects may also be considered to be photophobic. Soil moisture and soil texture effects (Lummus *et al.*, 1983; Marrone & Stinner, 1984) may also be needed to be considered in relation to larval movement. For example, all larval instars of the bean leaf beetle, *Cerotoma trifurcata* Foster (Coleoptera: Chrysomelidae), can move as far as 30.5 cm in the soil and they will move from unfavourable to favourable situations in response to food, soil texture, and moisture conditions (McConnell, 1915; Anderson & Waldbauer, 1977; Levinson, Waldbauer, & Kogan, 1979; Marrone & Stinner, 1983).

First instar larvae of *L. jacobaeae* are certainly attracted towards ragwort roots because significantly more moved towards these roots than towards the control (Table 6.1) but the stimulus of attraction was not determined. Carbon dioxide was shown to attract 1st instar larvae of another chrysomelid, the western corn rootworm (Strnad & Bergman, 1987) so CO_2 produced by the ragwort roots may also attract *L. jacobaeae* 1st instar larvae.

Strnad and Bergman (1987) reported that upward movement by western corn rootworm larvae resulted in a relatively even distribution above the release point after 20 h, whereas downward movement resulted in only 20% of the larvae moving greater than 10 cm. In contrast, *L. jacobaeae* 1st instar larvae prefer to move horizontally rather than vertically. In the field, more larvae are found in root crowns than in the soil but attraction towards root crowns was not tested. It is, however, possible that those larvae that move vertically through soil preferentially move downwards because there is a higher proportion of adventitious roots deeper down than towards the surface.

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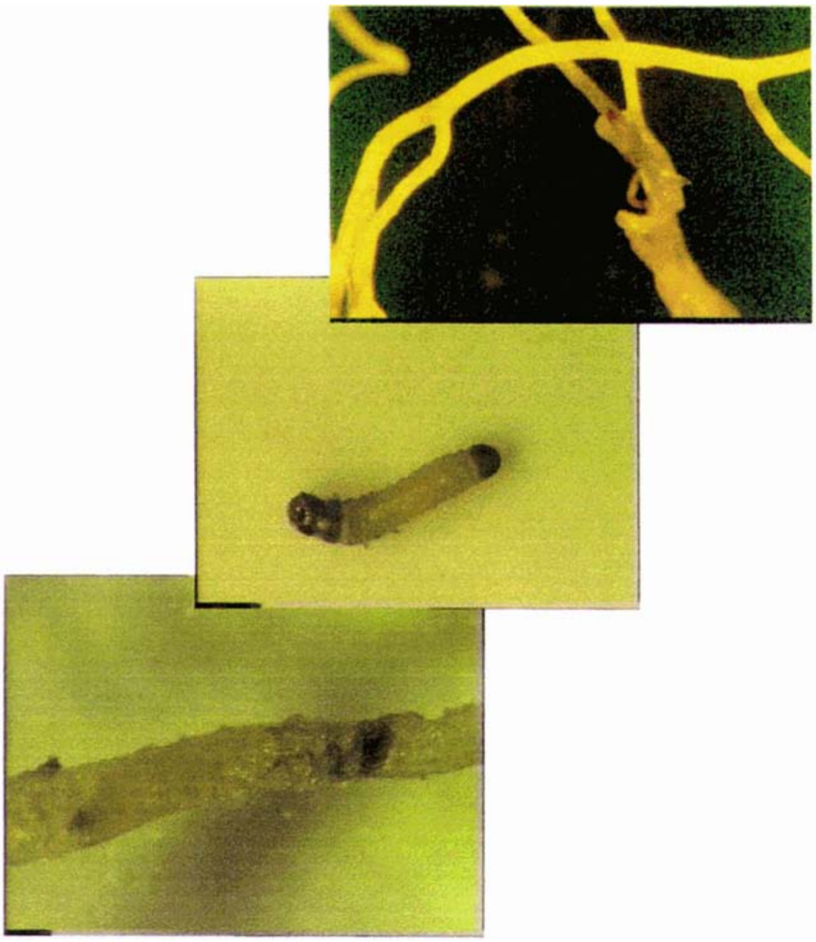
Chapter seven: Larval Interplant Movement



Experimental determination of the effect of interplant distance on the ability of *Longitarsus jacobaeae* larvae (Coleoptera: Chrysomelidae) to transfer between ragwort plants.

7.1 Abstract

The ability of ragwort flea beetle (*Longitarsus jacobaeae*) larvae to transfer between ragwort plants (*Senecio jacobaea*) was tested using four densities of newly hatched larvae (0, 10, 20, and 40 per plant) over three durations (15, 30, and 45 days). Overall, after the plant was killed, an average of 2.6% of the larvae moved 50 mm from a central plant to surrounding plants. When the plants were killed 45 days after the larvae were introduced, 4.3% of the larvae moved 50 mm. Only 0.71% of larvae moved 100 mm between plants when the plant was killed after 15 days but no larvae moved this distance when the plant was killed after 30 or 45 days.

7.2 Introduction

First instar larvae of ragwort flea beetle (*Longitarsus jacobaeae* L.) move more readily horizontally through three different soil mixtures than vertically when this was tested in the laboratory (Chapter Six). However, of those larvae that did move vertically, more moved downward than upward. Many 1st instar larvae of *L. jacobaeae* have to move from where they hatch to ragwort roots or root crowns when the eggs are laid in soil (Frick, 1971). Full-grown larvae also move from their feeding sites inside ragwort plants to the soil for pupation. If a ragwort plant dies while larvae of *L. jacobaeae* are feeding inside it, what will the surviving larvae do? Can they move from the plant to another one or do they die with the plant? This experiment was carried out to find if *L. jacobaeae* larvae can move between plants and to determine the effect of interplant distance on their ability to transfer between plants.

7.3 Materials and Methods

L. jacobaeae adults were collected from AgResearch's hill country research station at Ballantrae, near Woodville using a vacuum apparatus (Chapter 2) on 7 April 1998. Three hundred and eighty-eight beetles of both sexes were collected. The method used to obtain first instar larvae was described in Chapter 5. This was done between 7 April 1998 to 26 May 1998.

Ninety-six plastic trays (external measurements: 34.5 cm x 47 cm x 6 cm deep) were filled with soil. One ragwort plant was planted in the center of each plastic tray and a further four plants were planted equidistant around it. The outer plants were either 50 mm or 100 mm from the central plant.

When the plants were about 4 months old (26 May 1998), four densities of first instar larvae less than 24 h old (0,10, 20, and 40 larvae) were collected in small collection vials using a '000' "Haydn" fine brush. The purpose of introducing '0' larvae was to check that the plants were not contaminated with larvae. The larvae were then carefully picked up one by one from the collection vials and placed carefully between the stem and root crown of the center plant in each tray. This transfer was done in shade under an umbrella in still dry conditions because newly hatched larvae are very small and delicate. The larvae were then left on the ragwort plants in the field for three different durations (15 days, 30 days, and 45 days) then each centre plant was killed by using "Primus Gardener" flame gun. Before using Primus Gardner flame gun, a thermometer was placed under the root zone in the soil to check underground temperature after using the flame gun. The temperature of the underground root zone was about 16 ± 2.0 °C and was therefore safe for the survival of the larvae. A week after the centre plant was killed, the peripheral plants were removed and washed with water to remove the soil from around roots and root crowns. The plants were stored at 4°C in a controlled temperature room and the larvae were extracted and counted from 10 plants at a time. The extraction method and apparatus are described in Chapter 2. Analysis was by ANOVA using SAS 6.0 (1987) (Appendix 7.1).

7.4 Results

Some *L. jacobaeae* larvae moved successfully from their central plants to other plants 50 mm and 100mm away. However, the distance between plants had a significant effect on this (p=0.0012;Table 7.1) with 2.62% of larvae moving 50 mm and only 0.24% moving 100 mm (Table 2). Larval densities also significantly affected movement between plants (p=0.0129; Table 7.1). The results showed that the greater the number of larvae inside a plant initially, the greater the number that emerged from the plant when it was killed. The age of the larvae (15, 30 or 45 days old) had no significant effect on the number of larvae that emerged after the plant was killed (p=0.3693).

Table 7.1 TW-ANOVA (Two way analysis of variance) for the effects of interplant distance on ability of larvae to transfer between plants, the effects of larval durations and larval densities on larval movement.

Source of variation	df	Type III SS	MS	F	P
Distances	1	4.17	0.38	11.20	0.0012
Larval density	3	4.25	1.42	3.81	0.0129
Larval age	2	0.75	4.17	1.01	0.3693
Block	3	0.83	0.28	0.75	0.5273
Error	86	32.00	-	-	-

The percentage of larvae that emerged from a dead plant and then moved to other plants was also very low (Table 7.2). Overall, 2.62% of the larvae emerged and moved one week after the centre plant was killed. The highest overall percentage (4.3%) was larvae that moved 50 mm after being in the plant for 45 days before the plant was killed.

Table 7.2 Movement of larval *L. jacobaeae* between ragwort plants 50 mm and 100 mm apart.

Time	Introduced larvae per replicate (n=4)	50 mm No. of larvae	%	100 mm No. of larvae	%
15 days	0 x 4	0	0	0	0
	10 x 4	2	5	1	2.5
	20 x 4	1	1.25	1	1.25
	40 x 4	1	1.25	0	0
	Total = 280	4	1.43	2	0.71
30 days	0 x 4	0	0	0	0
	10 x 4	1	2.5	0	0
	20 x 4	2	2.5	0	0
	40 x 4	3	1.87	0	0
	Total = 280	6	2.14	0	0
45 days	0 x 4	0	0	0	0
	10 x 4	1	2.5	0	0
	20 x 4	1	1.25	0	0
	40 x 4	10	6.25	0	0
	Total = 280	12	4.29	0	0
Overall average			2.62	0.67	0.24

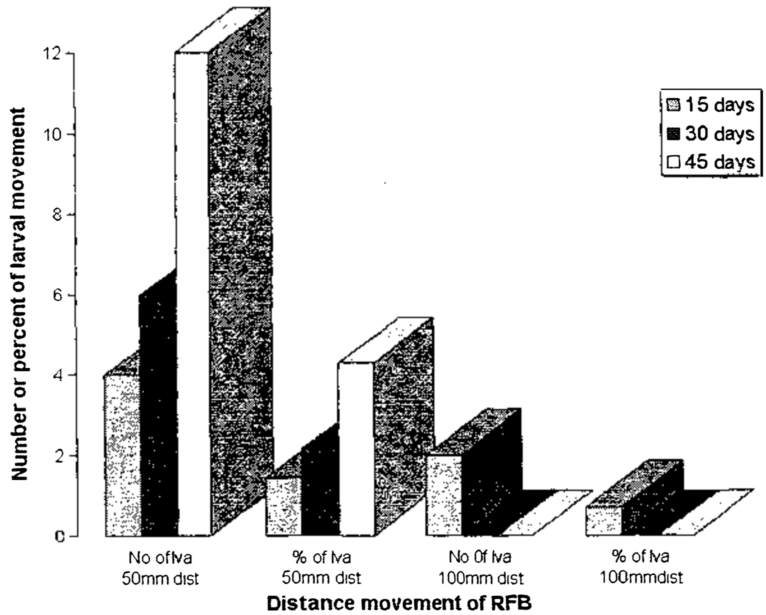


Figure 7.1 Number and percentage of *L. jacobaeae* larval movement from centre plant to the plants around the center plant which were 50mm and 100 mm far from center plant between three larval durations.

Although larval durations did not have a significant effect on larval inter-plant movement, it is clear that the older larvae could leave dead ragwort plants and move into the nearest plants (Tables 7.1, 7.2). The percentage of old larvae that moved was much higher than that of younger larvae but despite this no 30-day or 45-day old larvae moved 100 mm whereas 0.71% of 15-day old larvae did move 100 mm (Table 7.2).

7.5 Discussion

These results show that *L. jacobaeae* larvae can move to other ragwort plants when their original host plant dies, but few larvae do move and these only move short distances of generally less than 100 mm. Two other insect larvae that feed within roots are known to move between host plants in the field. Larvae of the western corn rootworm, (*Diabrotica virgifera* LeConte: Chrysomelidae) can migrate 254 mm to 1016 mm in soil (Suttle, *et al.,* 1967; Short & Luedtke, 1970). In this case all ages of larvae are equally able to move through soil (Suttle, *et al.,* 1967; Short & Luedtke, 1970). Larvae of bean leaf beetle, *Cerotoma trifurcata* Foster (Coleoptera: Chrysomelidae) also are reported to move 305 mm through soil between host plant roots (McConnell, 1915; Anderson & Waldbauer, 1977; Levinson, Waldbauer, & Kogan, 1979; Marrone & Stinner, 1983). Apart from the fact that such larvae can leave plant roots and move to the roots of adjacent plants nothing is known about the sensory perceptions, if any, that might be involved and how they might locate new hosts. I have shown that larvae of *L. jacobaeae* will leave the roots of ragwort plants after they have been killed but this has been observed before by Windig (1991). We still do not what causes *L. jacobaeae* larvae to leave such plants or even if they will leave healthy roots. Certainly other insect larvae are known to move through soil to feed on the roots. Examples are the root aphid *Pemphigus trehernei* Foster (Hemiptera: Aphidae) which lives in saltmarshes and sucks plant sap from the roots of the sea aster (Foster, 1975) and larvae of the grass grub *Costelytra zealandica* White (Coleoptera: Scarabaeidae) which feed on grass roots (Sutherland & Hillier, 1974). Both are external feeders and little is known about how they locate new roots.

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Chapter Eight: Larval Survival

The relationship between larval survival and larval and plant densities in the field.

8.1 Abstract

Survival of *L. jacobaeae* was highest (12.25%) at a mean soil water content of 12% ± 0.29 (S.E.) to 76% ± 1.81. Ragwort plant weight was highest (3.95 g/day) at a mean soil water content of 36% ± 1.1 to 82% ± 0.99. Larval density (0, 10, 20, 40 per plant), plant density (1, 2, 4, 8 plants/(0.5 m)^2) and feeding period (0-15, 16-30, 31-45 days) also significantly affected ragwort growth. Survival of *L. jacobaeae* was highest at a density of 10 larvae per plant (18.5%), and at plant densities of 8 per (0.5 m)^2, 0-15 days after introduction to the plants (10.76%). An age-specific life table for *L. jacobaeae* was constructed that gives survival rates of different life history stages of *L. jacobaeae*.

8.2 Introduction

The relationship between ragwort (*Senecio jacobaea* L.) and cinnabar moth (*Tyria jacobaeae* L., Lepidoptera: Arctiidae) has been studied intensively. Indications are that the population density of *T. jacobaeae* is food limited (Cameron, 1935; Crawley & Gillman, 1989; Dempster, 1971; 1982; van der Meijden, 1979; van der Meijden & van der Waals-Kooi, 1979; Myers, 1978). However, what effect *T. jacobaeae* has on the population density of ragwort plants is not clear according to these studies. A second biological control agent of ragwort, the ragwort flea beetle (*Longitarsus jacobaeae* Waterhouse) was also introduced to the Netherlands (Windig, 1991), USA (Hawkes, 1968; Hawkes & Johnson, 1978, McEvoy *et al.*, 1991), Australia (Cullen & Moore, 1980), Canada (Harris *et al.,* 1984), and New Zealand (Syrett, 1983). The adults of ragwort flea beetle feed on the leaves of ragwort and cause a characteristic pattern of holes (Frick, 1970) but Windig (1993) showed that this does not affect the mortality of ragwort plants. In contrast, the larvae of *L. jacobaeae* feed inside the roots or root crowns and can affect ragwort mortality (Windig, 1991, 1993; Hawkes & Johnson, 1978; and McEvoy *et al.*, 1989). A third biological control agent, the ragwort seed fly (*Botanophila seneciella* Meade (Diptera: Anthomyiidae)) is also present in New Zealand but its effect on ragwort plants is negligible here (Dymock, 1985).

The effects of temperature and relative humidity (van der Meijden & van der Waals-Kooi (1979); Baker-Kratz & Maguire (1984); McEvoy, 1984) and rainfall (Poole & Cairns, 1940; Dempster & Lakhani, 1979; Crawley & Gillman, 1989) on the population fluctuations of ragwort are known but there are no reports of how soil water content may affect the numbers of either ragwort plants or *L. jacobaeae.*

The life cycle of *L. jacobaeae* was studied in Australia (Cullen & Moore, 1980), New Zealand (Syrett, 1986), and in the Netherlands (Windig, 1991), but there is no published age-specific life table for *L. jacobaeae*. The aim of this chapter was therefore to determine

(i) the effects of soil water on populations of *L. jacobaeae* and ragwort and

(ii) the effects of larval density and ragwort density on the mortality of larvae and on the weight losses of ragwort and

(iii) to construct an age-specific-life table.

8.3 Materials and Methods

This study was conducted in a 0.5 ha area situated near the north side of Ecology building, Massey University. Relative humidity was measured 2.5 cm above the ground with a "Tinytalk2" Relative humidity data logger (range 0-95%) and the temperature was measured at the same place with a "Hobo" data logger (range -5 to 37°C) (Appendix 8.1). The area was initially free of ragwort plants and ragwort flea beetle, cinnabar moths and other organisms that feed on ragwort.

The effect of three larval feeding durations (15 days, 30 days, and 45 days) on the weight loss of ragwort was investigated by partitioning the study area into three 64 m^2 plots and dividing each of these into two 3.25 m x 3.25 m plots (details of the layout are provided in Appendix 8.2). The treatments were allocated at random to 64 $(0.5\ m)^2$ subplots using a 4 x 4 factorial design with 4 replicates. There were four blocks each with four densities of ragwort rosette plants (1, 2, 4, and 8 plants) and each with four densities of larvae per plant (0, 10, 20, and 40 per plant) (Appendix 8.2). The experiment was designed to measure both the effect of ragwort density on the survival of *L. jacobaeae* larvae and ragwort growth (measured as weight change) and the effects of *L. jacobaeae* larval density on ragwort growth and larval survival. In addition, information was obtained on the larval feeding duration inside roots or root crowns of ragwort single rosettes.

8.3.1 Effects of soil water on population of L. jacobaeae *and ragwort*

Soil samples were collected every three days from the study site between 15 May 1998 and 29 June 1998 (Appendix 8.3). On each occasion, one soil sample was collected from each of 10 randomly chosen $(0.5\ m)^2$ sub-plots using a soil corer (85mm in diameter by 140 mm long). The procedure for measuring soil water content in the soil samples is described in Chapter 4.

8.3.2. Effects of L. jacobaeae *larval density and ragwort density on survival of larvae and growth of ragwort*

Ragwort plants were grown at Massey University from seeds collected from Ballantrae, the AgResearch hill country research station, and near Woodville on 15 January 1998. The seeds were mixed with fine sand and a sample of the mixture was then scattered in each of the $(0.5\ m)^2$ sub-plots. The sub-plots were watered daily with a fine spray gun. The sub-plots were weeded and the ragwort plants thinned out on 11 March 1998 to obtain the required number of ragwort plants (1, 2, 4, or 8 plants per $(0.5\ m)^2$) for the experimental design.

First instar larvae of *L. jacobaeae* were reared from 388 adult beetles that were collected from ragwort plant at Ballantrae with a vacuum apparatus on 7 April 1998. The methods used to collect eggs and to rear first instar larvae are given in Chapter 5.

When the plants were about 4 months old (15 May 1998), four densities of < 24 hr old larvae (0, 10, 20, and 40) were collected in small vials by using a '000' Haydn finest brush. The exact number of larvae was transferred to the area between the root crown and stem of ragwort rosettes very carefully and individually using the finest brush as described in chapter 7. They were then left for 15 days, 30 days, or 45 days. Each of these three durations was allocated to different 64 m^2 plots. 4200 larvae were introduced into 240 ragwort rosettes in each of the three 64 m^2 plots.

After 15, 30 or 45 days as appropriate, each ragwort plant was carefully dug up to obtain the complete root system. Each plant was washed with water to remove the soil from its root system, placed on a plastic tray with drainage holes for two hours to dry, then weighed on an electronic balance (Appendix 8.4). The plants were then stored at 4°C in a controlled temperature room and the *L. jacobaeae* larvae were extracted from 10 of them each day because the extraction process took 24 hours at 45 ± 2.0°C as described in Chapter 2.

The entire extraction process took 72 days and the longest any plant was kept in the controlled temperature room was 24 days. After the larvae had been extracted they were counted and the plants were dried in an oven at 60°C for 12 hours, then weighed (Appendix 8.3).

Analysis of variance (4 x 4 factorial design) GLM programme (Appendix 8.5) was done by using SAS/STAT version 6.12. The objectives and hypotheses were described in Chapter 2 (B).

8.3.3 Fertility rate and age -specific life table

8.3.3.1 Fertility rate

Fertility was determined from 194 pairs of *L. jacobaeae* adults collected from Ballantrae. These were kept in 7 groups of 25 pairs and one group of 19 pairs in plastic-rearing cages at 20°C in a temperature controlled cabinet as described in Chapter 2. All eggs laid over 24 days were collected and counted each morning as described in Chapter 2. The eggs were then kept in the same 20°C temperature controlled cabinet and checked each morning 12 days after they were laid (Chapter 2) (Appendix 8.6).

The number of larvae that hatched subsequently over 17 days was recorded (Appendix 8.7).

8.3.3.2 Age - specific life table for L. jacobaeae

An age - specific life table or cohort life table (Table 8) was constructed for ragwort flea beetle according to Seber (1973), Southwood (1978), Zalucki and Kitching (1982), and Kyi, Zalucki, and Timash (1993)

x = age interval (days or week or etc.), of stage,

$\mathbf{N_x}$ = final number within the age mentioned in column x,

$\mathbf{l_x}$ = number alive at beginning of x

$\mathbf{d_x}$ = the number dying within the age interval stated in column x,

$\mathbf{q_x}$ = the proportion dying,

($q_x = d_x / lx$, where $d_x = l_x - l_{x+1}$)

$\mathbf{S_x}$ = survival rate within the age mentioned in column x,

($S_x = 1 - q_x$)

$\mathbf{d_xF}$ = factor responsible for d_x

The fertility rate and survival rates were calculated according to the different durations (15 days, 30 days, and 45 days), the numbers of plants per sub-plot (1, 2, 4, or 8 plant /(0.5 m)^2), and the numbers of larvae inserted into the root zones (0, 10, 20, and 40 larvae per plant).

8.4 Results

8.4.1 Environmental conditions during the study

Temperatures and relative humidity experienced during this study are given in Appendix 8.1. The mean monthly relative humidity ranged from 50.1% to over 95%, and averaged 91.3% during this study (January 1998 to August 1998) and the mean monthly temperature ranged from 5.3°C to 23.2°C.

8.4.2 Effect of soil water on L. jacobaeae *and ragwort populations*

The mean soil water content varied from a minimum of 12 ± 0.29% to a maximum of 82 ± 0.99% during the experiment (Table 8.1) while the survival rate for larval *L. jacobaeae* and weight of ragwort plants fluctuated (Table 8.1, Figures 8.1 & 8.2).

Table 8.1 Relationship between duration of *L. jacobaeae* larvae in ragwort rosettes and soil water (%), larval survival (%), and plant growth (g/day). (Raw data is given in Appendix 8.3 & 8.4).

Duration of larvae in ragwort (days)	Minimum & Maximum % soil water (with standard error)	Mean % soil water (with standard error)	% larval survival	Plant growth (g/day) (time from germination given in brackets)
15	12 ± 0.29 - 76 ± 1.81	47.99 ± 3.17	12.25	1.85 (115 days)
30	36 ± 1.10 - 82 ± 0.99	61.20 ± 2.42	9.98	3.95 (130 days)
45	35 ± 0.76 - 65 ± 1.78	42.20 ± 1.72	11.68	1.51 (145 days)

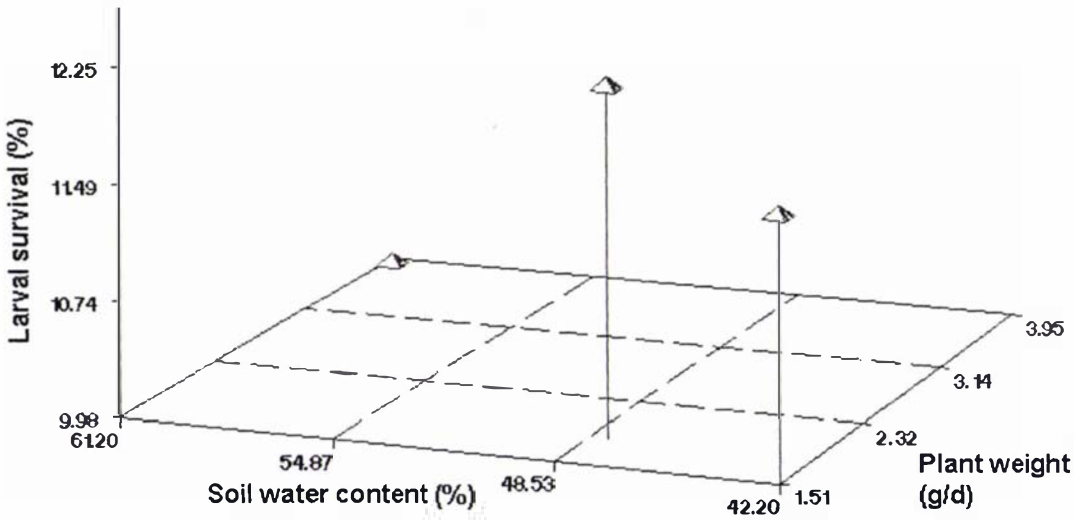

Figure 8.1 The relationship between soil water content (%), the survival of *L. jacobaeae* larvae (%), and mean plant weight (g/day).

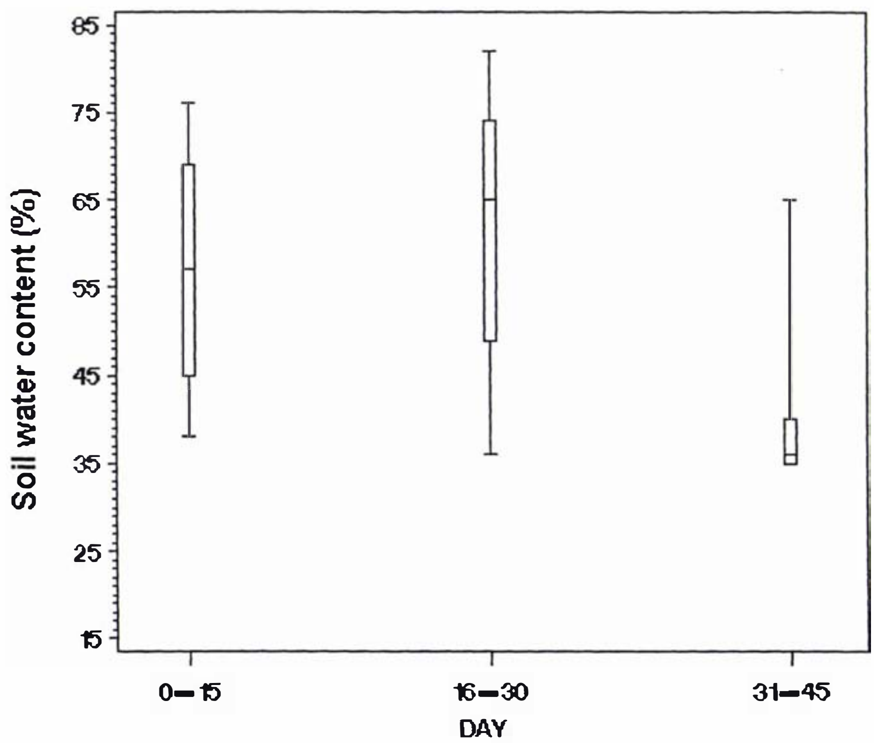

Figure 8.2 Variation in soil water content during the experiment.

8.4.3 Effects of larval density and ragwort density on survival of larval L. jacobaeae

Larval survival of *L. jacobaeae* depended on the density of ragwort rosettes. The highest larval survival (10.76%) occurred during 0 to 15-days when there were 8 plants/(0.5 m)^2 (Table 8.2) and when there were few larvae (10 larvae/plant) (Table 8.3). The lowest larval survival (6.61%) also occurred during 30-day period when there were 2 plants/(0.5 m)^2 (Table 8.3) and when there were high numbers of larvae (40 larvae/plant). The overall survival of larvae was 8.41%, 5.87%, and 6.7 % for 15 days, 30 days, and 45 days in ragwort respectively.

Table 8.2 Survival of *L. jacobaeae* larvae in relation to density of ragwort rosettes and time.

	Density of ragwort (plants/(0.5 m)^2)			
Duration	1	2	4	8
15 days	9.64%	10.00%	9.23%	10.76%
30 days	8.93%	6.61%	7.95%	8.62%
45 days	9.29%	8.21%	8.66%	9.87%

Table 8.3 Survival of *L. jacobaeae* larvae in relation to density of ragwort flea beetle larvae and time.

	Density of larvae (/plants)		
Duration	10	20	40
15 days	17.50%	10.83%	8.41%
30 days	14.33%	9.75%	5.87%
45 days	18.50%	9.83%	6.70%

8.4.4 Effects of L. jacobaeae *larval density and ragwort density on growth of ragwort rosettes*

The numbers of *L. jacobaeae* larvae within ragwort rosettes (0, 10, 20, and 40 larvae/plant), the density of ragwort (1, 2, 4, 8 plants/ (0.5 m)^2), and the period when larvae fed inside of the roots or root crowns (15-days, 30-days, and 45-days duration) all significantly and differently affected the growth of ragwort (*p*=0.0001 each) (Table 8.4). In addition, there were significant relationships between larval density and feeding duration (*p*=0.0026) (Table 8.4), ragwort density and feeding duration, and larval density and ragwort density (*p*=0.0001 each) (Table 8.4). Block effects on plant weight gain were not significant (*p*=0.6573) (Table 8.4).

Table 8.4 ANOVA for the effect of *L. jacobaeae* larval density (0, 10, 20, 40/plant), plant density (1, 2, 4, 8 plant 0.5m^2), and larval feeding duration (15 days, 30 days, and 45 days) on ragwort rosette weight gain (dependent variable).

Source of variations	DF	Sum of Square	Mean square	F Value	Pr > F
RFB larval density	3	261122.74	87040.91	336.80	0.0001
Ragwort density	3	52667.84	17555.95	67.93	0.0001
Feeding duration	2	233104.97	116552.48	451.00	0.0001
Blocks	3	416.40	138.80	0.54	0.6573
Larval density x feeding duration	6	5487.85	914.64	3.54	0.0026
Ragwort density x Feeding duration	6	38287.57	6381.26	24.69	0.0001
Larval density x Ragwort density	9	14157.21	1573.02	6.09	0.0001
Error	158	40832.22	258.43	-	-
Corrected total	190	646076.81	-	-	-

Most weight loss (93.4 g/plant) occurred when 40 larvae were introduced per plant at a plant density of 8 plant/(0.5 m)^2 and left for 15 days (Table 8.5 a and 8.6 a). However, similar weight loss patterns were caused by 10, 20, and 40 larvae/plant at the same plant density (8 plant/(0.5 m)^2) during all three time periods (0-15, 16-30, 31-45 days) (Tables 8.5. a & b, 8.6 a & b, and 8.7 a & b, Figures 8.43 & 8.4). Here, both the fresh and dry weights of ragwort were inversely proportional to the number of larvae within the plant and the density of ragwort plants. In most cases the patterns for fresh and dry weights were very similar although the fresh weights for 10 larvae/plant at densities of 1 plant/(0.5 m)^2 and 12plants/(0.5 m)^2 had slightly lower relative weights than the other treatments in comparison with the dry weights (Figure 8.3 & 8.4).

Table 8.5 (a) Mean fresh ragwort weight (g) with standard error in relation to plant density and *L. jacobaeae* larval density 15 days after larval introduction.

Larvae per plant	Plant per $(0.5m)^2$			
	1	2	4	8
0	212.86 ± 4.91	223.92 ± 0.32	158.35 ± 1.79	147.93 ± 1.42
10	145.95 ± 2.66	168.88 ± 5.5	150.81 ± 1.18	104.03 ± 2.03
20	142.47 ± 4.52	111.50 ± 1.54	119.17 ± 1.3	101.51 ± 2.65
40	103.56 ± 9.82	101.76 ± 3.37	104.94 ± 0.57	93.40 ± 1.51

Table 8.5 (b) Mean dry ragwort weights (g) with standard errors in relation to plant density and *L. jacobaeae* larval density 15 days after larval introduction.

Larvae per plant	Plant per $(0.5m)^2$			
	1	2	4	8
0	28.29 ± 1.31	29.61 ± 0.92	21.14 ± 0.08	20.89 ± 0.52
10	20.09 ± 0.77	25.18 ± 0.94	20.50 ± 0.12	12.60 ± 0.41
20	19.41 ± 0.94	14.20 ± 0.35	14.62 ± 0.19	12.04 ± 0.38
40	12.61 ± 1.44	13.05 ± 0.31	12.03 ± 0.07	10.98 ± 0.17

Table 8.6 (a) Mean fresh ragwort weight (g) with standard error in relation to plant density and larval of *L. jacobaeae* density 30 days after larval introduction.

Larvae per plant	Plant per $(0.5m)^2$			
	1	2	4	8
0	272.04 ± 12.63	267.89 ± 2.58	221.18 ± 3.19	206.68 ± 5.51
10	195.44 ± 3.61	225.48 ± 10.36	155.73 ± 1.96	118.48 ± 7.17
20	147.57 ± 3.02	176.04 ± 7.63	147.88 ± 3.29	115.28 ± 10.5
40	128.85 ± 7.2	165.80 ± 5.29	126.23 ± 3.17	118.14 ± 8.6

Table 8.6 (b) Mean dry ragwort weights (g) with standard errors in relation to plant density and larval of *L. jacobaeae* density 30 days after larval introduction.

Larvae per plant	Plant per $(0.5m)^2$			
	1	2	4	8
0	37.75 ± 2.25	34.82 ± 0.92	29.85 ± 0.68	28.92 ± 0.98
10	27.30 ± 0.62	29.77 ± 0.55	18.76 ± 0.18	16.59 ± 1.11
20	20.18 ± 0.32	22.43 ± 0.54	19.41 ± 0.44	14.61 ± 1.38
40	17.23 ± 1.17	20.93 ± 1.76	15.59 ± 0.50	13.95 ± 1.10

Table 8.7 (a) Mean fresh ragwort weights (g) with standard errors in relation to plant density and larval of *L. jacobaeae* density 45 days after larval introduction.

Larvae per plant	Plant per $(0.5m)^2$			
	1	2	4	8
0	258.53 ± 3.41	268.53 ± 24.04	310.29 ± 3.12	285.36 ± 2.25
10	223.47 ± 4.18	221.52 ± 2.13	266.95 ± 6.24	186.74 ± 1.67
20	197.07 ± 7.49	214.06 ± 3.93	232.01 ± 5.58	177.44 ± 4.18
40	134.90 ± 2.99	201.39 ± 3.78	218.16 ± 4.02	155.55 ± 3.02

Table 8.7 (b) Mean dry ragwort weights (g) with standard errors in relation to plant density and larval of *L. jacobaeae* density 45 days after larval introduction.

Larvae per plant	Plant per $(0.5m)^2$			
	1	2	4	8
0	31.46 ± 0.41	33.98 ± 3.76	39.56 ± 0.45	34.95 ± 0.71
10	30.28 ± 0.46	26.18 ± 0.3	32.04 ± 1.08	28.27 ± 0.86
20	27.09 ± 0.09	23.95 ± 0.99	30.05 ± 0.45	24.74 ± 0.65
40	17.13 ± 0.35	21.79 ± 0.72	26.57 ± 0.46	21.99 ± 1.09

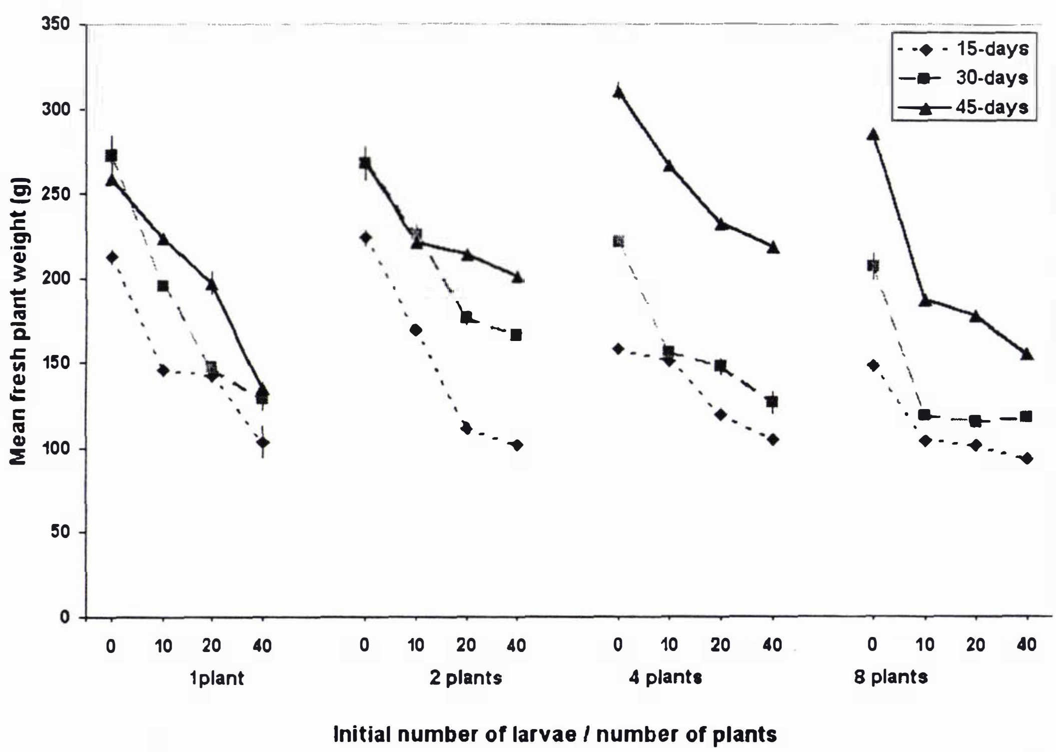

Figure 8.3 **The effects of *L. jacobaeae* larvae on single rosettes of ragwort (fresh weight) in relation to larval density and plant density. Bars indicate ± 1 standard error.**

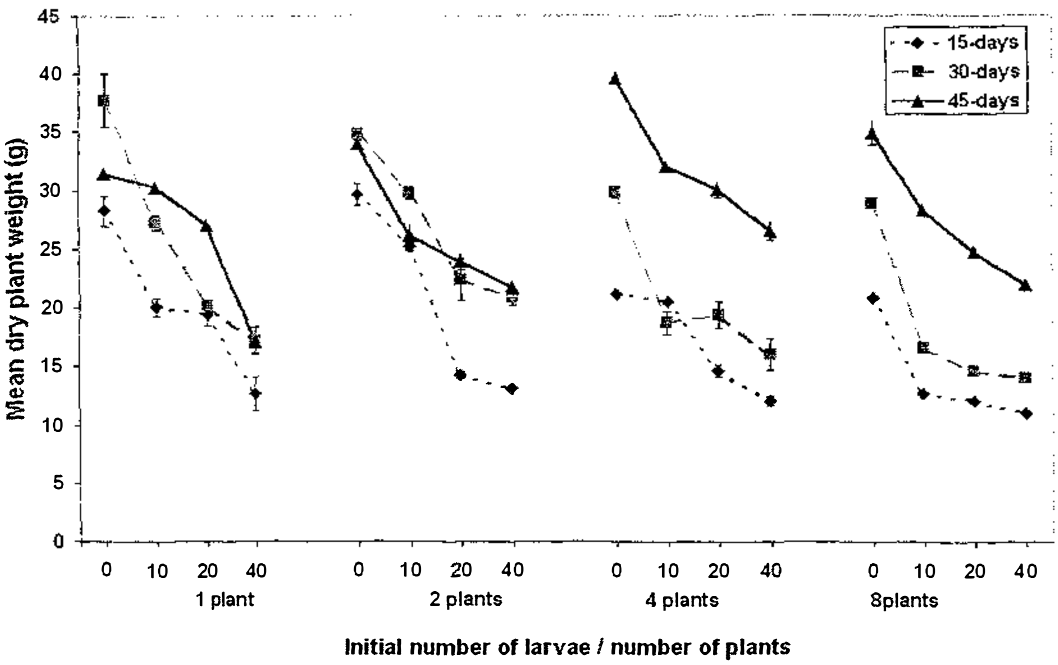

Figure 8. 4 The effects of *L. jacobaeae* larvae on single rosettes of ragwort (dry weight) in relation to larval density and plant density. Bars indicate ± 1 standard error.

Larval density significantly affected the growth of ragwort when the results of four different larval densities were compared (Tukey's studentized range test, Table 8.8.). The greatest different between the means was between no larvae and 40 larvae/plant and the smallest was between 20 larvae and 40 larvae/plant (Table 8.8.). Plant density also significantly affected growth rate of ragwort except at densities of 4 plants/0.5 m^2 and one plant/0.5 m^2 (Table 8.9.). The biggest difference was between 2 plants/0.5 m^2 and 8 plants/0.5 m^2 and smallest between 4 plants/0.5 m^2 and one plant/0.5 m^2 (Table 8.9).

Table 8. 8 Tukey's studentized range test for comparisons of larval densities effect on plant fresh weight (df = 167, MSE = 3329.2781, critical value of studentized range = 3.67, All comparisons are significant at the 0.05 level).

Larvae comparisons	Simultaneous lower confidence limit	Difference between Means	Simultaneous upper confidence limit
0 larvae- 10 larvae	47.82	56.38	64.95
0 larvae- 20 larvae	71.27	79.83	88.40
0 larvae- 40 larvae	90.40	98.97	107.53
10 larvae- 20 larvae	14.93	23.45	31.97
10 larvae- 40 larvae	34.07	42.59	51.11
20 larvae- 40 larvae	10.62	19.14	27.66

Table 8.9 Tukey's studentized range test for comparisons of ragwort densities effect on plant fresh weight (alpha = 0.05, confidence = 0.95, df = 167, MSE = 3329.2781, critical value of studentized range =3.67).

Ragwort comparisons	Simultaneous lower confidence limit	Difference between Means	Simultaneous upper confidence limit	Significant at 0.05 level
1 plant - 2 plants	-23.92	-15.40	-6.88	yes
1 plant - 4 plants	-12.08	-3.51	5.05	no
1 plant - 8 plants	20.81	29.33	37.85	yes
2 plants - 4 plants	3.32	11.87	20.45	yes
2 plants - 8 plants	36.20	44.73	53.25	yes
4 plants - 8 plants	24.27	32.84	41.40	yes

All three periods when *L. jacobaeae* larvae were in ragwort rosettes had significant effects on the fresh weight of ragwort rosettes (Table 8.10.). Here the biggest difference was the 0-45 day period and the 0-15day period and the smallest was between the 0-30 day and 0-15 day periods (Table 8.10).

Table 8.10 Tukey's studentized range test for comparisons of feeding duration effect on fresh weight of ragwort (alpha = 0.05, confidence = 0.95, df = 167, MSE = 3329.2781, critical value of studentized range =3.67). All comparisons were significant at the 0.05 level.

Feeding duration comparisons	Simultaneous lower confidence limit	Difference between Means	Simultaneous upper confidence limit
45-days - 30-days	41.58	48.33	55.08
45-days - 15-days	78.37	85.09	91.82
30-days - 45-days	-55.08	-48.33	-41.58

8.4.5 Fertility and age-specific life table

A life table for *L. jacobaeae* is presented in Table 8.13 starting from 194 pairs (388) adults and using a fertility rate of 3.8 eggs / day for each adult female (calculated from Appendix 8.2). The final numbers of individuals in each life history stage are given in column N_x. This shows that the lowest survival rate (0.279) occurred amongst 16- 30 day old larvae whereas both adult beetle mortality (0.083) and egg mortality (0.115) rates were both lowest during days 1 to 24 (Table 8.11). In addition, the mortality rate of newly hatched larvae (< 24 hours old) (0.168) was also low.

Table 8.11 Age specific life table for *L. jacobaeae* at 20°C (see 8.3.3.2 for definitions). (l_x and d_x are calculated from Appendix 8.4 - lva columns, and observed from Appendix 8.6 and 8.7).

x	N_x	l_x	d_x	q_x	S_x	d_xF
Adult- (24 days)	356	388	32	0.083	0.917	- fungi - unknown
Eggs (1-24days)	15136	17109	1973	0.115	0.885	- unhatched - fungi - unknown
larvae -1day	12600	15136	2536	0.168	0.832	- dehydration - unknown
larvae - 15 days	5093	12600	7507	0.596	0.404	- unknown
larvae - 30days	1422	5093	3671	0.721	0.279	- unknown
larvae - 45 days	450	1422	972	0.684	0.316	- unknown - fungi

In this experiment, mortality of 28 adult *L. jacobaeae* beetles was mainly caused by unknown factors. Six beetles were killed by fungi (*Beauveria bassiana*) and egg mortality from day 1 to day 24 was mainly caused by infertility. Pale orange coloured unhatched eggs were considered as infertile. *Beauveria bassiana* also killed eggs. The oviposition rate per female per day was 3.68 ((17109/24days)/194 females) at 20°C.

Mortality of one-day-old larvae was caused by dehydration and unknown factors. Dead larvae with shrunken bodies were considered to be dehydrated. Larval mortality between 0 – 15 days, 16 – 30 days, and 31 – 45 days was caused by unknown factors. Mortality of 16 – 30 days old larvae was higher than mortality rate of 0 – 15 days old larvae and mortality rate of 31 – 45 days old larvae. Mortality of pupae was not studied in this experiment because all inserted larvae were extracted after 45-days.

8.5 Discussion

Previous studies have emphasised the importance of weather (mostly rainfall and temperature) on the interaction between ragwort and its biocontrol agents, but none have mentioned the effect of soil water content. The only mention of soil water content was by van der Meijden *et. al.* (1988) who reported that 92.5% of ragwort seeds can germinate at 15°C when the soil moisture content was 29%. I have shown that soil water content is correlated with survival of *L. jacobaeae* larvae and ragwort rosette weight gain (Table 8.1). Soil water content was measured during my study. I did not investigate any artificial treatments of different soil water contents effect on the survival of *L. jacobaeae* larvae and ragwort plant growth. If someone want to know soil water effect on the survival of *L. jacobaeae* and ragwort plant growth, it is necessary to set up artificial treatments of soil water content (e.g. 40%, 50%, 60%, 70% soil water content), introducing different numbers of larvae (e.g. 10, 20, 40, 80/plant), and different stages of ragwort plant growth in the experiment. What physiological effect soil water content has on ragwort and how this affects *L. jacobaeae* is not known.

Poole & Cairns (1940) reported that ragwort grows well when rainfall exceeds 870 mm/ annum in New Zealand, and Dempster and Lakhani (1979) and Lakhani and Dempster (1981) also reported that at Weeting Heath, England, summer rainfall plays an important role in determining the number of ragwort plants present when the temperature is high in summer. They found that establishment of young ragwort plants depended upon rainfall rather than soil fertility, competition from other pasture plants, and feeding by cinnabar moth larvae. Weather and rabbit disturbance can also influence the growth of ragwort from seed and from perennial rootstocks, and spring rainfall and the timing of ragwort growth in spring are the key factors that cause population of ragwort and cinnabar moths to fluctuateat at Silwood Park, England (Crawley & Gillman, 1989). Cox and McEvoy (1983) found that summer moisture stress has a strong influence on the interactions between ragwort and either *L. jacobaeae* or cinnabar moth, but the variation in precipitation that occurs in western Oregon does not reduce the ability of cinnabar moth and *L. jacobaeae* combined to depress the ragwort population.

Windig (1993) reported that ragwort mortality is much higher when the density of *L. jacobaeae* is high and, when ragwort is dense, significantly more small plants are eaten by the adult beetles. Ragwort plant density and light intensity had no significant influence on the mortality of ragwort, but light intensity does affect *L. jacobaeae* because it is rarely present where ragwort is shaded (Windig, 1993). Interestingly, light intensity plays an important role in determining the distribution of ants, *Lasius alienius* L. (no shaded areas) and *Formica polyctena* Forster (shaded areas), both of which prey on ragwort flea beetle (Windig, 1993).

McEvoy, Cox, and Coombs (1991) found that high numbers of *L. jacobaeae* could cause heavy mortality of small, young ragwort plants and they can cause a sharp decline in successful reproduction by large ragwort plants. Syrett (1986) found that more ragwort plants died in cages when *L. jacobaeae* adults were present, indicating that some ragwort mortality was caused by the damage done by these beetles. She suggested that if *L. jacobaeae* adults can cause plant deaths in the field when beetle densities were high and where ragwort plants are subjected to competition from grass and other plant species, then high larval densities could also kill ragwort. My results clearly indicate that larvae of *L. jacobaeae* in the laboratory (Chapter 5) can kill ragwort rosettes and they also demonstrate that the presence of these larvae can at least reduce the growth rate of ragwort in the field. Interestingly, ragwort lost more weight when second instar larvae (30 days old) were present than when third instars (45 days old) were present.

There is no published information on larval survival of *L. jacobaeae* in the field. I found that larval survival was best between 0 and 15 days after being introduced onto ragwort as newly hatched larvae if there was a high density of ragwort rosettes. Survival was also good in 45 day old larvae when larval numbers were low. In the field, the number of larvae per plant had no effect on the feeding rates of individual larvae, but the number of larvae per plant and the density of ragwort plants clearly affected the growth rate of ragwort.

It is hard to compare the survival rate that I obtained from *L. jacobaeae* eggs with other published data because they were obtained under different conditions. I found that the survival rate from egg to third instar larvae was 9.9% (corrected for the extraction apparatus efficiency of 84%) at 20°C whereas Delpatchitra (1991) reported a survival rate of 46% from egg to third instar larvae. She also found that the oviposition period of *L. jacobaeae* at 15 and 18°C in the laboratory ranged from 24 - 91 days and the oviposition rate was 5.5 and 4.7 eggs per day respectively. I found that the oviposition rate was 3.8 eggs per day at 20 °C over 24 days but I did not determine the total oviposition period. This was well below the maximum daily rate of 9.6 eggs at 20°C reported by Delpachitra (1991). These differences could have arisen from several causes such as where the adults were collected, the way they were fed and the degree of crowding they experienced while they were held in captivity.

Little is known about the causes of egg mortality. Ireson and Teraud (1982) reported in Australia that carabids, staphylinids, arachnids, and acarines are the most common predators of *L. jacobaeae* in summer and early autumn when the bettles are ovipositing and when the eggs are hatching. Windig (1991) recorded in the Netherlands that two common ant species (*L. alienius and F. polyctena*) feed on eggs of *L. jacobaeae.*

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SECTION IV

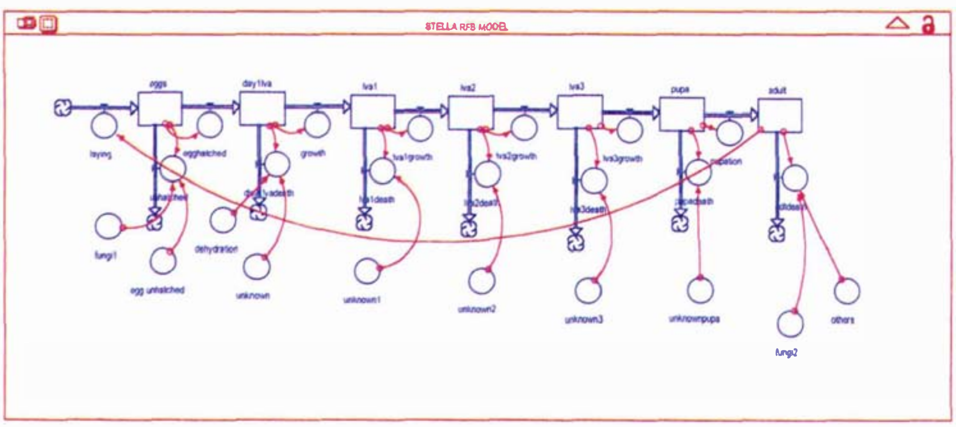
MODELLING *LONGITARSUS JACOBAEAE*

AND RAGWORT

Chapter Nine: Analysis of an insect-plant interaction Model

Chapter Nine: Analysis of an insect-plant interaction Model

(This paper was delivered to the 7th Australasian Conference on Grassland Invertebrate Ecology; 4-6 Oct. 1999, Perth.)



Using STELLA to model Ragwort Flea Beetle and Ragwort.

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9.1 Abstract

The population dynamics of ragwort flea beetle (*Longitarsus jacobaeae* Waterhouse; Coleoptera: Chrysomelidae) and its host plant, ragwort (*Senecio jacobaea* L.), were modelled using Stella Research Software (1996). The usefulness of this modelling approach to pest management is discussed, with the unsurprising conclusion that we need to collect significantly more data in order to make this technique attractive to resource managers.

9.2 Introduction

Engineers have used systems analysis and computer modelling for many years to solve problems in complex physical systems. Their success inspired biologists to use similar techniques in pest management and population ecology. Models can assist the development of pest management techniques. Computer modelling was used to describe the invertebrate functional response (Holling, 1965), to develop component models of movement among units of discrete habitats (Kitching, 1971), to model cowpea aphid population dynamics (Gilbert and Guitierrez, 1973), population growth in grain weevil (Hardman, 1976), movement processes and component analysis (Zalucki and Kitching, 1982), grass grub ecology (Logan, 1984), and so on.

Models can be used to estimate how key factors affect the population dynamics of pests and pest control agents, and to evaluate the effects of key components that are difficult to measure in the field e.g., the consumption rate of root-feeding larvae. Models are also useful for examining interrelationships between pests, their enemies, and natural ecosystem components. They can be used to predict which natural enemies are most effective in pest management programmes and to predict pest damage, economic losses, and variation in natural ecosystems.

Models can be classified into (1) statistical models such as regression models, (2) mechanistic models such as analytical and simulation models, and (3) optimization models which developed as the basis of procedures for finding optimal solutions (Norton, Holt, and Mumford, 1993). There is a fourth type of model, systems models, which we will describe. Systems models provide a way of viewing an interconnected world while allowing us to concentrate on particular subsystems of concern. Such models use a system of stocks, flows, connectors and converters and operate by assigning the appropriate values to each part of the model. We originally wanted to use this modelling approach to give us an overview of the insect-plant interaction involving *L. jacobaeae* (RFB ragwort flea beetle) and ragwort, using our own data on this beetle compiled with (mostly) data captured from previously published studies on ragwort. Our approach was to first construct separate models for population changes of ragwort flea beetle and ragwort, and then to combine them.

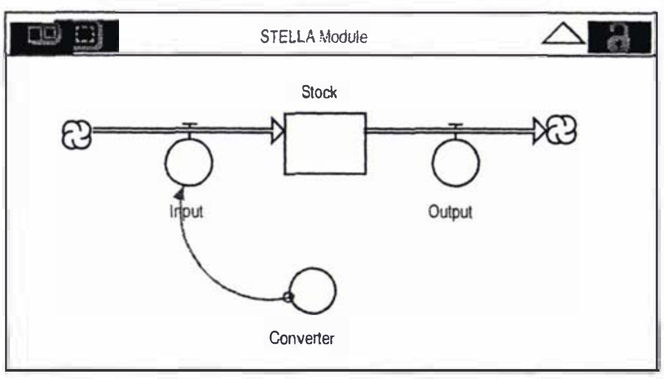


Figure 9.1 The elements of a STELLA module.

Ragwort flea beetle model

The model of the population dynamics of *L. jacobaeae* included the life cycle of *L. jacobaeae* and factors affecting the increase or decrease in numbers of each developmental stage. The model was constructed to estimate population fluctuations of *L. jacobaeae* for up to 13 years. Stages used in the life cycle of *L. jacobaeae* are the adult (male and females); eggs; newly eclosed 1st instar larvae; 1st, 2nd and 3rd instar larvae; and the combined prepupal and pupal period (Figure 9.2). Data from a cohort-life table analysis from "chapter 8" were incorporated into the model. We used data on pupal mortality from Windig (1991).

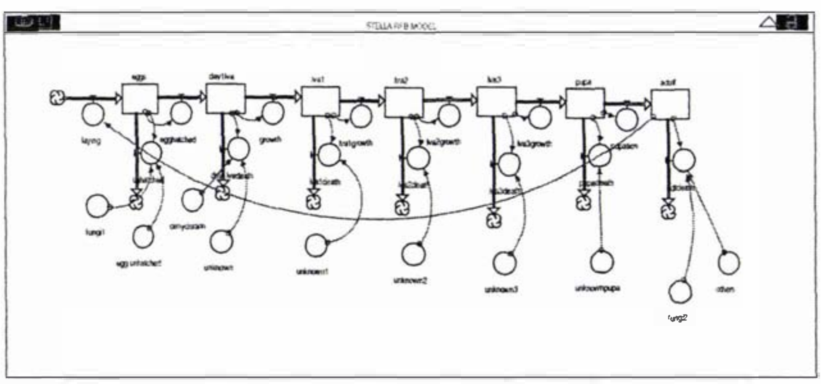
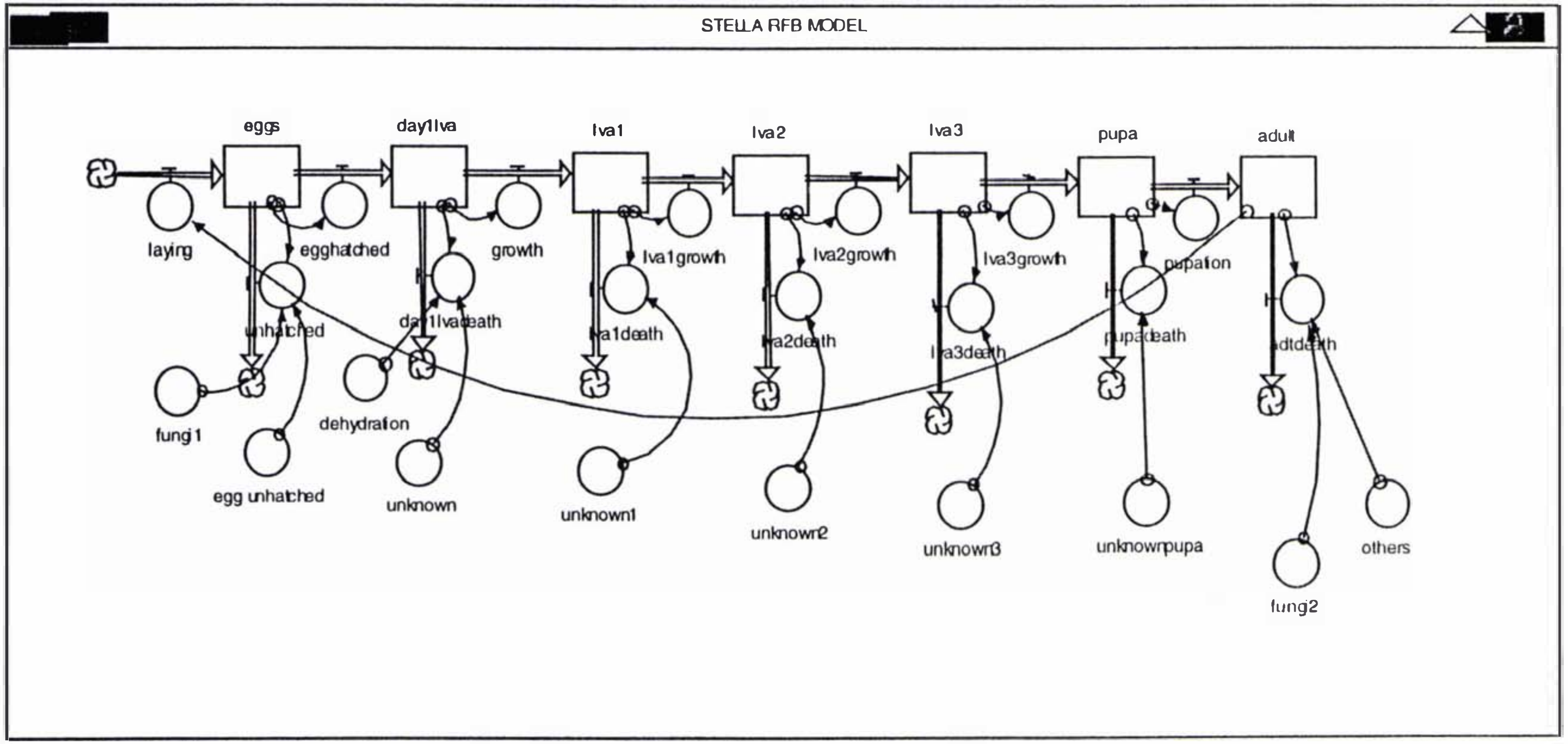


Figure 9.2 STELLA-RFB Model

Stocks, flows, converters, and connectors were used to construct the model (Figure9. 1). Stocks, represented by rectangles, are all of the above 7 life cycle stages. Flows are depicted by a thick line. All 7 stocks had inputs (increments at each stage) and outputs (decreases at each stage). There were 10 converters that converted inputs into outputs (Figure 9.2, Appendix 9.1 (formula)). Connectors linked stocks to converters, and converters to other converters. Arrows depicted the causal linkages between the 7 stages of *L. jacobaeae*, inputs and outputs, and converters. Population changes can be estimated by changing the value of the initial population of any stage of *L. jacobaeae*.



Enlargement of Figure 9.2 STELLA – RFB Model

Eggs laid by a female beetle during her lifetime are the first stock; while the adult stage is the last. The latter was assumed to be 50% male and 50% female. Eggs laid by live females at the start of the next generation are connected by a feedback loop between adult females and eggs.

The egg laying period was estimated to be 220 days (Syrett, 1986) for the data used to build the model.

Ragwort model

Our ragwort model incorporates the different growth stages of ragwort (Figure 9.3). It was built in a similar fashion to the RFB model using the data of Harper and Woods (1957), McEvoy (1984), McEvoy and Rudd (1993), and Thompson (1985). The rosette stage was divided into single and multiple rosettes and the single rosette stage was divided into 1st year rosettes (rosette1) and 2nd year rosettes (rosette2). The multiple rosette stage similarly is divided into plants that became multiple rosettes in the 2nd year (multi rosette 1) and in the 3rd year (multi rosette 2). Flowering ragwort was labelled as flowering-1, flowering-2, and flowering-3 depending on the year it flowered (Figure 9.3).

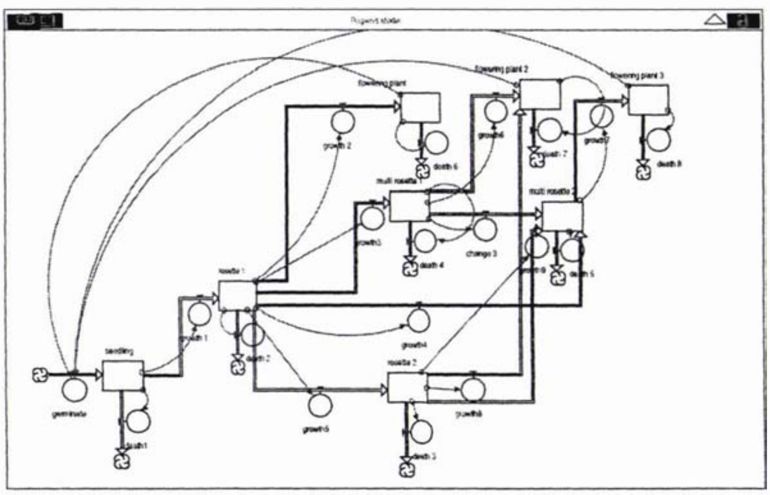
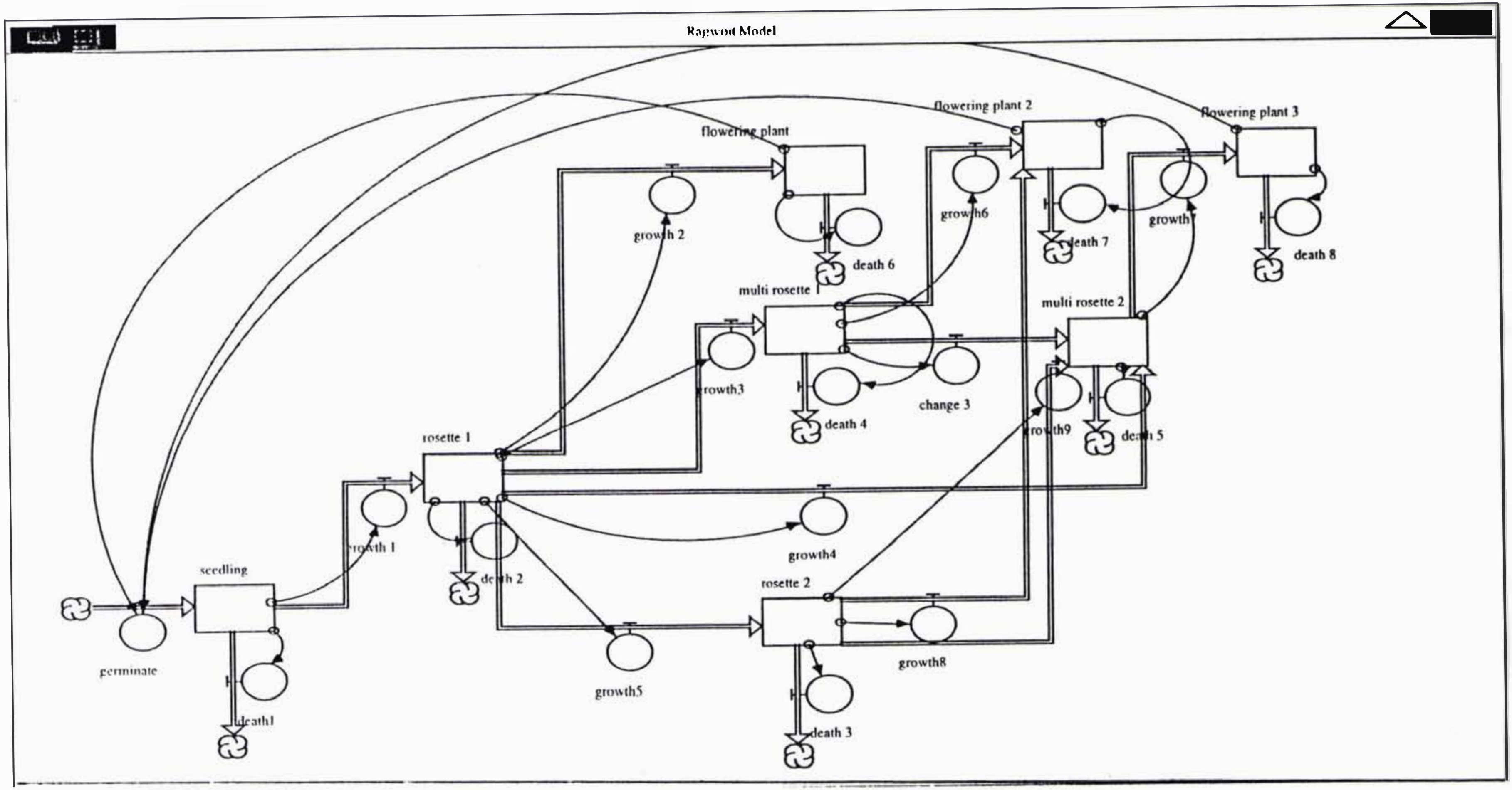


Figure 9.3 The RAGWORT Model

The first stock was seedlings and the end stocks were plants that flowered in the 1st, 2nd and 3rd years. The flowering stages produced seeds and some seeds germinated to



Enlargement of Figure 9.3 The RAGWORT Model

become seedlings. Three feedback loops were connected from flowering1, flowering2, and flowering3 to the germination of seedlings (Figure 9.3, Appendix 9.2 (formula)).

Combining ragwort flea beetle and ragwort models

This model (Figure 9.4, Appendix 9.3 (formula)) was constructed to assess the interrelationship between *L. jacobaeae* and its food, ragwort. The model was divided into two parts: (1) *L. jacobaeae* population dynamics and (2) different stages of ragwort. The RFB and ragwort models were connected by 6 converters; these were total number of larval instars 1, 2, and 3; and the daily feeding rate of each of these larvae. This accounts for larval survival and ragwort mortality and the data were obtained from Kyi's Ph.D. laboratory feeding experiment. The model takes into account the different effects that feeding by different larval instars has on different stages of ragwort. This combined model is based only on larval feeding which is the only density dependent factor in the model. The model was constructed to estimate population changes of ragwort flea beetle and ragwort for up to 13 years.

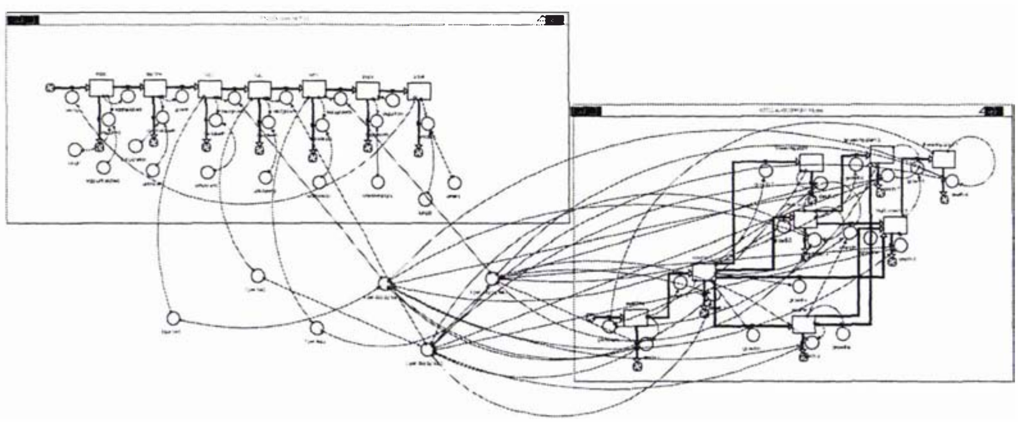
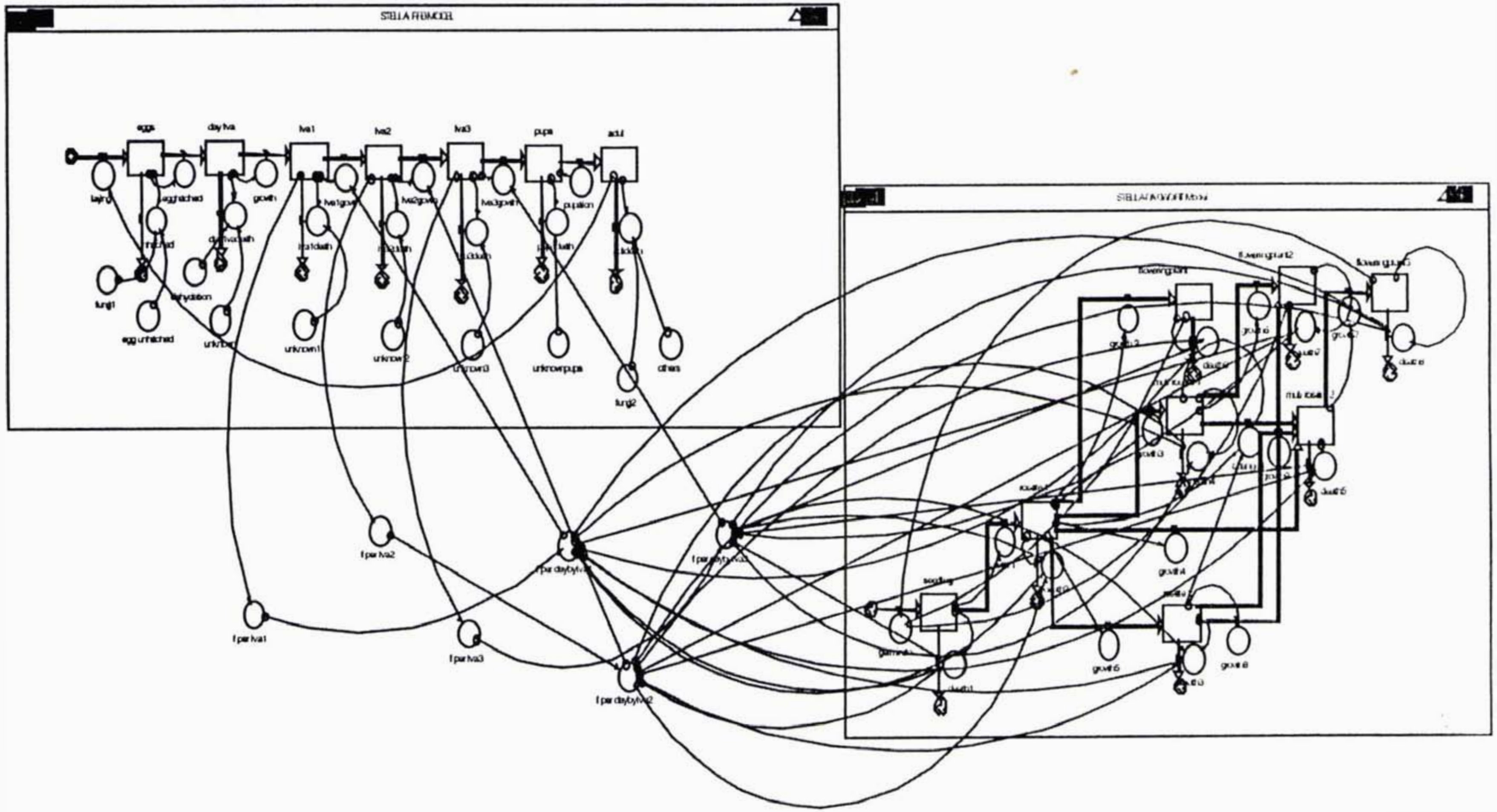


Figure 9.4 Interrelationship between RFB and ragwort, STELLA-RFB-RAGWORT Model.



Enlargement of Figure 9.4 Interrelationship between RFB and ragwort, STELLA – RFB - RAGWORT Model.

9.3 Results

Ragwort flea beetle model

The numbers of each stage of *L. jacobaeae* for each year are given in Figure 9.5. This shows that 17109 eggs are laid by 194 female *L. jacobaeae* (assuming a 50:50 sex ratio) during 24 days in the first year. A female *L. jacobaeae* can lay 808 eggs ((17109/24)/194 x 220) during her lifetime. These in turn would develop into 683 newly eclosed larvae, 568 1st instar larvae, 229 2nd instar larvae, 64 full grown larvae, 20 pupae, and 10 male and female adults during the first year (Figure 9.5). The RFB model can estimate population changes during 13 years. First instar, 2nd instar, and 3rd instar larvae populations increased from 568, 229, and 64 to 97842, 23781, and 3504 respectively within 13 years. This represents increases of 172 (97842/568), 104 (23781/229), and 55 (3504/64), respectively, during the 13 year period. At the end of year 13, the population of *L. jacobaeae* adults will become 882 (Figure 9.5). If 1000 fertilised female *L. jacobaeae* are released then there will be 882000 male and female adults at the end of 13 years. These types of population increment (especially larvae and adults) may be enough for a successful biological control programme of ragwort, but this population model treats only one component, ragwort flea beetle.

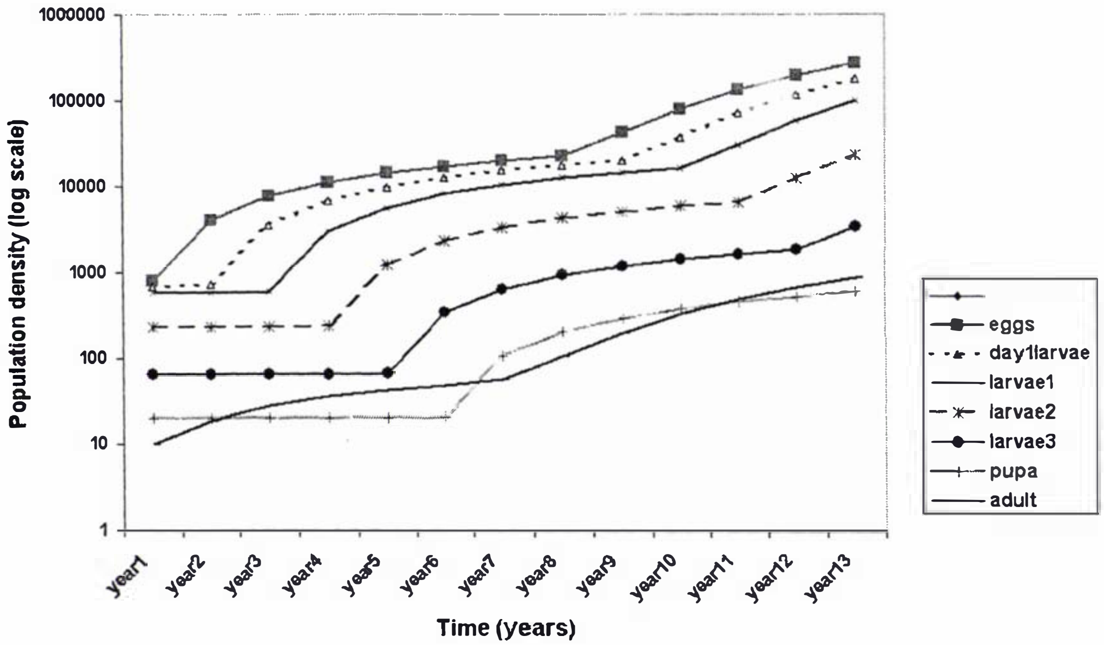


Figure 9.5 Population fluctuations of different stages of *L. jacobaeae* from 1 to 13 years from the output of the STELLA-RFB Model. The numbers of each stage are shown for each year.

Ragwort model

A starting cohort of 147 seedlings produced 76 single small rosette plants (Rosette1), and plants with 26 rosettes (Rosette 2), 6 multi rosettes (Multi rosette 1), 18 big multi rosettes (Multi rosette 2), as well as 12 flowering plants after one year and 11, and 13 plants that will flower after 2 and 3 years respectively. No density dependent factors were included in the model so it was not surprising that the population increased to an unrealistically large size by year 13 (Figure 9.6). The inclusion of density dependent factors such as recruitment from seedlings to rosettes would presumably alter this by causing the population to fluctuate, although the precise nature of those fluctuations would depend on the type and values of the density dependent parameters.

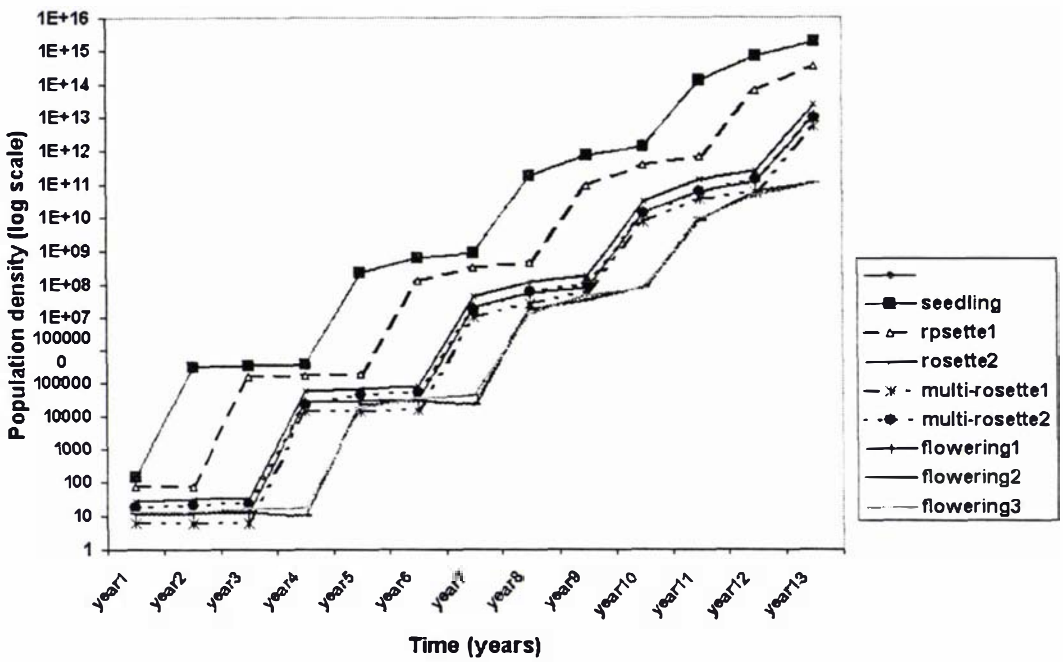


Figure 9.6 Output of STELLA-RAGWORT Model for population changes of different stages of ragwort during 13 years. The numbers of each stage are shown for each year.

Combined ragwort flea beetle and ragwort model

The example of the combined model was started with 10 adult *L. jacobaeae* (5 males and 5 females) and 147 ragwort seedlings. Both populations declined to extinction. At the end of year 2, no ragwort was left, but 1034 3[rd] instar larvae, 352 pupae and 19 adult beetles were still alive. All were dead at the end of year 3.

9.4 Discussion

Our combined model represents a first attempt at simulating the interactions between *L. jacobaeae* and ragwort. Population changes in *L. jacobaeae* have never been modelled before but Thompson (1985) has published a model for ragwort populations. Our model incorporates new data on ragwort (McEvoy and Rudd, 1993) and takes into account the different stages of ragwort growth and differences in the number of years that ragwort can flower. Despite these changes, our model produced similar results to that ragwort can flower. Despite these changes, our model produced similar results to that of Thompson (1985). Both models, however, suffer from a lack of density dependent factors but such data is not yet available. Our combined model therefore assumes that the ragwort flea beetle population would maintain the ragwort population below the level at which any density dependence might operate. This may be a reasonable assumption when ragwort crowding is low, but it is unlikely to be true when ragwort is at a level where it is a problem for land managers. Moreover, it tells us nothing about the influence that other factors such as environmental conditions or other organisms have on the population changes of *L. jacobaeae* and ragwort. It would be interesting to know, for example, how the effectiveness of both *L. jacobaeae* and other biological control agents introduced to control ragwort in New Zealand such as ragwort seedfly (*Pegohylemyia jacobaeae and P. seneciella*; Diptera: Anthomyiidae) and cinnabar moth (*Tyria jacobaeae*; Lepidoptera: Arctiidae) may change when they are present together. Incorporating environmental factors that affect the growth rates of both ragwort and *L. jacobaeae* into the model could also make the model applicable to different regions. Such a complete model could then be of use to resource managers for estimating the effectiveness of *L. jacobaeae* for managing ragwort.

We consider that our combined *L. jacobaeae*-ragwort model represents a starting point from which to explore population fluctuations of *L. jacobaeae* and ragwort for up to 13 years. It is a potential means of estimating the number of *L. jacobaeae* adults needed to control a known density of ragwort within a specified period. The latter use may prove to be its most useful feature, as we currently have no way to make these estimates other than from experience.

9.5 Acknowledgements

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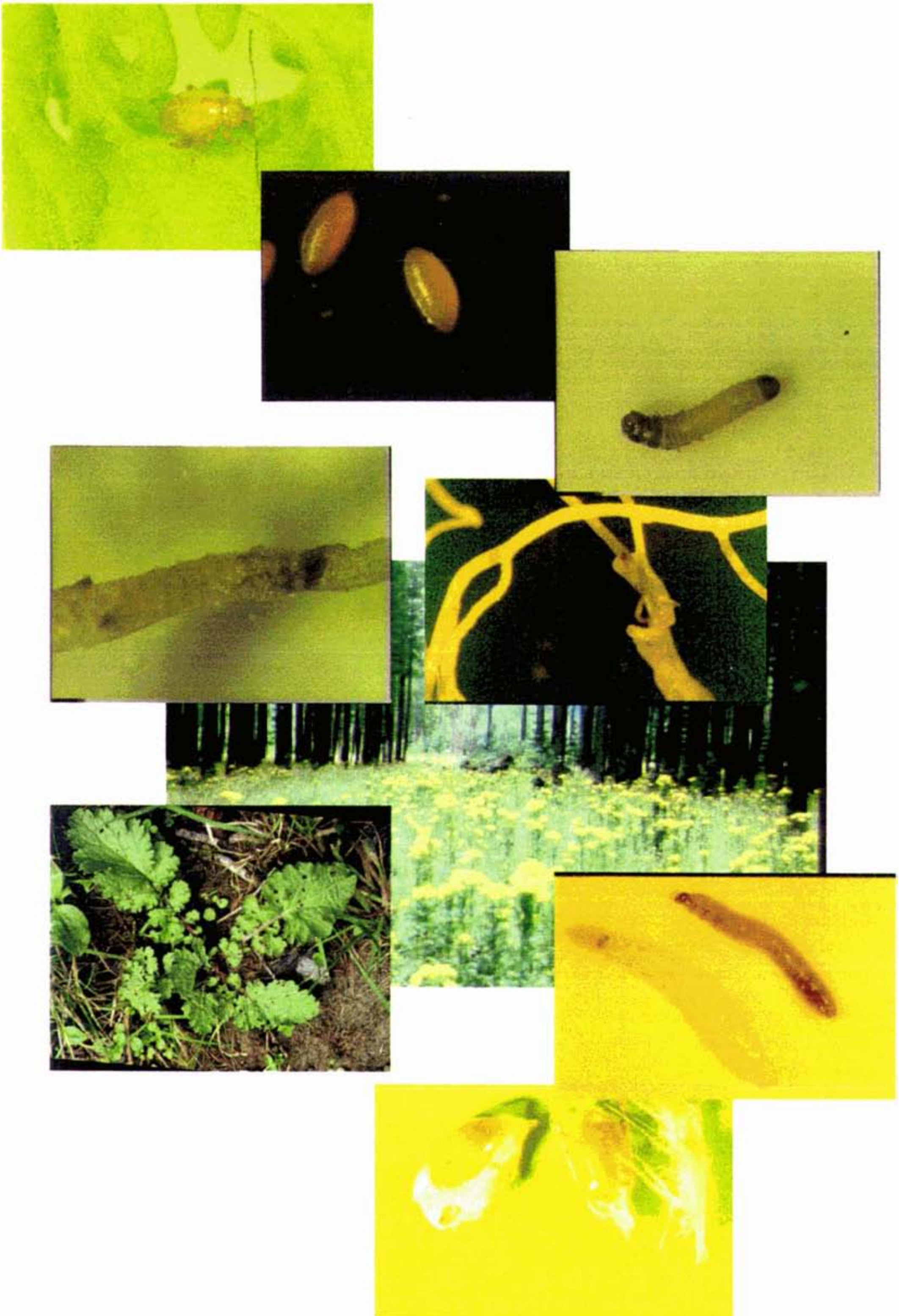
SECTION V

GENERAL DISCUSSION

Chapter Ten: General Discussion

Chapter Ten: General discussion



General Discussion

Ragwort is a noxious weed and ragwort flea beetle *Longitarsus jacobaeae* is a very effective biological control agent for ragwort (Schmidl, 1972; Isaacson, 1975; Syrett *et al.,* 1984; Syrett, 1986 Friend, 1987; Wardle, 1987; McEvoy, Cox, & Coombs, 1991). McEvoy *et al.* (1991) found in western Oregon in a 12 year survey of 42 ragwort populations, that the plants declined up to 99.9% and this was caused by the effectiveness of *L. jacobaeae* at different sites and different times. *L. jacobaeae* is the key factor which regulates population abundance of ragwort (McEvoy & Rudd, 1993). *Longitarsus jacobaeae* is well established throughout New Zealand and is being actively redistributed to control ragwort populations in various regions (Syrett *et al.,* 1991; Hayes, 1994; Harman *et al.,* 1996). Apart from the ways in which *L. jacobaeae* is spread by human intervention, what is it that makes *L. jacobaeae* such an effective biocontrol agent in New Zealand? *L. jacobaeae* is now a global biocontrol agent in temperate regions wherever ragwort is a weed. Studying the feeding ecology of *L. jacobaeae* has a major drawback which is that while some larvae occur in the root crown of the plant, most feeding activity is concentrated underground within the roots. An efficient method of extracting larvae from roots was an interesting and essential first step in this study.

The **MK I** extracting apparatus that was developed was 84% efficient for extraction of *L. jacobaeae* larvae from roots in soil. The efficiency may be a little reduced when extracting *L. jacobaeae* larvae from ragwort rosettes with soil. The highest percentage of movement out of the sample was found 15 hours after the beginning of the extraction process. MacFadyen (1962) extracted soil organisms by using a funnel-type water-cooled extractor. Hadley (1971) tested extraction of *Molophilus ater* Meigen (Diptera: Tipulidae) from soil by heating soil cores to a temperature of 46°C after 195 min. Peak extracted numbers occurred between 75 and 120 min after the start of the extraction process. Usher and Booth (1984) extracted soil micro-arthropods from soil at 35°C (for the longer extraction periods) or at 40°C (for shorter time of extraction). Pande and Berthet (1973) showed that the gradient extractors are likely to be reasonably efficient for small arthropods. The extracting apparatus used in this experiment was basically constructed to develop a temperature gradient and can be used successfully to extract *L. jacobaeae* larvae from the roots or root crowns of ragwort plants. If an extractor is performing inefficiently, only the most quickly moving organisms with various stages will be found in the extract (MacFadyen, 1962). My extracting apparatus can extract *L. jacobaeae* larvae and other soil organisms including rapidly moving or slowly moving organisms so that it is useful for extracting root-feeding insects and other soil organisms.

Temperature gradient and extraction duration are necessary factors to consider when improving apparatus efficiency. The construction of the original apparatus was constrained by lack of funding. A **MK II** extraction apparatus would include the following: The construction of the apparatus must be modified to enable it to be operated by one researcher; pump system for cold water circulation; much smaller sieve size (for example 0.5-μ mm) for collecting containers. Modifications to the heating system could enable more precise temperature gradients to be developed in the samples. Nevertheless the **MK I** extraction apparatus was efficient enough to give the researcher confidence in the accuracy of the numbers of larvae extracted.

Population fluctuations of *L. jacobaeae* vary according to the ages and stages of ragwort plant, study areas, and seasonality (Cullen & Moore, 1980; Harman & Syrett, 1989). It is possible that the population of *L. jacobaeae* at Ballantrae is somewhat delayed in adult emergence compared with Turakina, because the former is higher (300 m above sea level) than the latter (at sea level).

Herbivores can effect a sharp decline in the various stages of host plants (McEvoy *et al.*, 1991). *Longitarsus jacobaeae* can reduce vegetative ragwort densities by 95%, and reproductive flower production by 39% (James, McEvoy, & Cox, 1992). The rosette stage of ragwort is the most suitable for the adults and larvae of *L. jacobaeae* (Chap. 4-Ballantrae studies). High loads of *L. jacobaeae* larvae cause heavy mortality of the smaller, younger ragwort plants in the population (McEvoy *et al.*, 1991). It is likely that feeding of a number of larvae inside the roots and root crowns of ragwort can kill seedlings and small rosette plant. The killed plants may disappear in the area and cause a food shortage for the *L. jacobaeae*. Flowering plants may be less attractive for the *L. jacobaeae* adults. McEvoy *et al.* (1991) suggested studying whether large plants are more tolerant of *L. jacobaeae* herbivory. Attraction and response of *L. jacobaeae* adult beetles to ragwort and odour sources have been tested (Zhang & McEvoy, 1994,1995, & 1996). However, to fully understand the attractiveness of ragwort for *L. jacobaeae* larvae, it is essential to test the preference of *L. jacobaeae* for different stages of ragwort rather than just adult plants.

When extracting *L. jacobaeae* larvae from soil samples with ragwort, soil mites, earthworms, soil nematodes, and other soil organisms (e.g. Collembola, soil aphids, ants, Staphylinids, millipedes) were also extracted. The relationships between ragwort, *L. jacobaeae* and soil organisms are not known. It is necessary to study in more detail how ragwort, *L. jacobaeae*, and other soil organisms relate to each other to better understand how soil organisms affect population fluctuations of ragwort and *L. jacobaeae*.

Dempster (1971,1982) investigated the interaction between cinnabar moth, *Tyria jacobaeae* and ragwort in their native environments on dry soils in eastern England. Subsequent studies have investigated the interaction between cinnabar moth and its host plant in different environments in Canada (Harris *et al.* 1978) and on sand dunes in the Netherlands (Meijden & Waals-Kooi, 1979). McEvoy *et al.* (1991) studied the interaction between ragwort and its natural enemies (cinnabar moth, ragwort flea beetle, and ragwort seed fly) in abandoned pasture on the central Oregon coast in USA. Ireson *et al.* (1991) studied the effectiveness of *L. flavicornis* (Stephens) on its host ragwort in Australia. Ireson *et al.* (2000) reported that *L. flavicornis* is dispersed over all areas known to be infested by ragwort in southern Tasmania, and over about 90% of the infested areas in the north. In New Zealand, the distribution and establishment of cinnabar moth and *L. jacobaeae* have been studied (Syrett *et al.*, 1991). These studies did not emphasize the interaction between *L. jacobaeae* and ragwort nor mention details of extraction methods for *L. jacobaeae* from ragwort plants with soil. Consequently, I developed the new apparatus for extracting *L. jacobaeae* larvae from samples taken at Ballantrae with the aim of understanding the interactions between *L. jacobaeae* and ragwort plants.

There are suggestions that at higher *L. jacobaeae* larval density survival of larvae is reduced, and conversely at lower larval density survival is enhanced. The reasons for this may be that some larvae may die before reaching the roots and root crowns. Perhaps high larval densities can cause some, but not all, roots or parts of the roots to die. Larvae in these roots may therefore die also. I did not investigate how many larvae moved between different roots of the same ragwort plant but I did find that very few larvae moved between plants 10 cm apart. Larval crowding may also cause intraspecific competition for food and space. Regntere *et al.* (1981) found that intraspecific competition among Japanese beetle grubs, *Popillia japonica* Newman, (Coleoptera Scarabaeidae) occurred which caused higher mortality among smaller larval instars. Northern corn rootworm, *Diabrotica barberi* Smith & Lawrence is very sensitive to both intraspecific and interspecific competition (Woodson, 1994). Newly hatched larvae may compete outside the plant for entering into the roots or root crowns to get food and space. Second and third instars may also compete in the same manner.

The effect of plant density on the survival of larvae was a little different between the experimental plots and Ballantrae studies. The experimental plots showed that when rosette populations were high (8plants/m^2) *L. jacobaeae* larvae population was small. At Ballantrae, where rosette plant density was sometimes 17 plants/ m^2 (Appendix. 4.4), the larval population increased perhaps because they can get enough food from high population density of rosette. Larval feeding probably acted to decrease the rosette population at Ballantrae after which the larval population decreased because of shortage of host plants.

Some optimal foraging models (Rapport, 1971; Pulliam, 1974) are useful for exploring the relationship between feeding behaviour of organisms and food resources, but usually do not consider the effect of these factors on population growth. Rapport and Turner (1975) constructed a theoretical model, which is dependent on feeding rates, on food abundance and quality, and the effectiveness of these factors in influencing population growth. Practical observations on interrelationships between feeding rates and food sources are essential. It is also necessary to examine the density dependent factors or density independent factors affecting the different stages of *L. jacobaeae* and ragwort. It is important to understand survival of different stages of *L. jacobaeae* in relation to the composition of the nutrients in the different life-stages of ragwort.

The average feeding rate of *L. jacobaeae* larvae gradually increased from young larval to older stages on both laboratory and glasshouse experiments. Bigger larvae can eat more food than the younger larvae. In the laboratory experiment, some plants were wilting or nearly dried out in conditions caused by feeding of *L. jacobaeae* larvae. No plants were found wilting or dried out in the glasshouse experiment.

Weight losses of a ragwort plant subjected to feeding of *L. jacobaeae* larvae in the glasshouse experiment were higher than in the laboratory experiment. It is pertinent to ask why ragwort plants in the glasshouse experiment were not wilting or dried out by feeding activities of *L. jacobaeae* larvae? It may be that the nutrients and micronutrients in glasshouse plants were more amply supplied than in the laboratory petri dish-grown plants. The root system of ragwort also shows differences between glasshouse-grown and petri dish-grown plants. The glasshouse-grown plants have much stronger and more adventitious roots than the laboratory petri dish-grown plants. It is likely that the more extensive root system is much more efficient in gaining more nutrients from the soil. Glasshouse-grown plants were always much larger than plants of the same age grown in petri dishes.

Finally, glasshouse-grown plants obtain more sunlight than the laboratory grown plants, thus aiding in photosynthesis.

Larval survival and mean larval weight of *L. jacobaeae* larvae in the laboratory were higher than those in the glasshouse. While factors affecting the survival of larvae and their weight are not precisely known, it is likely that larvae can penetrate and feed more easily in laboratory grown plants. The root system and plant structure may be more succulent and softer in laboratory grown plants than glasshouse grown plants. The more larvae inside the roots the quicker they can kill the plants. The feeding rate of the larvae and their survival can affect the mortality of ragwort plants. Other factors such as softness of roots, penetration of larvae into the roots, movement ability of larvae and physiological changes of larvae are all factors, which affect the feeding rate of *L. jacobaeae* larvae. Higher densities of *L. jacobaeae* were needed to kill glasshouse-grown than laboratory-grown plants. The number of *L. jacobaeae* needed to kill a ragwort plant may be correlated with plant structure, feeding rate of larvae, and larval sizes. Thus, while James, McEvoy, and Cox (1992) found that damage by *L. jacobaeae* could reduce the ability of flowering plants to compensate for defoliation and defloration, the converse may be true, and plant structure and growth may affect the ability of ragwort plants to resist and compensate for attack by *L. jacobaeae* larvae.

Survival of larvae was measured from laboratory and glasshouse experiments. In both experiments, more larvae survived where original numbers introduced were low. The environmental conditions (temperature, humidity) were kept constant in the laboratory and glasshouse experiments and survival of larvae was thus only influenced by larval density and feeding duration.

It is also important to understand the pattern of larval movement when their food becomes scarce as a plant dies. Not surprisingly, the laboratory movement experiment showed that larvae prefer to move towards roots rather than towards cotton wool, perhaps being attracted to roots by root exudates. It was also found that *L. jacobaeae* 1^{st} instars were likely to move downward in soil near the food source or near the emergence areas. Female beetles lay eggs in the soil on the root crowns of ragwort or in the adjacent soil (Frick, 1970; 1971), and this oviposition behaviour therefore facilitates the movement of newly hatched larvae. *L. jacobaeae* eggs were found 40 mm into the soil (Cullen & Moore, 1980), and this places the eggs close to ragwort roots. This oviposition behaviour may therefore help newly hatched larvae because it reduces the distance these larvae have to move to find roots.

The results of the field experiment suggest that only small numbers of larvae move after a plant dies. A small percentage (4.29%) of large larvae at high population densities (40 larvae/plant) in the roots or root crowns move from plant to plant after the death of their host plant. Further, although larvae may exit the dying plant, some die before reaching other plants. At high densities of ragwort, larvae could move from plant to plant, but when the distance between root systems of dead plant and live plant is greater than 10 cm, it may be much more difficult for larvae to move from plant to plant. Moist soil is essential for the movement of *L. jacobaeae* larvae outside the plant.

Lummus *et al.* (1983) found that larvae and pupae of southern corn rootworms, *Diabrotica undecimpunctata howardi* Barber (Coleoptera: Chrysomelidae) could survive best in soil moisture with plant-available water ranging from 70 to 100%.

Soil texture is effective on the survival of larvae and pupae of southern corn rootworms in southern Virginia peanut field (Lummus *et al.*, 1983). In this experiment, the soil texture, rainfall, and evaporation were constant, but larval survival within the plant may be affected by any of these variables.
The highest larval survival was found in plants with a low larval density. This means that larval crowding appears to increase larval mortality, which is a similar result to those from laboratory and glasshouse studies. Environmental conditions (mean temperature, humidity, and soil temperature) fluctuated during the experimental period and the correlation between these gross environmental conditions and larval survival is not statistically significant.
The highest survival of larvae was found on the highest density of ragwort. From these survival data, a cohort life table was constructed and used to calculate population fluctuations of *L. jacobaeae*. Pupal stage information was not included in this life table, but was gleaned from Windig (1991).

Modelling populations of *L. jacobaeae* and ragwort using STELLA models provided information which can be used to determine the optimal times of sequential releases of *L. jacobaeae* adults in a biological control programme. However, the combined model included feeding rates of different stages of larvae so the model was incomplete in that it lacked crucial data on density dependence factors such as predation and parasitism on *L. jacobaeae* and both intra and inter specific competition between all of the species involved. Additionally, because my study concentrated on data collection regarding the larval stage of *L. jacobaeae* certain key data about the dynamics of whole *L. jacobaeae* populations in relation to physical environmental parameters are lacking, but these data would form the basis for ongoing research.

My experimental studies emphasized that the interaction between ragwort and its insect herbivore (*L. jacobaeae*) was mainly dependent on the availability of food (ragwort population) and feeding activities of *L. jacobaeae* larvae. A preliminary study, Ballantrae study, laboratory and glasshouse studies, and field experiments have been carried out during a three year period. According to these studies, density dependent processes (food and feeding) play a major role on the population dynamics of ragwort and *L. jacobaeae*. Without a longer period of study it is difficult to tell whether density dependent processes always govern the population fluctuation of ragwort and *L. jacobaeae*. Long term studies on collection of field population of *L. jacobaeae* larvae and adults and ragwort, population of predators, parasites, and microorganisms (e.g. fungi), weather conditions, and different established areas of ragwort and *L. jacobaeae* are really necessary to estimate the success of a biological control programme. It is also important to measure dispersal and abundance of adult populations in the field to unravel the long term relationship between *L. jacobaeae* and its food, ragwort.

Beauveria bassiania (Balsamo) *Vuillimen* is an entomopathogenic fungus which infects over 100 different species of various order of insects (Ferron, 1977) such as Coleoptera (Gottwald & Tedders, 1982), Lepidoptera (Cheung and Grula, 1982), Diptera (Clark *et al.,* 1968), and Hemiptera (Ramoska, 1984; Latge *et al.,* 1987). More detail about the biology of adult *L. jacobaeae* and the relationship between *L. jacobaeae* and native soil microorganisms (e.g. fungi) may prove important.

For the biological control of ragwort, inundation or mass-release of *L. jacobaeae* adults may cause higher density of larvae hatching in the field with consequent crowding of newly hatched larvae that could increase mortality of *L. jacobaeae* larvae. Releasing of suitable *L. jacobaeae* adult population from time to time (year after year) may be a better method of producing high larval numbers and hence good control of ragwort.

Do annual releases of flea beetles increase the growth rate of flea beetle populations? My results (Chapter 8) suggest that one female beetle may, on average, produce 36 larvae. I assume that only about 10% of these become adults, and half of those are females, then we would expect each female to produce, on average, about two female adults; in other words the population approximately doubles each year. With these assumptions it is clear that releasing similar numbers of beetles year after year would substantially increase the population's growth rate in the first few years. For example, if an initial release comprised 250 beetles and this doubled in the first year, then a second release of 250 beetles would give a total population of 750 beetles, giving an apparent growth rate of 3x. However, a third release of 250 beetles would increase the apparent growth rate from 2x to just 2.33x, and a release in the fourth year would increase the apparent growth to 2.14x. By year five, an unaugmented population would have reached 8,000 beetles, but a population augmented with yearly releases of 250 adults would be almost twice that size (15,750). Thus, after five years, the augmented population would be almost one year ahead of the single-release population.

However, if 90% of the 36 larvae produced by a female survived to become reproducing adults, then the finite rate of population growth would be about 16x. In that case, augmentation with 250 beetles per year would have only a trivial effect on the apparent population growth rate, with the augmented population after five years being only about 6.7% larger than the single-release population. At the other extreme, if the finite rate of population growth was 1, so that the population neither grew nor declined, augmentation would give a population six times greater than the single-release population, which would still comprise just 250 adults.

In conclusion, augmentation has its greatest effect when population growth rates are low, and is probably pointless for populations that are growing rapidly.

Biological control of ragwort must be considered on a global scale by distributing new techniques and knowledge to the farmers, constructing environmentally sound computer models for the biologists, and exchanging new information between countries for sustainable agriculture. It is also necessary to develop strategies to exploit the combinatorial ecology of top-down (herbivore limitation) and bottom-up (resource limitation) forces (McEvoy & Coombs, 1999) for the success of biological control programme in the management of ragwort populations.

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APPENDICES

Appendices for Chapter 3.

3.1 Preliminary survey of *L. jacobaeae*, ragwort and other soil organisms on the different stages of ragwort plant in three study areas.

Pahiatua								
adtRFB	Seedl	Rosett	Flowerin	RFBlva	Mites	E.Worm	Nematod	Others
8	0	4	1	14	32	4	134	0
0	0	2	0	16	6	4	121	5
16	1	4	2	12	60	8	140	3
4	2	1	0	4	48	12	147	6
8	1	3	1	4	20	4	112	0
12	2	4	2	8	16	16	121	9
4	1	2	0	12	0	12	123	0
8	2	4	1	10	0	20	156	3
4	0	2	0	20	12	28	164	0
4	1	2	0	12	0	12	123	7
8	0	4	1	18	20	4	200	0
0	0	2	0	16	6	4	84	0
8	1	4	2	12	60	8	154	3
4	2	1	0	4	47	12	188	21
8	1	3	1	4	21	4	122	0
12	2	4	2	8	16	12	121	2
4	1	2	0	12	0	13	123	0
8	2	4	1	12	0	20	246	16
4	0	2	0	21	12	28	92	0
4	1	2	0	12	0	12	92	5
4	2	4	2	8	16	16	84	6
4	1	2	0	12	0	12	93	0
8	2	4	1	6	0	20	206	3
4	0	2	0	7	21	25	175	0
4	1	2	0	5	0	16	92	8
0	0	2	0	0	4	4	129	10
0	0	1	0	4	5	16	248	15
2	0	2	0	4	42	3	258	0
0	0	1	0	0	60	8	147	21
0	0	1	0	3	22	16	160	9
0	0	1	0	0	4	8	278	24
4	0	2	0	0	0	12	242	6
0	0	1	0	4	65	13	272	4
0	0	0	0	0	25	15	156	7
3	0	2	0	2	18	12	188	0
4	0	2	0	5	17	10	228	0
0	0	1	0	0	61	8	254	21
0	0	1	0	3	75	17	130	11
0	0	1	0	0	12	12	148	19
4	0	2	0	0	45	16	252	7
3	0	2	0	4	16	11	238	0
0	0	1	0	0	15	8	274	21
0	0	1	0	6	22	16	140	9
0	0	1	0	0	12	8	158	18
4	0	2	0	0	0	16	254	6
0	0	2	0	0	11	4	354	15
0	0	1	0	5	1	16	149	9
3	0	2	0	5	0	12	448	0
0	0	1	0	0	5	8	285	21
0	0	1	0	6	12	20	260	9
0	0	0	0	0	8	24	278	33
5	0	1	0	5	7	21	246	0
0	0	1	0	0	10	24	122	3
0	0	0	0	0	12	33	328	12
0	0	1	0	0	4	18	124	0
1	0	1	0	4	12	32	225	9
0	0	0	0	4	27	7	252	0
0	0	2	0	0	54	4	147	15

0	0	1	0	0	0	12	135	3
2	0	1	0	6	24	21	305	11
0	0	0	0	0	36	8	251	4
4	0	1	0	3	0	5	116	0
0	0	1	0	0	12	20	202	3
0	0	0	0	0	13	21	360	15
0	0	1	0	0	11	16	254	0
0	0	0	0	0	12	21	142	11
0	0	1	0	0	4	15	264	0
4	0	1	0	3	12	20	235	9
0	0	0	0	4	47	12	152	0
0	0	2	0	0	0	11	147	9
0	0	1	0	0	46	12	135	0
1	0	1	0	5	36	20	125	9
0	0	0	0	0	25	24	171	5
2	0	1	0	3	52	21	146	0
0	0	1	0	0	27	12	121	5
0	1	1	0	6	21	10	144	26
0	2	1	0	5	36	4	152	13
0	0	0	0	0	11	8	236	11
4	3	2	0	3	10	12	337	31
0	2	1	0	0	4	8	256	0
0	2	2	0	0	8	4	232	15
0	0	1	0	0	12	4	216	8
0	3	1	0	6	0	10	231	0
0	0	0	0	0	0	8	124	0
3	3	2	0	5	0	12	176	6
9	2	1	0	5	12	20	237	3
2	3	1	0	3	20	28	135	6
0	1	1	0	7	0	20	44	16
0	2	1	0	0	36	4	72	15
0	0	0	0	0	38	8	36	0
4	3	2	0	4	0	23	167	11
4	2	1	0	0	4	8	36	0
0	2	2	0	0	40	11	32	14
0	0	1	0	0	52	4	20	18
0	3	1	0	5	63	8	86	0
6	0	0	0	0	35	21	10	20
2	3	2	0	4	27	3	102	21
7	2	1	0	3	3	5	67	3
1	3	1	0	2	5	4	35	22
0	1	1	0	2	0	7	34	16
240	69	150	17	417	1890	1303	17263	790
4.752475	1.366336	2.970297	0.336633	8.257425	37.42574	25.80198	341.8415	15.64356
3.281413	1.011998	1.077782	0.493493	4.958524	19.14933	7.144193	83.22874	8.020806
1.64	0.505	0.538	0.246	2.479	9.574	3.572	41.614	4.01
Ballantrae								
4	0	2	0	19	39	8	245	0
4	0	1	0	20	22	4	121	0
0	0	0	0	18	56	0	247	25
0	0	0	0	11	49	4	62	0
8	1	2	2	16	35	5	112	8
8	2	4	1	18	34	16	121	0
4	0	2	0	14	0	12	123	0
12	4	1	1	8	0	12	111	8
8	1	4	1	12	20	16	156	0
8	2	5	3	16	13	20	54	5
8	2	4	1	12	14	16	120	0
4	0	2	0	7	0	12	67	0
8	4	1	1	7	0	12	124	0
8	1	4	1	16	12	16	46	0
12	2	5	3	16	3	20	154	5
4	0	2	0	18	39	8	94	0
4	0	1	0	17	2	4	81	0
0	0	0	0	13	50	0	145	25
0	0	0	0	11	29	12	60	0
8	1	2	2	14	15	5	221	12
0	0	0	0	4	29	5	152	0

8	1	2	2	4	15	4	112	22
8	2	4	1	6	14	4	121	0
4	0	2	0	2	0	12	123	0
12	4	1	1	3	0	12	105	0
8	0	4	0	8	22	12	103	12
4	0	2	0	11	33	12	123	11
0	0	0	0	12	0	8	256	0
5	2	1	0	10	42	20	243	5
0	0	1	0	6	31	8	131	0
3	0	7	1	12	0	5	125	5
3	1	2	0	7	15	16	132	4
0	0	1	0	10	16	9	200	10
9	1	2	0	12	15	36	287	12
1	1	3	2	8	32	24	253	4
5	0	7	1	13	0	4	125	1
4	1	2	0	7	12	16	132	1
0	0	1	0	10	16	8	225	10
8	1	2	0	8	15	24	278	2
9	1	3	2	15	13	24	273	1
0	0	0	0	12	0	8	256	0
5	2	1	0	10	14	20	173	1
0	0	1	0	10	3	9	232	0
4	0	7	1	16	0	12	200	11
4	1	2	0	8	16	4	132	1
0	0	1	0	6	18	8	201	10
8	1	2	0	12	15	39	321	2
5	1	3	2	5	6	24	173	12
3	0	7	1	9	0	6	225	1
5	1	2	0	12	8	14	132	1
4	0	2	0	6	0	28	112	0
0	0	1	0	11	13	12	123	11
3	0	13	0	8	0	8	85	0
0	0	0	0	10	7	24	166	5
0	0	1	0	10	0	24	122	0
5	0	2	0	9	9	15	129	0
0	0	0	0	10	0	15	127	15
0	0	0	0	9	6	16	261	0
5	0	2	0	13	12	16	148	12
12	0	5	0	14	23	12	135	8
0	0	0	0	10	0	15	267	0
0	0	0	0	11	13	24	261	0
3	0	2	0	12	12	16	242	5
3	0	5	0	16	13	18	235	12
0	0	1	0	6	14	12	212	5
4	0	13	0	10	0	8	125	0
0	0	0	0	15	0	24	176	4
0	0	1	0	7	0	24	122	0
5	0	2	0	16	12	16	142	4
5	0	3	0	14	13	12	135	8
4	0	13	0	12	0	8	125	0
0	0	0	0	0	0	24	196	4
0	0	1	0	11	0	25	212	0
6	0	2	0	6	15	20	229	0
0	0	0	0	14	0	12	187	5
6	0	1	0	16	57	0	278	5
0	0	0	0	15	74	8	462	0
4	0	2	0	12	0	16	395	12
0	0	0	0	9	28	4	448	0
0	0	1	0	12	3	4	421	0
4	0	3	0	15	0	0	230	10
6	0	1	0	11	0	20	184	0
0	0	0	0	16	0	16	335	10
0	0	0	0	12	0	28	118	0
5	0	3	0	16	0	0	230	0
5	0	1	0	0	0	20	184	12
0	0	1	0	15	26	0	278	0
0	0	0	0	9	24	8	262	0
7	0	2	0	17	0	16	396	16
0	0	0	0	16	18	5	348	18
0	0	1	0	0	3	5	421	0

6	0	3	0	15	0	0	330	0
6	0	1	0	12	0	20	164	12
0	0	0	0	12	0	16	235	15
0	0	0	0	0	0	28	187	16
4	0	3	0	11	0	0	129	0
5	0	1	0	15	0	20	134	20
0	0	0	0	9	0	16	135	1
7	0	0	0	16	16	16	87	25
5	0	3	0	15	0	0	153	0
366	41	211	30	1107	1243	1303	18826	497
7.247524	0.811881	4.178217	0.594059	21.92079	24.61386	25.80198	372.7920	9.841584
3.423684	0.865675	2.573740	0.674199	4.431874	15.32757	8.340548	90.51844	6.598829
1.711	0.432	1.286	0.337	2.215	7.663	4.17	45.259	3.299
Turakina								
16	4	5	2	0	0	0	0	0
8	1	1	0	0	0	0	0	0
24	5	8	1	0	0	0	0	0
36	3	10	1	0	0	0	0	0
48	7	11	1	0	0	0	0	0
42	2	8	0	0	0	0	0	0
16	1	6	1	0	0	0	0	0
18	3	2	1	0	0	0	0	0
28	10	3	0	0	0	0	0	0
5	10	3	2	0	0	0	0	0
13	2	5	1	9	13	39	256	0
12	1	4	3	3	23	15	386	3
0	0	1	0	4	18	18	257	12
15	0	2	1	4	12	27	365	12
12	4	4	3	6	0	36	356	0
5	0	1	0	6	31	9	306	17
10	3	4	2	4	18	18	387	18
8	0	1	0	0	0	6	310	12
13	0	2	0	6	18	30	247	12
7	2	2	1	3	18	18	257	14
15	3	5	3	3	112	27	256	6
8	0	1	0	1	0	36	335	0
5	0	1	0	0	31	9	382	36
10	4	4	2	8	108	27	377	15
4	0	1	0	0	0	6	210	12
3	0	1	0	9	32	39	406	0
2	0	1	0	0	32	15	465	6
0	0	1	0	4	18	18	357	5
5	0	2	1	4	114	27	450	6
2	0	1	0	4	22	36	355	0
5	0	1	0	0	31	9	362	17
0	0	0	0	5	124	27	407	8
5	0	1	0	0	0	6	325	12
3	0	2	0	8	18	30	447	12
12	4	4	3	4	18	36	365	0
5	0	1	0	0	31	9	354	17
10	3	4	2	4	8	27	387	8
5	0	1	0	0	0	6	335	7
3	0	1	0	9	26	39	106	0
2	0	1	0	0	16	15	126	8
12	0	2	0	6	18	30	147	25
7	0	3	0	6	24	18	187	16
0	0	0	0	0	24	6	250	20
5	0	0	0	6	5	3	280	21
8	0	2	0	5	12	15	332	32
5	0	1	0	5	15	9	125	49
0	0	0	0	4	35	9	183	21
4	0	0	0	3	18	21	154	57
4	0	1	0	4	12	9	245	9
0	0	0	0	1	35	9	383	31
0	0	0	0	5	8	21	354	27
13	0	2	0	5	7	15	365	0
12	0	3	0	9	11	18	387	19

0	0	0	0	0	12	6	195	0
5	0	0	0	6	27	3	280	1
7	0	2	0	7	0	15	262	33
13	0	1	0	6	15	9	256	9
0	0	0	0	5	35	9	185	18
4	0	2	0	2	18	21	154	37
2	0	1	0	5	15	9	215	26
10	0	5	0	5	35	9	163	25
5	0	3	0	5	18	21	254	17
7	0	1	0	4	8	21	243	27
5	0	1	0	4	12	9	265	12
6	0	3	0	0	15	9	283	16
8	0	2	0	6	26	15	332	15
5	0	1	0	7	10	9	325	9
0	0	0	0	5	35	9	283	8
0	0	0	0	5	18	21	254	57
4	0	2	0	5	45	12	250	0
0	0	0	0	0	12	27	120	10
10	0	1	0	0	0	9	138	12
5	0	1	0	4	0	3	57	0
0	0	0	0	4	36	9	63	21
0	0	1	0	6	5	3	139	12
5	0	1	0	5	0	9	78	0
4	0	1	0	5	0	3	45	0
0	0	0	0	5	29	8	66	22
5	0	1	0	6	62	5	69	15
0	0	1	0	0	36	4	75	15
7	0	2	0	0	45	12	145	35
0	0	0	0	0	0	27	120	0
5	0	1	0	0	54	9	110	18
5	0	1	0	4	0	3	147	0
4	0	2	0	0	70	12	45	16
0	0	0	0	0	0	21	140	0
4	0	1	0	4	0	6	78	33
1	0	1	0	4	0	3	57	0
0	0	0	0	6	16	9	69	5
16	15	0	53	12	16	75	165	54
2	0	0	0	0	20	27	87	10
5	0	1	0	0	0	9	138	0
5	0	1	0	4	0	9	57	0
0	0	0	0	4	16	7	63	12
5	0	1	0	2	5	7	39	1
12	0	1	0	5	0	5	78	10
11	0	1	0	5	0	25	67	0
10	0	0	0	7	16	23	66	17
0	0	1	0	7	30	21	69	21
11	0	1	0	10	12	23	75	15
723	87	177	84	353	1940	1463	20190	1266
14.31683	0.861386	1.821782	0.831683	6.990099	38.41584	28.97029	399.8019	25.06930
8.255827	2.351251	2.068791	5.321426	2.935175	24.50149	12.22570	133.9672	13.23464
4.127	1.175	1.034	2.66	1.467	12.25	6.112	66.983	6.617
Sites	adults	larvae	seedling	rosette	flowering			
Pahiatua	4.75	8.26	1.36	2.97	0.34			
stderr	1.64	2.48	0.51	0.54	0.25			
Ballantrae	7.25	21.92	0.81	4.18	0.59			
stderr	1.71	2.22	0.43	1.29	0.34			
Turakina	14.32	6.99	0.86	1.82	0.83			
stderr	4.13	1.47	1.18	1.03	2.66			

3.2 Simple correspondence SAS programme for 3 sites survey.

```
options ls=78 ps=60 nodate;
proc format;
   value $ ragwort 'Balan'='Balantr' 'Turaki'='Turakin' 'Pahia'='Pahiatu';
run;
data rfb;
   input ragwort $ fleadt flelva seedl rosett flower;
```

```
cards;
Balan   366 1107 41 211 30
Turaki 723   353 87 177  84
Pahia   240   417 69 150  17
;
run;
proc print;
run;
proc corresp data=rfb outc=rfb1 short rp cp;
    var fleadt flelva seedl rosett flower;
    id ragwort;
run;
proc print uniform data=rfb1;
run;

/*Annotate Data*/
data coor1;
   set rfb1;
   x=dim1; y=dim2;
   xsys='2'; ysys='2';
   text=ragwort;
   style='Swiss';
   size= 1;
   keep x y text xsys ysys style size;
run;
/*---to show graph on screen only---*/
axis1 label=(h=1.2 f=swissb 'First Princiapl axis 87.31%')
order=-0.8 to 1.4 by 0.2;
axis2 label=(h=1.2  a=90 f=swissb 'Second Principal axis 12.69%')
order=-0.8 to 0.8 by 0.2;
goptions device=win display colors=(black);
proc gplot data=coor1;
    plot y*x=1 / annotate=coor1 frame href=0 vref=0 haxis=axis1 vaxis=axis2;
symbol1 v=none;
proc print;
run;
```

Contingency Table

Sites	RFB adults	RFB larvae	Ragwort seedlings	Ragwort rosettes	Flowering plants
Pahiatua	240	417	69	150	17
Ballantrae	366	1107	41	211	30
Turakina	723	353	87	177	84

The numbers of all organisms were mentioned by average values.

The ordination of three sites (Ballantrae, Pahiatua, and Turakina) together with the mean number per sample of *L. jacobaeae* adults, larvae, are different (Appendix 3.1). Stages of ragwort (seedlings, rosettes, and flowering plants) were used as the variables. The row and column profiles were considered first during the analysis (Table 3.2).

Table 3.2 Simple correspondence analysis of *L. jacobaeae* and ragwort plants in three sites.

Row profiles

Study sites	adults	larvae	seedlings	rosettes	Flowering plants
Ballantrae	0.21	0.63	0.23	0.12	0.08
Turakina	0.51	0.25	0.61	0.12	0.06
Pahiatua	0.27	0.47	0.08	0.17	0.02

Column profiles

Study sites	adults	larvae	seedlings	rosettes	Flowering plants
Ballantrae	0.28	0.59	0.21	0.39	0.23
Turakina	0.54	0.19	0.44	0.33	0.64
Pahiatua	0.18	0.22	0.35	0.28	0.13

Correspondence analysis confirmed that the majority of adults were found at Turakina, but larger numbers of larvae were found at Ballantrae and Pahiatua than Turakina (Row and column profiles in Table 3.2). The number of *L. jacobaeae* larvae over the entire study was highest at Ballantrae. Both profiles also showed that seedling numbers were higher at Pahiatua and Turakina than at Ballantrae, rosette numbers were higher at Ballantrae and Pahiatua than at Turakina, and flowering plants were higher at Turakina than at either Ballantrae or Pahiatua (Table 3.2, Figure 3.3). Correspondence analysis shows that *L. jacobaeae* larvae are most associated with Ballantrae (they are close together in Figure 3.3) suggesting that larvae are favoured at Ballantrae in comparison with Turakina and Pahiatua. Similarly, ragwort rosettes are most associated with Pahiatua and ragwort flowering plants are most associated with Turakina (Figure 3.3).

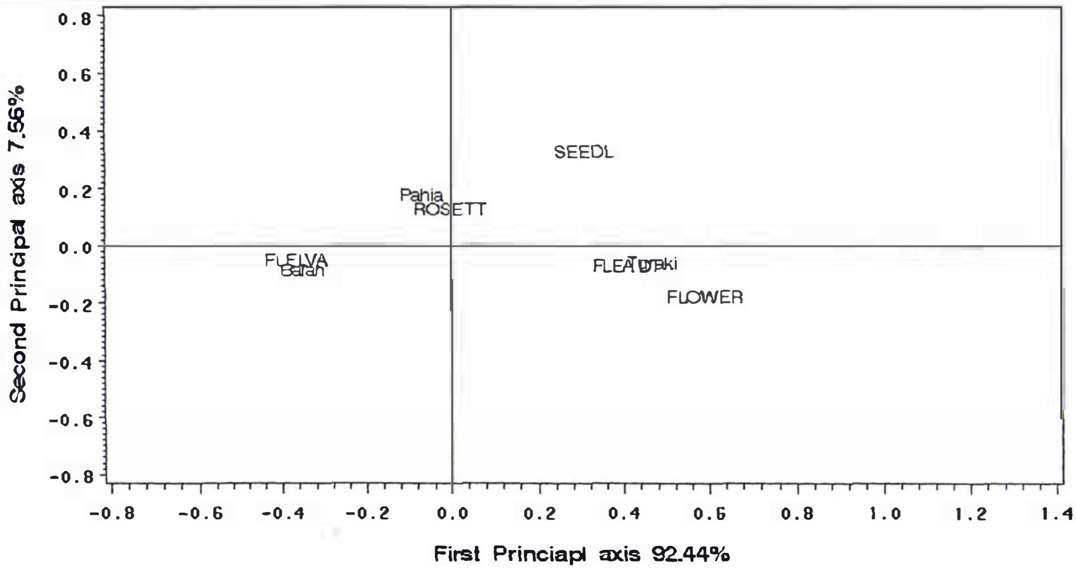

Figure 3.3 Correspondence analysis of *L. jacobaeae* and different stages of ragwort plants at three study sites.

This relationship between the numbers of *L. jacobaeae* and ragwort plants, and the three sites is confirmed by an examination of the contribution they make to the "total inertia" in the correspondence analysis (Table 3.3).

Table 3.3 Inertia and chi-square components of a simple correspondence analysis between *L. jacobaeae,* ragwort plants, and the three sites of Ballantrae, Pahiatua and Turakina.

Singular value	Principal Inertias	Chi-squares	Percent
0.36	0.13	536.58	92.44
0.10	0.10	43.87	7.56
	0.25	580.45	100.00
		(Degree of freedom = 8)	

For a clearer picture, it should be considered the decomposition of the total inertia of the correspondence among *L. jacobaeae* larvae, adults, different stages of ragwort and three sites. The SAS out put of total inertia and Chi square decomposition were shown in Table 3.3. 'Total inertia' is a measure of the total association between the rows and columns of the given contingency table. The first principal axis (Figure 3.3) explains 92.44% of the total inertia and indicates the strength of the association between *L. jacobaeae* larvae, ragwort rosettes and the different sites. However, the second principal axis accounts for 7.56% of the total inertia and shows that *L.*

jacobaeae adults, ragwort seedlings, and flowering plants also have some contribution to make to the relationship (Table 3.3). The positions of the column categories on the plot (Figure 3.3) indicate that the first principal dimension may regard as a contrast between *L. jacobaeae* larvae and adults. The second principal dimension, on the other hand was dominated mainly by different stages of ragwort. Hence, it may be concluded that about 92% of the information in the given data can be accounted for by a contrast between *L. jacobaeae* larvae and adults. The remaining information may be influence by the different stages of ragwort (about 8%). The distance of adult beetle and Turakina were quite closed and it may be concluded that the populations of adult beetles were much more in Turakina than other two sites (Pahiatua and Ballantrae). It may be concluded that populations of larvae were much higher in Ballantrae than other two sites (Turakina and Pahiatua).

Appendices for Chapter. 4.

4.1 Data collection from Ballantrae.

Date	larvae	Adult	Seedlin	Rosett	Flower	Mites	E.Worm	Nematd	Others
140596	19	1	2	2	.	13	2	21	.
140596	5	1	3	1	.	2	1	21	.
140596	3	2	.	3	.	56	4	47	19
140596	1	2	.	3	2	42	1	62	.
140596	10	2	1	2	2	5	1	12	2
140596	4	2	2	4	1	4	4	21	.
140596	4	1	.	2	.	2	3	23	.
140596	1	3	4	1	1	2	3	11	.
140596	2	2	1	4	2	2	4	16	.
140596	6	2	2	5	1	3	5	54	1
	55	18	15	27	9	131	28	288	22
	5.5	1.8	2.14	2.7	1.5	13.1	2.8	28.8	7.3
290696	3	2	.	4	.	2	3	43	2
290696	1	1	.	2	.	3	3	45	1
290696	2	.	.	5	.	3	2	56	4
290696	3	1	2	1	.	4	2	43	1
290696	1	.	.	1	.	3	2	31	3
290696	6	.	.	7	1	2	3	25	1
290696	1	2	1	2	.	2	4	32	1
290696	1	.	.	1	.	1	2	50	10
290696	2	2	1	2	.	5	9	78	2
290696	8	1	1	3	2	3	6	53	1
	28	9	5	28	3	28	36	456	26
	2.8	1.5	1.25	2.8	1.5	2.8	3.6	45.6	2.6
250796	4	1	.	2	.	.	4	62	.
250796	1	1	.	1	.	3	3	53	1
250796	8	1	.	13	.	.	2	34	.
250796	4	1	.	7	.	.	6	66	1
250796	1	1	.	1	.	.	6	22	.
250796	1	1	.	2	.	.	5	29	.
250796	4	1	.	3	.	.	3	27	.
250796	1	1	.	4	.	3	4	61	.

250796	3	1	.	2	.	3	4	42	1
250796	4	2	.	5	.	3	3	35	2
	31	11		40		12	40	431	5
	3.1	1.1		4		3	4	43.1	1.25
240896	5	1	8	7	.	8	.	25	4
240896	7	2	12	11	.	6	.	32	.
240896	1	.	3	2	.	2	.	22	.
240896	3	.	5	5	.	2	1	31	.
240896	.	.	2	4	.	2	1	2	.
240896	6	.	11	13	.	.	1	.	
240896	.	.	3	2	.	.	.	45	4
240896	6	1	13	8	.	.	.	28	2
240896	8	2	14	11	.	3	1	34	7
240896	4	.	5	5	.	1	.	42	.
	40	6	76	68	.	24	4	261	17
	5	1.5	7.6	6.8	.	3.4	1	29	4.25
190996	6	.	.	1	.	57	6	78	.
190996	1	.	.	.	.	74	2	101	.
190996	2	1	.	2	.	48	4	93	1
190996	2	.	.	.	.	128	1	48	.
190996	1	.	.	1	.	3	1	121	.
190996	5	1	.	2	.	48	3	30	.
190996	1	1	.	1	.	50	5	84	.
190996	2	.	.	.	.	46	4	35	1
190996	1	.	.	.	.	52	7	87	.
190996	1	1	.	3	.	44	3	30	.
	22	4		10		550	36	707	2
	2.2	1		1.67		55	3.6	70.7	1
171096	1	.	4	2	.	1	5	65	.
171096	5	.	12	8	.	.	4	60	.
171096	5	.	12	9	.	.	.	3	1
171096	12	.	11	17	.	.	4	3	.
171096	3	.	5	12	.	.	3	.	.
171096	.	.	3	3	.	.	1	.	.
171096	5	.	16	10	.	.	2	.	.
171096	.	.	1	.	.	.	4	.	.
171096	2	.	6	4	.	.	1	.	.
171096	2	.	5	3	.	.	1	.	.
	35		75	68	.	1	25	131	1
	4.4		7.5	7.6	0	1	2.8	32.75	1
151196	4	.	7	2	4	3	1	75	3
151196	7	.	5	1	.	.	2	120	3
151196	9	.	7	4	3	5	1	65	2
151196	5	.	4	3	2	12	.	2	3
151196	8	.	.	1	5	15	4	150	.
151196	3	.	5	5	4	.	60	2	.
151196	2	.	2	4	2	2	1	60	.
151196	1	.	4	.	.	3	2	56	1
151196	3	.	1	1	2	4	2	86	1
151196	4	.	.	4	3	.	3	54	.
	46		35	25	25	44	76	670	13
	4.6		4.38	2.78	3.13	6.29	8.44	67	2.17
41296	7	.	13	2	.	.	2	110	.
41296	7	.	12	1	.	2	3	74	.
41296	3	.	4	7	.	.	3	76	.
41296	.	.	11	.	.	4	6	65	2
41296	3	.	13	8	.	2	14	125	.
41296	4	.	12	11	.	.	2	136	
41296	8	.	11	11	.	2	9	148	
41296	8	.	12	.	.	.	7	86	.
41296	2	.	8	1	.	2	2	67	.
41297	.	.	7	.	.	.	1	30	
	42		103	41		12	49	917	2
	5.25		10.3	5.86		2.4	4.9	91.7	2

90197	4	3	2	8	1	3		77	1
90197	5	4	2	8	3		4	50	
90197	3	2	4	11	1	10	3	51	1
90197	8	5	5	13				50	
90197	7	6	3	12	3	8		4	3
90197	7	5	6	11	2	2		63	2
90197		2	3	4				25	1
90197	6	3	3	7	3	3	1	72	
90197	5	2	3	6	4	15		86	
90197			1	6	1	2	1	79	12
	45	32	32	86	18	43	9	557	20
	5.63	3.6	3.2	8.6	2.25	6.14	2.25	55.7	3.3
280197	6	4	2	12	2		5	45	
280197	4	2	2	12	3		2	36	
280197		1		1	3	4		42	16
280197	5	4	3	14	1	1	5	32	
280197	7	5	4	11		3		56	11
280197		1		1				30	
280197	8	5	3	17	3	4		41	1
280197	5	5		8	1	2	1	42	
280197	5	3		9	3	9	3	32	7
280197				1		2	1	56	
	40	30	14	86	16	25	17	412	35
	5.7	3.3	2.8	8.6	2.29	3.57	2.83	41.2	8.75
130297	3	4	1	11	1	56	0	6	2
130297	2	5	1	10	2	2	2	7	
130297	4	5	2	12	2	0	1	3	
130297	5	3	2	6	1	4	2	30	1
130297		2		8	1	5	2	10	7
130297	5	4	2	4		19	0	35	
130297	3	1	1	12	2	4	1	22	1
130297	1	5	1	13	3	6	4	41	
130297	2		1	5	2	39	0	20	1
130297		6		4	1	0	2	65	
	25	35	11	85	15	135	14	239	12
	3.13	3.9	1.4	8.5	1.7	13.5	1.4	23.9	2.4
270297	3	6		10	1	25		9	33
270297	5	10		11	2	3		3	
270297	1	5		8	1	32		13	6
270297	3	6		8	2	16		14	
270297	1	3		9		13	2	25	7
270297	4	8		11	1	2	3	7	1
270297	6	4		7	1	17	1	26	
270297		4		8		1		35	
270297	3	2		4	3	2	3	35	1
270297	4			5	2	14		20	
	30	48		81	13	125	9	187	48
	3.3	5.3		8.1	1.63	12.5	2.25	18.7	9.6
40397	3	7		9	1	0	2	21	1
40397	2	5		6		54	5	85	0
40397	3	5		8		57	3	64	17
40397	1	8		14	2	15	4	125	0
40397		11		14	1	42	0	4	4
40397	4	4		8		2	3		0
40397	1	10		10	2	11	3		2
40397	2	1		7	3	8	0		4
40397	5	1		4		3	2		3
	21	52		80	9	192	22	299	31
	2.63	5.8		8.9	1.8	21.3	3.14	59.8	3.4
170397		3		7		1	2	26	11
170397	4	8		4	1	1	6	32	
170397	3	10		12	2	3		21	
170397	1	5		11	1	54	2	7	
170397	6	8		10	1	6	6	46	
170397	1	5		12	1	145		23	2

170397	2	4		9		68	1	33	3
170397	5	9		5	1	7	1	3	
170397	3	4		5		1		40	1
170397		3		5	2	36	1	23	1
	25	59		80	9	322	19	254	18
	3.13	5.9		8	1.3	32.2	2.7	25.4	3.6
200397	1	5		7	1	9	4	9	2
200397	1	6		9		8	2		1
200397	2	7		7	2	168	1	16	11
200397	3	7		6	1	13	5	17	2
200397	1	11		13		82	3	19	2
200397		4		5	1	1	3	72	
200397		4		7	1	45			3
200397	1	10		11	1	87		18	11
200397	1	4		6	1	39		12	2
200397		7		9	1	8	1	20	
	10	65		80	9	460	19	183	34
	1.43	6.5		8	1.13	46	2.7	22.88	4.25
80497	2	11		6		27	1	48	7
80497	1	10		8	1	4		8	11
80497	1	6		7	1	5	5	42	
80497	2	8		7	2		3	35	1
80497	1	12		9		2	2	56	4
80497		6		12	2		2	24	2
80497		7		8		62		4	9
80497	1	4		7		42	2	24	5
80497		3		8	1	11	2	28	
80497	1	4		6		7	2	58	5
	9	71		78	7	160	19	327	44
	1.29	7.1		7.8	1.4	20	2.38	32.7	5.5
150497	3	10		6		21	3	33	20
150497	2	9		8	1	77	2	25	8
150497	4	12		7	1	11	3	16	1
150497	4	11		7	2	55	2	32	1
150497	1	10		9		4	1	20	2
150497	1	8		12	2	3	1	16	
150497	1	10		8		35	2	21	9
150497		12		7		45	1	17	5
150497	2	6		8	1	75	3	15	
150497		6		6		74	4	21	2
	18	94		78	7	400	22	216	48
	2.25	9.4		7.8	1.4	40	2.2	21.6	6
240497	3	14		6			4	23	10
240497	4	15		7	1	2	5	35	2
240497	2	12		7	1	116	5	42	29
240497	2	11		7	2		4	35	
240497	3	10		9			2	45	1
240497	3	9		11	2	53	3	21	1
240497	1	15		8		24	5	35	2
240497	4	10		7		97	3	47	1
240497	1	8		8	1	57	2	23	7
240497	1	6		6		122	1	36	2
	24	110		76	7	471	34	342	55
	2.4	11		7.6	1.4	67.3	3.4	34.2	6.1
120597	11	12		6		48		33	5
120597	15	15		7	1	5	2		2
120597	11	13		6	1	6	2	63	2
120597	11	13		7	1	24	1	36	6
120597	9	12		9		45	2	17	3
120597	12	9		10	1	5	2	27	2
120597	12	9		7		42	2	27	5
120597	10	12		7		35		16	5
120597	13	12		6	1	18		18	15
120597	14	10		6		8	2	15	2
	118	117		71	5	236	13	252	47

	11.8	11.7		7.1	1	23.6	1.85	25.2	4.7
290597	16	11	.	6	.	5	4	.	6
290597	6	10	.	7	1	1	2	.	2
290597	14	9	.	6	1	1	1	.	75
290597	10	9	.	7	1	3	2	16	5
290597	8	6	.	8	.	2	.	.	.
290597	12	12	.	10	1	1	3	31	6
290597	9	11	.	7	.	.	5	18	40
290597	12	12	.	7	.	8	1	21	47
290597	10	8	.	6	1	7	2	27	50
290597	8	7	.	6	.	2	3	35	.
	105	95		70	5	30	23	148	231
	10.5	9.5		7	1	3.3	2.5	24.67	28.87
30697	4	8	.	4	.	0	9	15	6
30697	6	9	.	4	1	0	2	45	.
30697	3	10	.	3	1	0	6	72	3
30697	3	8	.	6	.	0	6	52	23
30697	7	8	.	7	.	156	2	38	3
30697	5	10	.	9	.	0	7	15	4
30697	5	7	.	6	.	0	4	78	2
30697	5	6	.	6	.	76	4		7
30697	2	2	.	4	1	2	3	53	3
30697	5	.	.	5	.	4	2	12	57
	45	68		54	3	238	45	380	108
	4.5	7.6		5.4	1	23.8	4.5	42.2	12
210697	3	7	.	5	.	7	1	156	2
210697	5	7	.	6	.	3	.	.	3
210697	5	8	.	6	.	2	2	12	2
210697	7	6	.	7	.	19	.	4	.
210697	2	11	.	9	.	1	1	7	
210697	4	6	.	3	.	2	1	123	.
210697	4	7	.	6	.	3	.	4	.
210697	3	8	.	4	.	0	1	.	13
210697	.	.	.	4	.	32	.	12	1
210697	2	2	.	3	.	3	.	8	.
	35	62		53		72	6	326	21
	3.9	6.9		5.3		7.2	1.2	40.75	4.2
110797	6	8	.	3	.	1	1	32	.
110797	8	8	.	5	.	16	2	12	.
110797	9	9	.	5	.	.	1	9	12
110797	4	5	.	6	.	9	2	26	.
110797	5	4	.	7	.	15	.	11	20
110797	5	4	.	4	.	11	1	53	.
110797	6	7	.	4	.	11	3	87	.
110797	3	3	.	2	.	3	.	2	1
110797	6	5	.	3	.	1	2	85	1
110797	4	4	.	3	.	28	.	52	2
	56	57		42		95	12	369	36
	5.6	5.7		4.2		10.6	1.7	36.9	7.2
310797	11	7	.	3	.	6	.	42	3
310797	9	6	.	3	.	4	.	47	3
310797	8	9	.	4	.	11	1	68	3
310797	12	7	.	3	.	4	.	11	2
310797	7	4	.	2	.	.	1	15	2
310797	9	4	.	5	.	6	1	38	3
310797	8	8	.	5	.	.	2	16	1
310797	6	6	.	3	.	28	2	73	2
310797	11	3	.	4	.	6	2	22	1
310797	10	4	.	6	.	5	1	68	5
	91	58		38		70	10	400	25
	9.1	5.8		3.8		8.75	1.43	40	2.5
210897	12	3	.	4	.	17	.	19	3
210897	10	3	.	3	.	6	.	17	20
210897	11	6	.	3	.	14	1	48	6

210897	11	6	.	4	.	1	4	15	1
210897	12	4	.	3	.	17	4	57	3
210897	10	2	.	4	.	44	.	16	4
210897	9	2	.	5	.	12	.	7	2
210897	9	4	.	5	.	.	3	12	.
210897	6	3	.	2	.	5	1	14	1
210897	8	2	.	2	.	24	2	6	3
	98	35		35		140	15	211	43
	9.8	3.5		3.5		15.6	2.5	21.1	4.8
110997	15	2	.	4	.	6	1	20	4
110997	14	.	.	5	.	24	1	14	6
110997	12	4	.	3	.	.	1	35	2
110997	12	5	.	3	.	.	.	21	4
110997	10	2	.	2	.	7	1	9	1
110997	13	2	.	2	.	5	1	6	38
110997	12	3	.	5	.	36	.	9	5
110997	15	3	.	.	.	9	.	9	50
110997	10	2	.	2	.	11	1	11	73
110997	7	5	.	2	.	4	.	.	36
	120	28		28		102	6	134	219
	12	3.1		3.1		12.75	1	14.9	21.9
101097	18	2	.	3	.	5	1	10	4
101097	15	1	.	3	.	6	1	11	2
101097	15	1	.	2	.	11	2	15	24
101097	12	1	.	2	.	7	5	35	7
101097	16	2	.	5	.	.	6	45	2
101097	12	1	.	2	.	7	2	16	6
101097	10	1	.	3	.	14	4	65	9
101097	12	.	.	3	.	7	8	126	60
101097	11	.	.	3	.	23	1	15	13
101097	13	.	.	2	.	2	1	14	7
	134	9		28		82	31	352	134
	13.4	1.3		2.8		9.1	3.1	35.2	13.4
61197	15	.	2	3	.	4	2	20	59
61197	19	.	1	4	.	1	.	31	3
61197	14	.	1	4	.	24	.	32	68
61197	13	1	2	2	.	2	.	12	28
61197	12	.	1	3	.	23	.	5	25
61197	11	.	1	3	.	3	.	12	4
61197	13	1	1	2	.	16	.	5	16
61197	12	.	3	2	.	7	2	7	2
61197	12	.	1	2	.	4	.	5	30
61197	15	1	.	2	.	.	.	5	.
	136	3	13	27		84	4	134	235
	13.6	1	1.4	2.7		9.3	2	13.4	26.1
121297	2	4	5	4	1	3	2	12	2
121297	3	4	6	3	1	28	3	15	.
121297	2	3	5	3	1	13	.	12	3
121297	1	3	7	2	1	55	.	.	12
121297	1	3	4	2	.	5	2	6	2
121297	4	2	8	2	3	12	1	.	.
121297	1	2	6	2	.	42	.	.	66
121297	1	2	6	4	.	.	3	20	9
121297	1	2	4	4	.	1	2	10	1
121297	.	3	4	3	.	72	1	6	32
	16	28	55	29	7	231	14	81	127
	1.8	2.8	5.5	2.9	1.4	25.7	2	11.6	15.9
150198	5	5	6	5	2	5	1	96	2
150198	4	4	6	5	.	2	.	5	4
150198	5	4	6	4	.	8	.	10	4
150198	6	3	7	4	3	2	1	3	19
150198	4	3	6	3	1	9	.	13	8
150198	4	4	7	4	.	16	3	25	45
150198	7	4	8	3	3	5	.	8	22
150198	7	3	10	3	2	18	.	9	16

150198	4	3	3	5	.	11	2	.	25
150198	5	2	4	6	3	5	2	49	2
	51	35	63	42	14	81	9	218	147
	5.1	3.5	6.3	4.2	2.3	8.1	1.8	24.2	14.7
50298	2	5	3	5	2	4	2	16	3
50298	1	5	2	3	.	.	1	8	.
50298	1	4	3	5	1	6	.	15	.
50298	2	3	2	6	2	12	1	22	2
50298	3	3	2	5	1	28	.	18	2
50298	.	1	1	3	1	2	1	6	.
50298	.	1	1	2	2	8	.	16	1
50298	2	5	4	4	1	.	.	6	2
50298	2	7	4	5	1	9	1	2	.
50298	1	8	3	2	.	11	.	18	.
	14	42	25	40	11	80	6	127	10
	1.8	4.2	2.5	4	1.4	10	1.2	12.7	2
50398	2	7	1	5	1	.	1	.	22
50398	2	7	.	3	1	.	1	15	.
50398	1	6	.	4	.	6	1	73	3
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50398	1	6	1	6	.	7	.	18	3
50398	2	5	.	4	.	.	1	15	1
50398	1	3	.	2	.	6	1	.	3
50398	3	5	1	4	1	11	2	.	2
50398	1	5	1	5	.	3	2	18	4
50398	1	4	.	2	.	10	2	125	6
	16	54	5	40	3	52	11	311	72
	1.6	5.4	1	4	1	7.4	1.4	44.4	8
80498	3	7	.	4	.	6	.	3	.
80498	4	7	.	4	.	3	.	.	1
80498	4	8	.	3	1	.	1	18	.
80498	2	5	.	3	.	.	1	8	.
80498	4	5	.	3	.	4	3	33	1
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80498	3	8	.	3	.	.	3	.	1
80498	3	6	.	4	.	2	.	15	1
80498	5	6	.	5	.	27	4	7	49
80498	2	4	.	4	.	64	2	.	33
	32	61		38	1	140	15	84	86
	3.2	6.1		3.8	1	20	2.1	14	14.3
80598	6	11	.	4	.	6	1	17	3
80598	7	10	.	5	.	2	3	21	1
80598	10	14	.	4	.	.	3	44	3
80598	5	8	.	3	.	.	2	22	2
80598	6	5	.	5	.	.	2	16	.
80598	6	8	.	4	.	13	1	30	67
80598	7	9	.	4	.	4	3	32	3
80598	8	7	.	4	.	.	2	.	1
80598	5	8	.	3	.	13	2	11	15
80598	5	7	.	2	.	.	2	56	2
	65	87		38		38	21	249	97
	6.5	8.7		3.8		7.6	2.1	27.7	10.8
50698	5	8	.	4	.	3	4	85	13
50698	6	10	.	4	.	.	6	52	9
50698	7	9	.	4	.	6	2	46	1
50698	5	8	.	2	.	11	.	21	11
50698	5	7	.	3	.	3	2	47	1
50698	5	6	.	3	.	16	1	13	42
50698	4	7	.	6	.	1	2	53	2
50698	4	5	.	5	.	9	2	22	18
50698	5	10	.	2	.	.	6	55	2
50698	4	6	.	2	.	.	2	57	.
	50	76		35		49	27	451	99
	5	7.6		3.5		7	3	45.1	11

100798	6	4	.	4	.	.	9	25	2
100798	7	5	.	4	.	4	3	252	23
100798	8	5	.	3	.	2	2	48	3
100798	5	2	.	3	.	.	5	23	2
100798	7	5	.	3	.	.	3	25	1
100798	6	3	.	3	.	.	3	42	3
100798	12	3	.	5	.	4	3	78	4
100798	6	3	.	3	.	.	4	38	3
100798	10	6	.	4	.	.	3	27	.
100798	7	5	.	3	.	.	1	.	.
	74	41		35		10	36	558	41
	7.4	4.1		3.5		3.3	3.6	62	5.1
60898	8	3	.	4	.	.	8	42	.
60898	9	3	.	3	.	4	5	35	2
60898	10	2	.	3	.	.	2	18	.
60898	8	2	.	3	.	.	3	75	.
60898	8	2	.	3	.	.	1	38	1
60898	11	4	.	4	.	.	7	5	.
60898	7	2	.	2	.	.	2	8	.
60898	7	2	.	4	.	.	.	15	.
60898	10	3	.	3	.	.	.	22	.
60898	8	.	.	3	.	.	4	157	.
	86	23		32	.	4	32	415	3
	8.6	2.6		3.2	.	4	4	41.5	1.5

4.2 Weather records for Ballantrae.

Month	rain	meantemp	soiltemp
May-96	163	9.7	14.9
Jun-96	83	7	10.3
Jul-96	198	7.4	11.7
Aug-96	118	7.4	11
Sep-96	90	12.8	13.6
Oct-96	112	12.3	15.4
Nov-96	139	11.5	16.1
Dec-96	138	14.5	19.4
Jan-97	66.3	14.7	22.5
Feb-97	59.4	16.5	17.3
Mar-97	127.9	14.6	15.5
Apr-97	134.6	11.3	14
May-97	38.2	11.7	12.8
Jun-97	84.6	7.7	9
Jul-97	51.9	7.2	9.8
Aug-97	61.8	8.4	9.5
Sep-97	92.1	8.5	11.6
Oct-97	113	11	12.2
Nov-97	60.5	13	14.8
Dec-97	148	14.3	15.7
Jan-98	77.7	15.9	16.6
Feb-98	61.9	10.5	17.5
Mar-98	38.8	17.1	19.5
Apr-98	113.7	13.9	17.3
May-98	96.6	10.5	12.8
Jun-98	139.8	9.3	9.9
Jul-98	200.8	10.2	11.7
Aug-98	.	.	8.9

4.3 Percentage of soil water at Ballantrae between 1996 and 1998.

```
/*Ballantrae 140596*/
DATA water1;
INPUT SAMPLE W1 W2 W3;
      DOI=(W2-W3)/(W3-W1)*100;
CARDS;
1 2.94 58.69 33.56
2 2.64 82.02 46.69
3 3.00 55.93 32.79
4 2.90 71.18 44.09
5 2.82 70.44 39.45
6 2.99 68.03 43.19
7 2.93 43.96 27.11
8 3.00 86.62 55.99
9 2.91 81.42 49.07
;
RUN;
    PROC PRINT;
    PROC MEANS;
    PROC MEANS STDERR;
RUN;

/*Ballantrae 290696*/
DATA water2;
INPUT SAMPLE W1 W2 W3;
      DOI= (W2-W3)/(W3-W1)*100;
CARDS;
 1 4.91  75.173 44.58
 2 4.89  56.606 33.68
 3 4.89  72.456 36.78
 4 4.90  96.718 58.47
 5 4.47  99.116 54.12
 6 4.91  68.730 41.97
 7 4.87  61.540 37.29
 8 4.87  63.337 33.45
 9 4.89 100.07  61.72
10 4.76 102.02  64.51
;
RUN;
    PROC PRINT;
    PROC MEANS;
    PROC MEANS STDERR;
RUN;

/*Ballantrae 250796*/
DATA water3;
INPUT SAMPLE W1 W2 W3;
DOI=(W2-W3)/(W3-W1)*100;
CARDS;
 1 4.89 254.97 170.61
 2 4.76 145.30 100.77
 3 4.84 195.42 145.27
 4 4.83 152.15 103.35
 5 4.89 185.66 131.56
 6 4.79 147.27 109.41
 7 4.79 224.22 165.44
 8 4.81 159.97 105.69
 9 4.77 204.23 137.79
10 4.77 155.78  98.81
;
RUN;
    PROC PRINT;
    PROC MEANS;
    PROC MEANS STDERR;
RUN;

/*240896 Ballantrae*/
Data water4;
     input sample w1 w2 w3;
           doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.7475 136.5877  96.5600
 2 4.8027 135.0341  88.4508
 3 4.7009 101.5471  71.7718
 4 4.7381 131.2521  90.7759
 5 4.7669 114.0260  75.7857
 6 4.6950 149.2938  99.3877
 7 4.7359 144.5224 100.6356
 8 4.7434 140.1035  96.1619
 9 4.8113 120.4637  87.0191
10 4.6924  92.0317  67.2681
;
proc print;
run;
proc means;
proc means stderr;
run;

/*190996Ballantrae*/
data water5;
     input sample w1 w2 w3;
      DOI=(W2-W3)/(W3-W1)*100;
cards;
 1 4.7152 145.45  77.7518
 2 4.7587 160.38  85.4492
 3 4.6341 118.16  58.7731
 4 4.7735 104.28  59.3553
 5 4.7094 171.23  93.6471
 6 4.8751 170.03  90.3258
 7 4.8125 100.53  61.4670
 8 4.8584 135.35  74.8272
 9 4.8162 133.61  83.9541
10 4.8233 142.68  74.6113
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*171096Ballantrae*/
data water6;
     input samples w1 w2 w3;
       DOI=(W2-W3)/(W3-W1)*100;
```

```
cards;
 1 4.8205 115.0177 68.6762
 2 4.7556 125.2724 75.8795
 3 4.7858 119.5800 65.6791
 4 4.7582 133.8612 83.0984
 5 4.7526 104.6589 67.0553
 6 4.7453 135.4533 79.7795
 7 4.8900 135.9075 76.7656
 8 4.7558 134.7200 88.2256
 9 4.8201 107.0058 64.0079
10 4.7021 131.7913 75.6883
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*Ballantrae 151196*/
data water7;
     input sample w1 w2 w3;
DOI=(W2-W3)/(W3-W1)*100;
cards;
 1 4.9109  82.7288  56.4871
 2 4.9130  90.6904  60.5232
 3 4.8648 129.5554  71.3585
 4 4.9099 139.3031  91.0716
 5 4.7256 136.5168  87.4655
 6 4.7738 132.9744  83.6221
 7 4.8061 129.1147  82.3423
 8 4.7891  96.2568  64.3842
 9 4.7758 183.7800 119.1516
10 4.7634 139.5612  89.2213
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*Ballantrae 041296*/
data water8;
     input sample w1 w2 w3;
        doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.8204  96.5100  69.5863
 2 4.7821 150.2535  96.0915
 3 4.8902 135.4200 101.1269
 4 4.8279 115.5810  76.8475
 5 4.7104 102.3825  64.5432
 6 4.8853 135.1691  83.8416
 7 4.7488 128.2543  94.3172
 8 4.7532 131.9212  86.3672
 9 4.6880 108.4662  71.1384
10 4.7820 149.2535 101.1028
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*090197 Ballantrae*/
data water9;
     input sample w1 w2 w3;
        DOI=(W2-W3)/(W3-W1)*100;
cards;
 1 4.3550 56.0191 39.1487
 2 4.4328 50.6013 37.5158
 3 4.3153 55.8755 42.6907
 4 4.4270 51.3731 40.6390
 5 4.3776 73.2884 50.1835
 6 4.4514 79.6551 57.7174
 7 4.3616 57.9653 40.5028
 8 4.4067 75.2884 57.3475
 9 4.3825 64.3100 48.8500
10 4.4884 69.1267 49.9340
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*280197 Ballantrae*/
data water10;
     input sample w1 w2 w3;
       DOI=(W2-W3)/(W3-W1)*100;
cards;
 1 4.8988 62.1850 46.6928
 2 4.9738 71.9376 53.7457
 3 4.8672 85.3482 59.3191
 4 5.0707 79.1160 58.6429
 5 4.8340 62.6431 44.6078
 6 4.9228 70.7167 51.0821
 7 4.7663 47.9921 35.7033
 8 4.9018 53.9396 41.2566
 9 4.8876 68.6574 51.7829
10 4.8531 54.0481 39.6279
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*130297 Ballantrae*/
data water11;
     input sample w1 w2 w3;
```

```
     DOI=(W2-W3)/(W3-W1)*100;
cards;
 1 4.6572  92.0054 65.6508
 2 4.6428  92.0156 66.3492
 3 4.6500 102.0208 76.0015
 4 4.7501  82.0002 55.9985
 5 4.5488  92.0195 65.8725
 6 4.6511  92.0405 66.1275
 7 4.6408 115.0210 89.0002
 8 4.6592  82.0025 56.3455
 9 4.7524  87.0375 54.2654
10 4.5475  87.0230 53.2709
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*270297 Ballantrae*/
data water12;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.65  84.29 60.15
 2 4.70 121.17 99.44
 3 4.61  67.63 43.12
 4 4.64  54.72 31.91
 5 4.60  93.41 68.89
 6 4.72  98.56 72.30
 7 4.56  56.12 33.84
 8 4.67  75.36 50.27
 9 4.63  84.11 59.66
10 4.72 105.21 80.72
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*40397 Ballantrae*/
data water13;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.80  91.89 66.64
 2 4.82  84.72 60.93
 3 4.78 115.76 81.52
 4 4.83 120.15 92.67
 5 4.81  85.12 61.35
 6 4.79  83.35 74.82
 7 4.77  71.14 48.67
 8 4.81  89.01 66.72
 9 4.80  76.83 55.70
10 4.79 103.61 71.56
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*170397 Ballantrae*/
data water14;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.80  83.87 60.25
 2 4.78 102.26 80.61
 3 4.81  65.71 43.43
 4 4.82  78.05 56.26
 5 4.79  82.63 58.47
 6 4.83  96.42 72.65
 7 4.78 103.88 81.12
 8 4.81  68.39 45.78
 9 4.80  85.92 61.05
10 4.78  64.61 41.01
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*200397 Ballantrae*/
data water15;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.8085 101.4146 74.1793
 2 4.7745  74.9617 60.5574
 3 4.7997  61.6134 45.7197
 4 4.6788  87.4478 69.7122
 5 4.8347 108.0041 86.9345
 6 4.6806  80.8845 62.6258
 7 4.6918  72.5143 60.2413
 8 4.7874  58.1744 42.8585
 9 4.8213  80.1311 65.1984
10 4.8576  84.2977 58.1354
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*80497 Ballantrae*/
data water16;
```

```
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.76 68.12 58.05
 2 4.76 47.36 36.43
 3 4.73 54.53 47.81
 4 4.78 45.25 39.77
 5 4.81 41.26 32.02
 6 4.76 53.28 42.14
 7 4.80 58.25 49.51
 8 4.81 61.24 52.35
 9 4.78 53.57 45.38
10 4.76 62.73 54.27
;
proc print;
run;
proc means;
proc means stderr;
run;

/*150497 Ballantrae*/
data water17;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.81 69.24 51.65
 2 4.75 65.71 48.61
 3 4.71 81.07 62.34
 4 4.77 92.76 65.47
 5 4.77 51.62 36.58
 6 4.78 60.11 47.70
 7 4.79 79.39 62.58
 8 4.80 70.51 52.73
 9 4.77 77.32 60.58
10 4.81 51.25 38.42
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*240497 Ballantrae*/
data water18;
     input sample w1 w2 w3;
       DOI=(W2-W3)/(W3-W1)*100;
cards;
 1 4.8019 76.5887 51.3794
 2 4.9509 95.3541 67.8691
 3 4.8497 71.6380 49.1103
 4 4.8153 64.8353 46.4368
 5 4.8336 68.2978 51.1448
 6 4.9141 68.2916 48.5526
 7 4.9445 88.4779 67.8336
 8 4.9317 91.0345 62.3998
 9 4.9135 73.1054 53.7015
10 4.9341 86.9505 60.7753
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*120597 Ballantrae*/
data water19;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.71 126.15  70.85
 2 4.65  85.75  48.72
 3 4.62  98.11  54.63
 4 4.60 134.27  81.47
 5 4.61 112.16  65.54
 6 4.62  68.44  42.33
 7 4.60 145.32  87.21
 8 4.58 125.80  68.16
 9 4.59 133.12  78.35
10 4.70 141.03 105.59
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*290597 Ballantrae*/
data water20;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.72 118.23  78.27
 2 4.65  95.16  52.18
 3 4.66 102.27  60.73
 4 4.64  98.54  57.28
 5 4.65 138.71 109.29
 6 4.59 127.31  86.15
 7 4.57  96.27  55.76
 8 4.71  92.61  47.64
 9 4.67 124.52  82.51
10 4.65 110.12  70.27
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*030697 Ballantrae*/
data water21;
```

```
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.78 124.82 75.15
 2 4.82  85.26 65.17
 3 4.80 110.38 70.33
 4 4.76 136.71 88.20
 5 4.77 127.16 78.21
 6 4.75  98.22 69.36
 7 4.79 132.54 85.12
 8 4.69 121.19 77.10
 9 4.81  89.28 67.24
10 4.83 115.26 74.27
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*210697 Ballantrae*/
data water22;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.85  91.33 66.75
 2 4.84 125.21 84.11
 3 4.86  82.75 56.20
 4 4.78 136.43 90.17
 5 4.86  64.26 52.35
 6 4.88  72.37 53.02
 7 4.81 131.14 87.08
 8 4.88  73.62 54.61
 9 4.87  68.58 45.21
10 4.87  64.94 37.01
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*110797 Ballantrae*/
data water23;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.83 117.11  78.72
 2 4.79 134.25  95.56
 3 4.87 100.16  61.15
 4 4.85 106.17  67.12
 5 4.81 127.32  88.25
 6 4.78 128.21  89.28
 7 4.88 105.27  65.19
 8 4.83 102.34  62.54
 9 4.90  99.55  59.35
10 4.76 149.73 113.12
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*310797 Ballantrae*/
data water24;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.83 105.47 67.36
 2 4.81 110.26 72.11
 3 4.85  98.38 62.20
 4 4.78 123.15 81.16
 5 4.86  89.83 60.25
 6 4.84 103.71 63.31
 7 4.85 112.24 73.01
 8 4.83 105.17 65.23
 9 4.82  91.29 58.06
10 4.83 111.44 68.00
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*210897 Ballantrae*/
data water25;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.80 159.21  85.52
 2 4.78 175.35  99.28
 3 4.79 143.74  74.64
 4 4.81 181.68 115.33
 5 4.80 186.81 118.71
 6 4.82 132.27  58.57
 7 4.79 124.69  55.25
 8 4.B3 171.54  92.11
 9 4.77 158.86 B0.B6
10 4.B1 156.06 79.25
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*110997 Ballantrae*/
```

```
data water26;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.80 135.64  82.15
 2 4.79 131.58  79.21
 3 4.82 143.70  91.37
 4 4.78 123.63  71.16
 5 4.83 102.65  50.45
 6 4.79 164.71 112.12
 7 4.81  98.57  46.16
 8 4.82 168.66 115.23
 9 4.69 132.62  80.31
10 4.93 134.64  81.84
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*101097 Ballantrae*/
data water27;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.74 151.75  82.02
 2 4.76 149.28  81.76
 3 4.81 160.64  92.29
 4 4.68 136.37  68.63
 5 4.69 174.49 106.45
 6 4.82 147.51  79.38
 7 4.75 155.67  87.48
 8 4.76 143.83  75.52
 9 4.74 121.44  53.68
10 4.75 169.77 101.83
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*061197 Ballantrae*/
data water28;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.8745  76.9052 55.1296
 2 4.8515  74.4103 46.0557
 3 4.8925  84.8211 55.5071
 4 4.7861  77.7631 45.4838
 5 4.8131  85.8553 52.4100
 6 4.8134  70.3220 45.7742
 7 4.8715  68.3285 40.1832
 8 4.9041  81.2751 47.2575
 9 4.9422  79.1130 50.4305
10 4.8737 107.3639 73.9342
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*121297 Ballantrae*/
data water29;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.85 65.59 51.01
 2 4.68 84.47 70.75
 3 4.77 76.58 62.25
 4 4.75 50.72 40.68
 5 4.67 85.56 70.32
 6 4.86 72.45 56.71
 7 4.72 53.76 39.39
 8 4.80 52.71 38.64
 9 4.67 74.59 55.36
10 4.87 69.47 50.99
;
proc print;
run;
proc means;
proc means stderr;
run;

/*150198 Ballantrae*/
data water30;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.75  79.35 61.75
 2 4.81  80.62 62.84
 3 4.78  75.71 58.62
 4 4.70  87.43 70.71
 5 4.73  62.25 42.43
 6 4.72  91.18 72.45
 7 4.75 101.87 75.58
 8 4.78  52.63 36.69
 9 4.74  70.26 57.81
10 4.74  89.40 72.62
;
run;
proc print;
run;
proc means;
proc means stderr;
run;
```

```
/*50298 Ballantrae*/
data water31;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.76 113.13  92.50
 2 4.78 120.82  99.11
 3 4.72 115.76  95.16
 4 4.76  86.51  74.05
 5 4.75  98.26  77.64
 6 4.68 135.33 113.23
 7 4.75  68.87  40.21
 8 4.77 138.91 121.10
 9 4.73  93.64 76.82
10 4.80 159.03 130.39
;
run;
proc print;
run;
proc means;
proc means stderr;
run;


/*50398 Ballantrae*/
data water32;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.78 54.29 45.25
 2 4.78 60.27 51.63
 3 4.72 51.04 42.15
 4 4.76 53.68 45.28
 5 4.82 61.71 53.11
 6 4.77 69.22 59.06
 7 4.80 48.47 37.54
 8 4.81 65.53 57.81
 9 4.78 45.21 36.72
10 4.78 31.16 22.01
;
run;
proc print;
run;
proc means;
proc means stderr;
run;


/*80498 Ballantrae*/
data water33;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.70  90.99 65.75
 2 4.72 101.16 68.88
 3 4.68  85.73 59.91
 4 4.73  76.71 54.72
 5 4.71 114.68 79.61
 6 4.69  88.02 64.65
 7 4.67 123.13 92.52
 8 4.71  72.24 47.57
 9 4.70  65.11 43.36
10 4.69  83.47 74.53
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*080598 Ballantrae*/
data water34;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.81 105.36  67.25
 2 4.82 124.72  87.77
 3 4.79 136.10  96.63
 4 4.81  92.25  55.28
 5 4.78  85.47  50.23
 6 4.83  82.63  45.17
 7 4.78 113.12  71.85
 8 4.80  74.85  37.40
 9 4.82  72.16  34.68
10 4.86 162.06 124.14
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*050698 Ballantrae*/
data water35;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.68 123.52 75.05
 2 4.70 110.66 62.12
 3 4.71 132.71 87.22
 4 4.80  98.73 52.32
 5 4.62 126.25 78.65
 6 4.61 122.16 76.71
 7 4.62 115.89 68.76
 8 4.65 135.75 84.81
 9 4.68 126.90 79.15
10 4.73 139.00 85.31
;
run;
proc print;
```

```
run;
proc means;
proc means stderr;
run;

/*100798 Ballantrae*/
data water36;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.75 172.23  95.50
 2 4.78 150.65  74.16
 3 4.82 165.43  90.74
 4 4.71 200.96 129.33
 5 4.72 138.12  61.95
 6 4.70 164.16  89.87
 7 4.77 175.13  96.12
 8 4.75 146.45  63.83
 9 4.76 182.87 104.08
10 4.74 224.46 145.42
;
run;
proc print;
run;
proc means;
proc means stderr;
run;

/*160898 Ballantrae*/
data water37;
     input sample w1 w2 w3;
      doi=(w2-w3)/(w3-w1)*100;
cards;
 1 4.65 130.95  80.25
 2 4.70 141.27  92.37
 3 4.61 125.63  74.21
 4 4.63 136.48  85.69
 5 4.65  99.79  50.77
 6 4.66 152.81 105.49
 7 4.68 134.80  83.82
 8 4.62 120.11  71.16
 9 4.64 137.26  86.02
10 4.66 122.80  71.06
;
run;
proc print;
run;
proc means;
proc means stderr;
run;
```

4.4 SAS programme for multiple correlation analysis. Balm.dat

```
May96 5.5  1.8 2.1 7.7 1.5 13.1 2.8 28.8  7.30 163   9.7 14.9 72.19
Jun96 2.8  1.5 1.2 7.8 1.5  2.8 3.6 45.6  2.6  83   7.0 10.3 81.25
Jul96 3.1  1.1  0  4.0  0   3.0 4.0 43.1  1.25 198   7.4 11.7 46.24
Aug96 5.0  1.5  0  6.8  0   3.4 1.0 29.0  4.25 118   7.4 11.0 47.14
Sep96 2.2  1.0  0  6.7  0  55.0 3.6 70.7  1.0  90  12.8 13.6 87.38
Oct96 4.4  0   0  7.6  0   1.0 2.8 32.78 1.0  112  12.3 15.4 71.96
Nov96 4.6  0  7.5 5.8 3.1  6.29 8.44 67.0  2.17 139  11.5 16.1 60.04
Dec96 5.25 0 10.3 5.86 2.7  2.4 4.9 91.7  2.0  138  14.5 19.4 51.84
Jan97 5.7  3.5 9.6 8.6 2.2  9.71 2.54 48.5  6.0  66.3 14.7 22.5 40.16
Feb97 2.2  4.6 4.5 8.3 1.6 13.0 1.8 21.3  6.0  59.4 16.5 17.3 48.56 /*13.02.97 &27.02.97*/
Mar97 2.4  6.1 0.7 8.3 1.4 33.2 2.8 36.02 3.75 127.9 14.6 15.5 38.67 /*17.03.97*/
Apr97 1.98 9.2  0  7.7 1.4 42.4 2.66 29.5  5.87 134.6 11.3 14.0 34.43 /*7.04.97 &24.04.97*/
May97 11.2 10.6 0  7.05 1.4 13.45 2.2 24.9 16.8  38.2 11.7 12.8 70.69 /*12.05.97 & 29.05.97*/
Jun97 4.2  7.25 0  5.35 1.0 15.5 2.85 41.5  8.1  84.6 7.7 9.0 52.40 /20.06.97*/
Jul97 7.4  5.75 0  4.0 0.5  9.7 1.6 38.5  4.85 51.9 7.2 9.8 58.78 /*10.07.98 & 31.07.97*/
Aug97 9.8  3.5  0  3.5  0  15.6 2.5 21.1  4.8  61.8 8.4 9.5 96.19 /*21.08.97*/
Sep97 12.0 3.1  0  3.1  0  12.75 1.0 14.9 21.9  92.1 8.5 11.6 75.54 /*11.09.97*/
Oct97 13.4 1.3  0  2.8  0   9.1 3.1 35.2 13.4 113.0 11.0 12.2 90.76 /*9.10.97*/
Nov97 13.6 0  6.8 2.7  0   9.3 2.0 13.4 26.1  60.5 13.0 14.8 65.11 /*6.11.97*/
Dec97 1.8  0  7.5 4.9  0  25.7 2.0 11.6 15.9 148.0 14.3 15.7 31.98 /*11.12.97*/
Jan98 5.1  3.5 6.9 4.2 1.4  8.1 1.8 24.2 14.7  77.7 15.9 16.6 33.41 /*15.01.98*/
Feb98 1.8  4.2 3.2 4.0 2.3 10.0 1.2 12.7  2.0  61.9 10.5 17.5 27.82 /*5.02.98*/
Mar98 1.6  5.4 0.3 4.0 1.4  7.4 1.4 44.4  8.0  38.8 17.1 19.5 24.94 /*5.03.98*/
Apr98 3.2  6.1  0  3.8 1.0 20.0 2.1 14.0 14.3 113.7 13.9 17.3 42.97 /*7.04.98*/
May98 6.5  8.7  0  3.8 1.0  7.6 2.1 27.7 10.8  96.6 10.5 12.8 72.59 /*7.05.98*/
```

```
Jun98 5.0  7.6  0  2.5  0   7.0 3.0 45.1  11.0 139.8 9.3 9.9 70.13 /*4.06.98*/
Jul98 7.4  4.1  0  1.5  0   3.3 3.6 62.0  5.1  200.8 10.2 11.7 92.14 /*9.07.98*/
Aug98 8.6  2.6  0  1.0  0   4.0 4.0 41.5  1.5    .   .  8.9 69.15 /*5.08.98*/


/*mean value of extraction and data collection from Ballantrae*/
/*larvae adult seedling rosette flower mites eartworm nematod others*/
options ls=78 ps=65 nodate;
data balamean;
    infile h:\user\'balm.dat';
    input mmyy $ larvae adult seedling rosette flower mites $ eartworm $ nematod $ others $
        rain $ meantem $ soiltemp $ sowater;
        sowasq=sowater**2;

/*date adult seedl rosett flower larvae mite eworm nemat other*/
/*Ballantrae*/
title1 'Extration from Ballantrae samples';
proc corr data=balamean out=balamea1;
   var larvae adult seedling rosette flower sowater;
run;
proc reg data=balamean;
model sowater=larvae adult;
model sowater=seedling rosette flower;
run;
proc reg data=balamean;
model sowater=larvae adult/ss1 ss2 stb;
model sowater=seedling rosette flower/ss1 ss2 stb;
run;
proc reg data=balamean;
    model sowater=larvae adult;
    model sowater=seedling rosette flower;
    output out=d p=plarvae r=rlarvae u95=up l95=down;
run;
```

4.5 Canonical correlation analysis SAS program

```
/*mean value of extraction and data collection from Ballantrae*/
/*larvae adult seedling rosette flower mites eartworm nematod others*/
options ls=78 ps=65 nodate;
data balamean;
infile h:\user\'balm.dat';
input mmyy $ larvae adult seedling rosette flower mites eartworm nematod others;
/*date adult seedl rosett flower larvae mite eworm nemat other*/
/*Ballantrae*/

proc cancorr data=balamean out=balamea1 all
    vprefix=RFB vname='Ragwort Flea Beetles'
    wprefix=RAGWORT wname='Three Stages of Ragwort';
  var larvae adult;
  with seedling rosette flower;
run;
/*To plot the canonical scores*/
proc plot data=balamea1;
   plot rfb1*ragwort1 rfb2*ragwort2;
run;
```

```
/*---to show graph on screen only---*/

axis1 label=(h=1.2 f=swissb 'Mean numbers of larvae');

axis2 label=(h=1.2  a=90 f=swissb 'Mean numbers of rosettes');

goptions device=win display colors=(black);
proc gplot data=balamea1;
   plot  rfb1*ragwort1/ frame href=0 vref=0 haxis=axis1  vaxis=axis2;
run;

axis1 label=(h=1.2 f=swissb 'Mean numbers of adults');

axis2 label=(h=1.2  a=90 f=swissb 'Mean seedlings and flowering plants');

goptions device=win display colors=(black);
proc gplot data=balamea1;
   plot  rfb2*ragwort2/ frame href=0 vref=0 haxis=axis1  vaxis=axis2;
run;
```

Appendices for Chapter. 5.

5.1 Laboratory studies to measure consumption rates of RFB larvae

Three different times of introduction of RFB larvae - T_1, T_2, and T_3

Four levels of RFB larvae numbers - 0, 10 ,20, 40

A 3 x 4 factorial design with 5 replicates will be used.

RFB larvae	numbers of larvae	numbers of rosett plant	Total larvae
no larvae		5	
T_1	10	5	50
T_2	10	5	50
T_3	10	5	50
no larvae		5	
T_1	20	5	100
T_2	20	5	100
T_3	20	5	100
no larvae		5	
T_1	40	5	200
T_2	40	5	200
T_3	40	5	200
Total		60	1050

A 3 x 4 Factorial Design

Treatments			Blocks				
larvae	level		1	2	3	4	5
1st stage	1	I	a11	b11	c11	d11	e11
			(3)	(12)	(6)	(1)	(4)
	2	II	a21	b21	c21	d21	e21
			(5)	(2)	(3)	(5)	(2)
	3	III	a31	b31	c31	d31	e31
			(7)	(1)	(12)	(9)	(3)

2nd stage	1	IV	a12 (4)	b12 (7)	c12 (11)	d12 (8)	e12 (3)
	2	V	a22 (1)	b22 (3)	c22 (7)	d22 (11)	e22 (6)
	3	VI	a32 (2)	b32 (5)	c32 (2)	d32 (4)	e32 (1)
3rd stage	1	VII	a13 (10)	b13 (9)	c13 (10)	d13 (3)	e13 (8)
	2	VIII	a23 (8)	b23 (4)	c23 (4)	d23 (7)	e23 (7)
	3	IX	a33 (12)	b33 (10)	c33 (9)	d33 (10)	e33 (9)
no larvae	1	X	a1t (6)	b1t (8)	c1t (5)	d1t (6)	e1t (10)
	2	XI	a2t (9)	b2t (6)	c2t (8)	d2t (12)	e2t (11)
	3	XII	a3t (11)	b3t (11)	c3t (1)	d3t (2)	e3t (12)

A 3 x 4 Factorial design for field layout.

1	**20 - T51-2**	**13**	**40 -T32 -3**	**25**	**0 -T123 -1**	**37**	**10 -T14 -3**	**49**	**40 -T65 - 2**
2	**40 - T61-2**	**14**	**20 -T22 -3**	**26**	**40 -T63 -2**	**38**	**0 -T124-1**	**50**	**20 -T25 - 3**
3	**10 - T11-3**	**15**	**20 -T52 -2**	**27**	**20 -T23 -3**	**39**	**10 -T74 -1**	**51**	**10 -T45 - 2**
4	**10 - T41-2**	**16**	**20 -T82 -1**	**28**	**20 -T83 -1**	**40**	**40 -T64 -2**	**52**	**10 -T15 - 3**
5	**20 - T21-3**	**17**	**40 -T62 -2**	**29**	**0 -T103-3**	**41**	**20 -T25 -3**	**53**	**40 -T35- 3**
6	**0 - T101-3**	**18**	**0 -T112-2**	**30**	**10 -T13 -3**	**42**	**0 -T104-3**	**54**	**20 -T55 - 2**
7	**40 - T31-3**	**19**	**10 -T42 -2**	**31**	**20 -T53 -2**	**43**	**20 -T84 -1**	**55**	**20 -T85 - 1**
8	**20 - T81-1**	**20**	**0 -T102-3**	**32**	**0 -T113-2**	**44**	**10 -T44 -2**	**56**	**10 -T75 - 1**
9	**0 - T111-2**	**21**	**10 -T72 -1**	**33**	**40 -T93 -1**	**45**	**40 -T34 -3**	**57**	**40 -T95 - 1**
10	**10 -T71-1**	**22**	**40 -T92 -1**	**34**	**10 -T73 -1**	**46**	**40 -T94 -1**	**58**	**0 -T105- 3**
11	**0 - T121-1**	**23**	**0 -T122-1**	**35**	**10 -T43 -2**	**47**	**20 -T54 -2**	**59**	**0 -T115- 2**
12	**40 - T91-1**	**24**	**10 -T12 -3**	**36**	**40 -T33 -3**	**48**	**0 -T114-2**	**60**	**0 -T125- 1**

5.2 SAS programme for analysis of laboratory feeding experiment.

```
options ls=78 ps=60 nodate;
title1 'Analysis of Laboratory Feeding Experiment';
data labfd2;
 do densit=0,10,20,40;
   do repl=1 to 5;
     do time='15days', '30days', '45days';
       do xx=1 to 3;
         input pltwt @@;output;
end; end; end; end;
cards;
26.1109 34.4526 0.1851 26.1799 41.5789 0.5793 27.5980 46.4919 0.6467
26.8538 34.3971 0.1330 25.3835 39.2352 0.3480 25.8015 42.9162 0.4166
27.8580 33.7589 0.1002 25.0860 40.4651 0.3577 25.8088 45.3033 0.6229
26.8699 32.9912 0.1070 26.3411 40.5789 0.5200 25.7924 45.1963 0.6078
25.2726 33.0033 0.1197 26.8889 38.9366 0.3552 26.8957 46.3709 0.7291
25.9019 27.4146 0.0921 26.0816 22.6537 0.0613 26.6068 16.7994 0.0401
26.4025 27.7721 0.1017 26.6968 20.5367 0.0549 26.3830 17.3821 0.0543
25.0868 26.8145 0.0854 26.6390 19.3567 0.0549 25.0384 18.5797 0.0562
26.4617 27.5596 0.0973 27.3835 21.0713 0.0563 26.5872 16.2574 0.0381
27.0118 25.5605 0.0826 25.0638 21.7257 0.0612 27.2103 17.4313 0.0625
26.7392 27.4991 0.0953 26.1797 21.8965 0.0533 26.8132 19.5272 0.0468
24.3099 27.4906 0.0947 25.7153 16.2240 0.0482 25.9733 15.6331 0.0321
25.6959 25.5742 0.0683 27.4813 20.0412 0.0516 28.6668 18.3032 0.0551
27.5470 26.2048 0.0845 28.4218 21.3526 0.0576 29.5122 16.7337 0.0428
26.7419 24.9299 0.0501 25.7832 19.2982 0.0429 25.0565 14.7070 0.0322
27.1273 19.5949 0.0485 25.5742 12.1867 0.0218 25.8113  6.0816 0.0102
27.7631 17.1573 0.0523 26.1084 16.2324 0.0397 27.4194  7.6691 0.0182
26.7567 19.2717 0.0471 25.0327 15.8318 0.0382 26.0098  7.5944 0.0176
26.4511 16.3006 0.0401 24.2496 13.6188 0.0301 27.3812 11.5309 0.0200
25.5244 16.1083 0.0392 29.7304 12.0168 0.0209 25.3734  4.6557 0.0098
;
 run;
/*Transposing the given data*/
proc sort data=labfd2;
    by densit time repl;
run;
proc print; run;
proc transpose data=labfd2  out=labfd22 prefix=pltwt;
     by densit time repl;
     id xx;
     var pltwt;
run;
proc print; run;
/*To delete unnecessary variables and to label the responses*/
data labfd22; set labfd22;
 keep densit time repl pltwt1 pltwt2 pltwt3;
 label pltwt1='initial wt of plant'
```

```
    pltwt2='fresh wt of plant'
    pltwt3='dry wt of plant';
run;

 proc glm data=labfd2;
   class densit time;
      model pltwt= densit time densit*time/ss1;
                output out=resid p=pred r=resid;
run;
proc plot data=resid;
plot resid*pred;
run;

/*Performing the Analysis of Variance*/
proc glm data=labfd22 order=data;
class densit time;
model pltwt1 pltwt2 pltwt3 = densit time densit*time/ss1;
    contrast 'densit_L' densit -3 -1 1 3;  /*Linear effect of Level*/
    contrast 'densit_Q' densit 1 -1 -1 1;  /*Quadratic effect of Level*/
    contrast 'densit_C' densit -1 3 -3 1;  /*Cubic effect of level*/
    manova h=densit time densit*time/htype=1 etype=1;
    lsmeans densit time densit*time/pdiff;
run;

/*Canonical Variate analysis*/
title2 'CVA with Density as group';
proc candisc data=labfd22 ncan=2 out=cand1
    anova distance wcov pcov bcov;
    class densit;
    var pltwt1 pltwt2 pltwt3;
run;
proc plot data=cand1;
   plot can2*can1=densit;
run;
/*High resolution graphs*/
goptions dev=aplplus nodisplay colors=(black) rotate=landscape
        hsize=10 in vsize=7.5 in;
axis1 label=(h=1.2 f=complex a=0 "Canonical Variate 1") minor=(n=3);
axis2 label=(h=1.2 f=complex a=90 "Canonical Variate 2") minor=(n=3);
footnote1";
symbol1 i=none v=square h=2 pct;
symbol2 i=none v=circle h=2 pct;
symbol3 i=none v=triangle h=2 pct;
symbol4 i=none v=star h=2 pct;
```

5.3. Glasshouse studies to measure consumption rates of RFB larvae

Three different times of introduction of RFB larvae - T_1, T_2, and T_3
Four levels of RFB larvae numbers - 0, 10 ,20, 40
A 3 x 4 factorial design with 5 replicates will be used.

RFB larvae	numbers of larvae	numbers of rosett plant	Total larvae
no larvae		5	
T_1	10	5	50
T_2	10	5	50
T_3	10	5	50
no larvae		5	
T_1	20	5	100
T_2	20	5	100
T_3	20	5	100
no larvae		5	
T_1	40	5	200
T_2	40	5	200
T_3	40	5	200
Total		60	1050

A 3 x 4 Factorial Design

Treatments			Blocks				
larvae	level		1	2	3	4	5
1st stage	1	I	a11	b11	c11	d11	e11
			(3)	(12)	(6)	(1)	(4)
	2	II	a21	b21	c21	d21	e21
			(5)	(2)	(3)	(5)	(2)
	3	III	a31	b31	c31	d31	e31
			(7)	(1)	(12)	(9)	(3)
2nd stage	1	IV	a12	b12	c12	d12	e12
			(4)	(7)	(11)	(8)	(3)
	2	V	a22	b22	c22	d22	e22
			(1)	(3)	(7)	(11)	(6)
	3	VI	a32	b32	c32	d32	e32
			(2)	(5)	(2)	(4)	(1)
3rd stage	1	VII	a13	b13	c13	d13	e13
			(10)	(9)	(10)	(3)	(8)
	2	VIII	a23	b23	c23	d23	e23
			(8)	(4)	(4)	(7)	(7)
	3	IX	a33	b33	c33	d33	e33
			(12)	(10)	(9)	(10)	(9)
no larvae	1	X	a1t	b1t	c1t	d1t	e1t
			(6)	(8)	(5)	(6)	(10)
	2	XI	a2t	b2t	c2t	d2t	e2t

		(9)	(6)	(8)	(12)	(11)
3	XII	a3t	b3t	c3t	d3t	e3t
		(11)	(11)	(1)	(2)	(12)

A 3 x 4 Factorial design for field layout.

1	20 - T51-2	13	40 -T32 -3	25	0 -T123 -1	37	10 -T14 -3	49	40 -T65 - 2
2	40 - T61-2	14	20 -T22 -3	26	40 -T63 -2	38	0 -T124-1	50	20 -T25 - 3
3	10 - T11-3	15	20 -T52 -2	27	20 -T23 -3	39	10 -T74 -1	51	10 -T45 - 2
4	10 - T41-2	16	20 -T82 -1	28	20 -T83 -1	40	40 -T64 -2	52	10 -T15 - 3
5	20 - T21-3	17	40 -T62 -2	29	0 -T103-3	41	20 -T25 -3	53	40 -T35- 3
6	0 - T101-3	18	0 -T112-2	30	10 -T13 -3	42	0 -T104-3	54	20 -T55 - 2
7	40 - T31-3	19	10 -T42 -2	31	20 -T53 -2	43	20 -T84 -1	55	20 -T85 - 1
8	20 - T81-1	20	0 -T102-3	32	0 -T113-2	44	10 -T44 -2	56	10 -T75 - 1
9	0 - T111-2	21	10 -T72 -1	33	40 -T93 -1	45	40 -T34 -3	57	40 -T95 - 1
10	10 -T71-1	22	40 -T92 -1	34	10 -T73 -1	46	40 -T94 -1	58	0 -T105- 3
11	0 - T121-1	23	0 -T122-1	35	10 -T43 -2	47	20 -T54 -2	59	0 -T115- 2
12	40 - T91-1	24	10 -T12 -3	36	40 -T33 -3	48	0 -T114-2	60	0 -T125- 1

5.4. SAS programme for analysis of glasshouse feeding experiment.

```
options ls=78 ps=63 nodate;
data fdglas1;
   do time=1 to 3;
   do level=1 to 4;
   do block=1 to 5;
      input pltwt @@;output;
   end; end;end;
cards;
42.6516 48.3675 45.6125 48.6137 48.2917
33.8269 33.6712 33.6217 33.4125 34.5176
28.6712 28.6415 28.6125 28.6154 29.6441
16.8848 17.6752 17.7125 17.8812 17.6611
50.9941 51.2514 50.6445 50.6120 50.8763
27.3387 27.6153 28.1265 26.3712 33.6543
23.6386 23.8712 21.6615 22.1654 23.6712
13.6939 14.6121 13.6117 12.8812 13.8875
71.4089 55.4401 55.4470 54.6701 57.4551
24.5541 24.1273 24.8732 25.5514 27.3491
```

```
19.8750 20.5103 19.8701 19.7780 23.6127
10.6713 11.2154 10.7612 11.1612 11.0015
;
run;
title 'Analysis of RFB larvae feeding data - Factorial Design';
proc format;
   value dft 1='15days' 2='30days' 3='45days';
   value lvl 1='nolarvae' 2='10larvae' 3='20larvae' 4='40larvae';
run;
proc print ;
run ;
proc glm data=fdglas1;
   format time dft. level lvl.;
   class block level time;
   model pltwt=block time level time*level/ss1;
   means time level time*level/ tukey;
   contrast 'time_l' time -1 0 1;  /*Time_linear effect*/
   contrast 'time_q' time 1 -2 1;  /*Time_quadratic effect*/
   contrast 'level_l' level -3 -1 +1 +3;  /*level_linear effect*/
   contrast 'level_l' level +1 -1 -1 +1;  /*level_quardratic effect*/
   contrast 'level_c' level -1 +3 -3 +1;  /*level_cubic effect*/
run ;

proc glm data=fdglas1;
   class block level time;
     model pltwt=block time level time*level/ss1;
           output out=resid p=pred r=resid;
run;
proc plot data=resid;
plot resid*pred;
run;


/*To create new data set with treatment means to interpret significant
  interaction effects - to tabulate and plot the means*/
proc tabulate data=fdglas1 f=7.2; /*To obtain two-way table of means*/
    class time level;
    var pltwt;
    table (time all),(level all)*pltwt*mean;
run;
proc means data=fdglas1 noprint;
   class time level;
   var pltwt;
output out=mfdglas1 mean=mpltwt;
run;
options ps=30;
proc plot data=mfdglas1;
```

```
    format time dft. level lvl.;
title2 h=1.5 f=swissi j=c 'Plot of factorial means: level vs time';
    plot mpltwt*level=time;
title2 h=1.5 f=swissi j=c 'Plot of factorial means: time vs level';
    plot mpltwt*time=level;
run;
```

5.5 Output from SAS/STAT programme (laboratory and glasshouse experiment).

Table 5.1 Analysis of Laboratory Feeding Experiment, General Linear Models Procedure. Dependent Variable: PLTWT1 (initial weight of ragwort).

Source	DF	SS	MS	F	P
Density_L	1	0.39	0.39	0.28	0.5981
Density_Q	1	0.29	0.29	0.21	0.6513
Density_C	1	0.93	0.93	0.67	0.4166
Time	2	0.82	0.41	0.30	0.7436
Density * time	6	3.64	0.61	0.44	0.8490
Error	48	66.25	1.38	0.40	
Correct total	59	72.32			

Table 5.2 Analysis of Laboratory Feeding Experiment, General Linear Models Procedure. Dependent Variable: PLTWT3 (dry weight of ragwort)

Source	DF	SS	MS	F	P
Density_L	1	0.88	0.88	389.43	0.0001
Density_Q	1	0.33	0.33	144.25	0.0001
Density_C	1	0.08	0.08	35.98	0.0001
Time	2	0.86	0.04	18.91	0.0001
Density * time	6	0.05	0.08	37.15	0.0001
Error	48	0.11	0.002		
Correct total	59	2.31			

Table 5.3 MANOVA Test Criteria and F Approximating for the Hypotheses of no overall Density Effect; no overall Time effect; and no overall * Time Effect.

Source	Wilks' Lambda	F- Value	Numerator df	Denominator df	P
Density	0.0098199	70.7966	9	112.1025	0.0001
Time	0.1964862	19.2583	6	92.0000	0.0001
Density* Time	0.08595	10.0210	18	130.5929	0.0001

Table 5.4 MANOVA Test Criteria and F Approximating for the Hypothesis of no overall Density_L Effect; no overall Density_Q effect; and no overall Density_C Effect.

Source	Wilks' Lambda	F- Value	Numerator df	Denominator df	P
Density_L	0.0234817	637.6558	3	46	0.0001
Density_Q	0.1828412	68.5281	3	46	0.0001
Density_C	0.2118232	57.0541	3	46	0.0001

Table 5.5 Canonical Discriminant Analysis of Laboratory Feeding Experiment of RFB larvae.

Eigenvalue	Difference	Proportion	Cumulative
1. 4.7974	4.5788	0.9532	0.9532
2. 0.2186	0.2016	0.0434	0.9966
3. 0.0170	.	0.0034	1.0000

Table 5.5 shows that the first two canonical variates were sufficient to explain almost all the differences among four densities of RFB larvae with the first dimension accounting for about 95% of the variation. The eigenvalue of the first canonical variate was 4.7974 and was much larger than the second eigenvalue (0.2186) so that the eigenvalue also strongly support the separation between the different four densities of RFB larvae.

Table 5.6 Canonical Discriminant Analysis of Laboratory Feeding Experiment of RFB larvae, Pooled within-class standardized Canonical coefficients.

Variates	CAN1	CAN2
PLTWT1	0.035	-0.137
PLTWT2	1.031	-0.763
PLTWT3	-0.049	1.273

The standardised canonical coefficients (Table 5.6) indicated that the first canonical dimension (CAN1) showed the dominance of PLTWT2. PLTWT1 and PLTWT3 were positively correlated with CAN1, but the PLTWT3 was negatively correlated with CAN1. It was a contrast between PLTWT2 (1.031) and PLTWT3 (-0.049).

The plot of canonical scores (Figure 5.1) showed that 10 and 20 densities of RFB larvae were overlapped and scattered groups. The other two (0, and 40 densities of RFB larvae) were separated positively and negatively from the first canonical dimension. The zero density of RFB larvae was positively affected so that the feeding of RFB larvae cannot be found. The 40 density of RFB larvae was negatively affected, that is the 40 density of RFB larvae were effectively caused damaging to ragwort plant by their feeding. This effect can also be verified by looking at the class means on canonical variables (Table 5.7).

Analysis of Laboratory Feeding Experiment

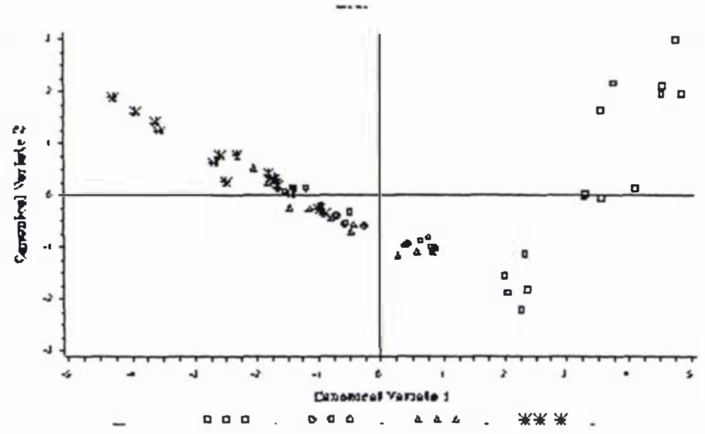


Figure. 5.1 The plot of canonical scores, which showed, scattered groups of four densities of RFB larvae.

Table 5.7 Canonical Discriminant Analysis of Laboratory Feeding Experiment of RFB larvae, class means on canonical variates.

Density	CAN1	CAN2
0 larvae/plant	3.421	0.280
10 larvae/plant	-0.444	-0.422
20 larvae/plant	-0.599	-0.453
40 larvae/plant	-2.378	0.595

Further examination of the difference between the 'four densities of RFB larvae' can be carried out using General Linear Models with canonical variable analysis (CVA) (Table 5.8 and Table 5.9). According to the results of CAN1 and CAN2, the densities of RFB larvae effects were highly significant in CAN1 ($p=0001$) and significant at $p=0.0109$ level at CAN 2 (Table 5.8 and Table 5.9). CAN1 provided a much larger R-sq value the modelling of CAN2 (R-sq value of CAN1=0.82 > R-sq value of CAN2= 0.56). It indicated that the overall variation of density effects along the first canonical dimension was smaller than along the second canonical dimension. The larger F value for CAN1 suggested that the effects of different densities on plant weight might be more prominent along first dimension than that of the second dimension. Note also that in both cases, the Root MSE value was 1 and the overall Mean was 0. That was expected that because the response variables CAN1 and CAN2 were both standardised scores.

The relationships among four densities of RFB larvae can be examined by means of 'contrasts'. The tests associated with contrasts showed linear, quadratic, and cubic effect of densities of RFB larvae feeding on plant weight. It means feeding by various densities of RFB larvae can affect differently on the ragwort plant weights.

Table 5.8 Analysis of Laboratory Feeding Experiment, Canonical Variable Analysis (CVA) with density group using General Linear Models Procedure. Dependent Variable: CAN1.

Source	DF	SS	MS	F	P
Density_L	1	230.10	230.10	231.00	0.0001
Density_Q	1	16.31	16.31	16.31	0.0002
Density_C	1	21.35	21.35	21.35	0.0001
Error	56	56.00	1.00		
Correct total	59	324.66			

Table 5.9 Analysis of Laboratory Feeding Experiment, Canonical Variable Analysis (CVA) with density group using General Linear Models Procedure. Dependent Variable: CAN2.

Source	DF	SS	MS	F	P
Density_L	1	0.63	0.63	0.63	0.4313
Density_Q	1	11.49	11.49	11.49	0.0013
Density_C	1	0.13	0.13	0.13	0.7240
Error	56	56.00	1.00		
Correct total	59	68.24			

Table 5.10 Tukey's Multiple Range Test for variable: CAN1, Alpha=0.05 df=56, MSE=1, Critical value of Studentized Range=3.745, Minimum Significant Difference=0.9669. Means with the same letter are not significantly different.

Density	Mean	N	Turkey Grouping
0 larvae/plant	3.4206	15	A
10 larvae/plant	-0.4442	15	B
20 larvae/plant	-0.5986	15	B
40 larvae/plant	-2.3778	15	C

Table 5.11 Tukey's Multiple Range Test for variable: CAN2 Alpha=0.05 df=56, MSE=1, Critical value of Studentized Range=3.745, Minimum Significant Difference=0.9669. Means with the same letter are not significantly different.

Density	Mean	N	Turkey Grouping
40 larvae/plant	0.5954	15	A
0 larvae/plant	0.2798	15	A B
10 larvae/plant	-0.4219	15	B
20 larvae/plant	-0.4533	15	B

Tukey's test was chosen to show the results from a typical multiple comparison procedure (Table 5.10 and Table 5.11).

Tukey's test showed that all four densities were significant at 5% level. But, the effectiveness by ten and 20 larval introductions were not different and the effectiveness of zero and 40 larval introductions were clearly different from each other with respect to CAN1 while no significant difference can be accounted for between 0 and 40 larval introductions and 10 and 20 larval introductions with respect to CAN2.

Table. 5.12 ANOVA table for factorial design of consumption rates of ragwort by RFB larvae in glasshouse experiment.

Dependent Variable: PLTWTT

Source	DF	SS	MS	F	p
Block	4	26.24	6.56	1.11	0.3625
Time	2	98.48	49.24	8.35	0.0008
Larval density	3	11676.03	3892.01	660.30	0.0001
Density * time	6	753.57	125.60	21.31	0.0001
Error	44	259.35	5.89		
Corrected total	59	12813.67			

Table 5.13 Tukey's Studentized Range (HSD) Test for variable: PLTWT (plant weight), means with the same letter are not significantly different.

(a) Comparison between time (feeding duration) and plant weight.

Time	Mean (g)	N	Tukey Grouping
15 – days	31.73	20	**A**
30 – days	29.06	20	**B**
45 days	28.97	20	**B**

(b) Comparison among Level (RFB larvae densities)

Larval density	Mean (g)	N	Tukey Grouping
No larvae	52.16	15	**A**
10 larvae/plant	29.24	15	**B**
20 larvae/plant	24.19	15	**C**
40 larvae/plant	14.09	15	**D**

5.6 RFB larval feeding rate

RFB larval feeding rate was calculated by modification of the Holling's disc equation (Holling, 1959 – see Chapter 5 references). The construction of the model was based on the laboratory experimental design so that three feeding duration and four larval densities were considered in the model.

Ragwort rosette weight without RFB larval effect during fifteen days feeding.

The average rosette plant weight without RFB larval infestation was calculated from the following formula.

$$\mathbf{FT = n^{-1} \Sigma a_i} \text{ ----------------(1)}$$

where FT was the average rosette weight, n - number of ragwort plants, and a_i - rosette plant weight (i= 1 to n).

Ragwort rosette weight with ten RFB larval effect during fifteen days feeding.

The average rosette plant weight with ten RFB larval infestation was calculated from the following formula.

$$\mathbf{FT_{10} = n^{-1} \Sigma d_i} \text{ ----------------(2)}$$

where FT_{10} was the average rosette weight, n - number of ragwort plants, and d_i - rosette plant weight (i= 1 to n).

One day average reduced plant weight ten larval effect or one day feeding amount or consumption rate per introduced larvae can be calculated by equation (1) - (2)/ t, that is,

$$\mathbf{FTX_{10} = (FT - FT_{10}) / t} \text{ ----------- (3)}$$

where FTX_{10} - one-day average reduced plant weight by ten larval effects, t - feeding duration. One RFB larva feeding amount over time or consumption rate can be calculated as equation (3) / N, that is,

$$\mathbf{FT\,(E)_{10} = FTX_{10} / N} \text{ -----------(4)}$$

where FT $(E)_{10}$ - one RFB larva consumption rate per day, N - number of RFB larvae introduced for the treatment. In this treatment 5 replicates were used with ten larvae introduced for each replicate.

The previous formulae were followed, inserting the appropriate values for the density of larvae or the duration of the experiment. The formulae were repeated for densities of 10, 20, 40 larvae/plant and durations of 15-days, 30-days, and 45-days larvae feeding inside roots or rootcrowns. The resulted values from calculating these formulae were used to calculate the mean feeding rate of RFB larvae, as follows:

$$\mathbf{FR\,(E) = n^{-1}\,[FR\,(E)_{15} + FR(E)_{30} + FR(E)_{45}\,]} \text{ -------------(5)}$$

where FR (E) - average feeding rate of a RFB larvae, n = 3, and $FR(E)_{15}$ – average feeding rate of a 15-days old larva, $FR(E)_{30}$ – average feeding rate of a 30-days old larva, and $FR(E)_{45}$ – average feeding rate of a 45-days old larva.

Ragwort plant weight gained from 15 to 75 days

Ragwort plants free of attack by RFB larvae were weighed at 15 days, 30 days, 45 days, 60 days, and 75 days. Mean values of plant weight and plant weight gained per day were calculated as follow:

$PWX_{15} = n^{-1} \sum Sd_i$ -----------------(1)

$PWX_{30} = n^{-1} \sum Rd_i$ -----------------(2)

$PWX_{45} = n^{-1} \sum Rtd_i$ -----------------(3)

$PWX_{60} = n^{-1} \sum Rsd_i$ -----------------(4)

$PWX_{75} = n^{-1} \sum Rsfd_i$-----------------(5)

where PWX_{15} to PWX_{75} were average plant weight from 15 days to 75 days, n - number of plants measured, Sd_i, Rd_i, Rtd_i, Rsd_i, $Rsfd_i$ were ragwort plant weight from 15 to 75 days,

$G15 = PWX_{15} / N$ -----------------(6)

$G30 = PWX_{30} / N$ -----------------(7)

$G45 = PWX_{45} / N$ -----------------(8)

$G60 = PWX_{60} / N$ -----------------(9)

$G75 = PWX_{75} / N$ -----------------(10)

where G15 to G75 were plant weight gained per day from 15 to 75 days, N - number of measurements. The average weight gained per day from 15 to 75 days (WtG) can be calculated as:

$WtG = n^{-1} [G15 + G30 + G45 + G60 + G75]$ ------------- (11)

The weight gained from 15 to 75 days is shown in Table 3.15 and Figure 3.3. The highest average weight gain of ragwort can be found between 15 to 30 days, after which weight gain stabilising before rising again from 45 days.

How many larvae need to kill a ragwort plant (in the laboratory)?

The numbers of RFB larvae needed to kill a single rosette ragwort plant can be estimated from the following equation.

Numbers of larvae = **WtG / FR** (E) ---------------------------(12)

where WtG - the ragwort plant mean weight gained per day and FR (E) - mean RFB larvae feeding rate.

Appendices for Chapter. 6.

6.1. Split plot design for movement of RFB larvae

Block 1			Block 2			Block 3			Block 4		
sand	silt	sa + si	sand	silt	sa + si	sand	silt	sa + si	sand	silt	sa + si
B	C	C	E	A	C	B	D	F	E	H	C
A	F	H	B	D	I	F	F	E	C	D	I
D	G	A	F	H	H	E	H	B	D	A	G
F	B	E	D	C	E	G	B	H	A	G	E

I	A	J	G	E	A	I	C	I	G	C	A
C	H	B	A	I	D	C	A	C	B	F	D
G	E	G	H	B	F	D	I	A	F	E	H
E	I	D	C	G	B	H	G	D	H	I	F
H	D	F	I	F	G	A	E	G	I	B	B

SAS programme for analysis of movement of RFB larvae.

```
options ls=78 ps=63 nodate;
data movet2;
     do soil=1 to 3;
        do sect=1 to 9;
           do block=1 to 4;
     input number @@; output;
   end;end;end;
cards;
30 41 38 42
 0  1  2  1
 9 12 10 11
30 25 21 24
 0  1  2  2
 6  5  7  5
22 12 17 12
 0  1  0  1
 3  2  3  2
32 35 30 30
 1  2  1  2
10  6  8 11
30 40 32 29
 0  1  1  1
 5  3  7  5
21 10 20 20
 0  0  1  0
 1  3  0  2
32 30 33 32
 3  2  2  2
 8 12 11 12
32 38 36 35
 1  1  0  1
 5  6  7  6
17  9  8 10
 0  1  2  1
 2  1  1  1
;
run;

title 'Movement of RFB - Split Plot Design';
proc format;
     value soil 1='Sand' 2='Silt' 3='SaSi';
     value sect 1='A' 2='B' 3='C' 4='D' 5='E' 6='F'
                7='G' 8='H' 9='I';                 /* A= mid- centre, B= cotton wool,
                                                    C= ragwort roots, D= down-
centre,
                                                    E= down cotton wool, F= down
ragwort roots, G=up- centre, H= up                                          cotton
wool, I
```

```
                                                         =ragwort roots.*/
run;
proc glm data=movet2;
     format soil soil. sect sect.;
     class block sect soil;

    /*To fit the full split plot model*/
     model number=block soil block*soil
                  sect soil*sect block*sect(soil)/ss1;
run;

/*To obtain main plot ANOVA*/
test h=block soil e=block*soil/htype=1 etype=1;
/*To obtain split plot ANOVA*/
test h=sect soil*sect e=block*sect(soil)/htype=1 etype=1;

/*To perform MCPs*/
means soil/duncan tukey e=block*soil etype=1;
means sect/duncan tukey e=block*sect(soil) etype=1;
proc tabulate data=movet2;
     format soil soil. sect sect.;
     class soil sect;
     var number;
     table (soil all),(sect all)*number*mean;
run;

proc means data=movet2 noprint;
     class soil sect;
     var number;
output out=mmovet2 mean=mnumber;
run;

proc glm data=movet2;
     format soil soil. sect sect.;
     class block sect soil;

    /*To fit the full split plot model*/
     model number=block soil block*soil
                  sect soil*sect block*sect(soil)/ss1;
                     output out=resid p=pred r=resid;
run;
proc plot data=resid;
plot resid*pred;
run;
```

Appendices for Chapter.7.

7.1 SAS programme for analysis of RFB larvae distance movement.

```
options ls=78 ps=63 nodate;
data distmov;
   do dist=1 to 2;
   do time=1 to 3;
   do larv=1 to 4;
   do block=1 to 4;
```

```
    input larvae @@;output;
  end; end;end;end;
cards;
0 0 0 0
0 1 1 0
0 1 0 0
0 0 1 0
0 0 0 0
0 0 0 1
0 0 1 1
1 1 1 0
0 0 0 0
0 0 1 0
0 0 0 1
1 4 4 1
0 0 0 0
1 0 0 0
0 0 0 1
0 0 0 0
0 0 0 0
0 0 0 0
0 0 0 0
0 0 0 0
0 0 0 0
0 0 0 0
0 0 0 0
0 0 0 0
;
run;
title 'Analysis of RFB larvae distance movement - Factorial Design';
proc format;
   value dis 1='50mm' 2='100mm';
   value dft 1='15days' 2='30days' 3='45days';
   value lvl 1='nolarvae' 2='10larvae' 3='20larvae' 4='40larvae';
run;
proc print ;
run ;
proc glm data=distmov;
   format dist dis. time dft. larv lvl.;
   class block larv time dist;
   model larvae=block time larv dist;
   contrast 'dist_l' dist -1 1; /*dist_linear effect*/
   contrast 'time_l' time -1 0 1; /*time_linear effect*/
   contrast 'time_q' time 1 -2 1; /*time_quadratic effect*/
   contrast 'larv_l' larv -3 -1 1 3; /*larv_linear effect*/
   contrast 'larv_q' larv 1 -1 -1 1; /*larv_quadratic effect*/
   contrast 'larv_c' larv -1 3 -3 1; /*larv_cubic effect*/
run ;
proc glm data=distmov;
   format dist dis. time dft. larv lvl.;
   class block larv time dist;
   model larvae=block time larv time*larv/ss1;
   means time larv time*larv/ tukey;
run ;
proc glm data=distmov;
   format dist dis. time dft. larv lvl.;
```

```
    class block larv time dist;
    model larvae=block time dist time*dist/ss1;
    means time dist time*dist/ tukey;
run ;
proc means data=distmov noprint;
    class dist time larv;
    var larvae;
output out=mdistmov mean=mlarvae;
run;

options ps=30;
proc plot data=mdistmov;
    format dist dis. time dft. larv lvl.;
    title2 'Plot of factorial means: dist vs time';
        plot mlarvae*dist=time;
    title2 'Plot of factorial means: time vs dist';
        plot mlarvae*time=dist;
run;
proc means data=distmov noprint;
    class dist time larv;
    var larvae;
output out=mdistmov mean=mlarvae;
run;

options ps=30;
proc plot data=mdistmov;
    format dist dis. time dft. larv lvl.;
    title2 'Plot of factorial means: larv vs time';
        plot mlarvae*larv=time;
    title2 'Plot of factorial means: time vs larv';
        plot mlarvae*time=larv;
run;
```

Appendices for Chapter 8.

8.1 Weather records for experimental plots at Massey Campus.

Data information
Logger
Type TinytalkII Humidity
Minimum reading 50.1 (%RH)
Maximum reading 99.1 (%RH)
Average reading 79 (%RH)

25/1/1998	58.1	23.15	5/2/1998	73.24	22.69
26/1/1998	60.18	20.94	6/2/1998	75.17	21.76
27/1/1998	66.08	22.36	7/2/1998	75.3	21.45
28/1/1998	65.64	23.29	8/2/1998	73.52	21.92
29/1/1998	68.17	21.77	9/2/1998	74.33	22.01
30/1/1998	76.19	22.11	10/2/1998	76.83	19.94
31/1/1998	77.7	19.88	11/2/1998	73.26	21.13
1/2/1998	73.99	19.59	12/2/1998	71.84	22.88
2/2/1998	77.55	19.14	13/2/1998	69.48	23.21
3/2/1998	74.84	21.16	14/2/1998	71.11	22.72
4/2/1998	74.31	22.48	15/2/1998	75.98	21.62

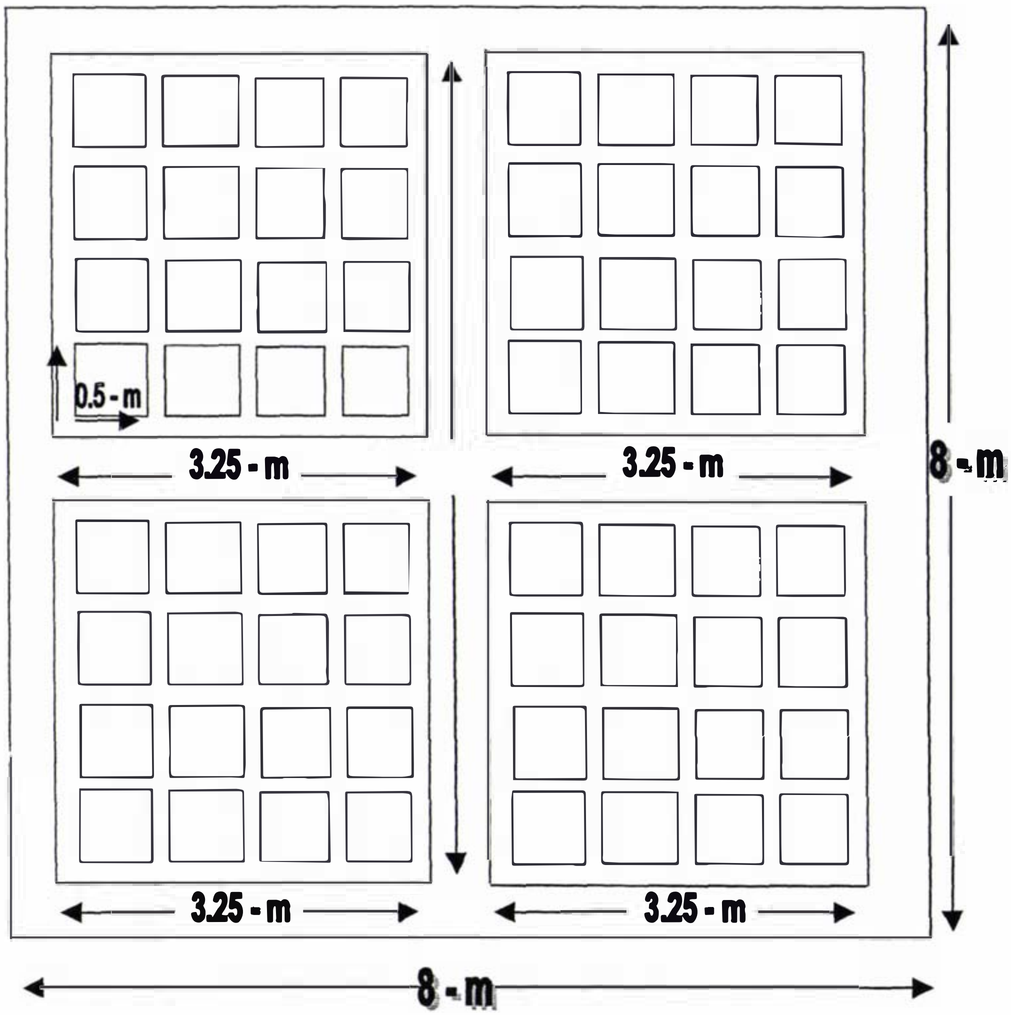
16/2/1998	74.23	22.69	1/4/1998	80.23	17.03
17/2/1998	71.52	23.23	2/4/1998	86.99	11.71
18/2/1998	75.03	18.55	3/4/1998	83.43	16.42
19/2/1998	72.98	20.32	4/4/1998	81.25	14.15
20/2/1998	67.73	21.95	5/4/1998	83.44	14.11
21/2/1998	69.78	20.94	6/4/1998	76.94	15.53
22/2/1998	73.26	18.85	7/4/1998	78.45	15.74
23/2/1998	74.46	18.25	8/4/1998	77.48	16.55
24/2/1998	81.75	13.61	9/4/1998	81.39	16.32
25/2/1998	78.19	17.13	10/4/1998	85.03	15.40
26/2/1998	74.28	17.37	11/4/1998	83.22	15.28
27/2/1998	73.66	19.96	12/4/1998	83.63	15.59
28/2/1998	75.58	18.81	13/4/1998	82.79	17.45
1/3/1998	77.83	17.59	14/4/1998	82.03	18.11
2/3/1998	74.25	17.54	15/4/1998	86.45	12.59
3/3/1998	74.81	18.46	16/4/1998	85.63	13.18
4/3/1998	71.86	19.09	17/4/1998	80.33	14.81
5/3/1998	74.52	19.10	18/4/1998	82.14	11.74
6/3/1998	74.03	20.03	19/4/1998	85.69	12.27
7/3/1998	74.01	20.25	20/4/1998	82.93	13.62
8/3/1998	74.83	20.88	21/4/1998	80.88	14.93
9/3/1998	73.62	20.41	22/4/1998	80.38	15.51
10/3/1998	73.79	21.12	23/4/1998	82.42	17.23
11/3/1998	77.99	19.33	24/4/1998	85.42	14.96
12/3/1998	81.4	17.41	25/4/1998	88.43	13.24
13/3/1998	78.54	19.37	26/4/1998	85.85	14.45
14/3/1998	76.19	21.23	27/4/1998	84.11	16.80
15/3/1998	82.93	14.48	28/4/1998	85.27	17.07
16/3/1998	80.83	15.43	29/4/1998	90.95	14.38
17/3/1998	76.82	15.50	30/4/1998	90.48	14.11
18/3/1998	75.75	16.75	1/5/1998	89.78	14.55
19/3/1998	74.36	17.58	2/5/1998	90.29	11.96
20/3/1998	72.91	17.83	3/5/1998	90.65	13.39
21/3/1998	71.68	19.15	4/5/1998	90.96	10.12
22/3/1998	70.69	16.39	5/5/1998	90.54	10.17
23/3/1998	73.43	17.22	6/5/1998	90.9	11.21
24/3/1998	71.03	18.56	7/5/1998	89.34	13.50
25/3/1998	69.98	19.35	8/5/1998	89.35	13.84
26/3/1998	76.33	16.80	9/5/1998	91.93	13.40
27/3/1998	78.15	17.09	10/5/1998	93.11	10.97
28/3/1998	80.06	18.62	11/5/1998	95.63	11.03
29/3/1998	80.63	17.24	12/5/1998	93.86	12.64
30/3/1998	80.74	16.75	13/5/1998	93.13	14.38
31/3/1998	80.28	14.09	14/5/1998	93.45	14.58

First	reading	time	"Thursday,"		May	"14,"	1998	14:48:15
Last	reading	time	"Thursday,"		August	"13,"	1998	10:48:15
Readings		1091						
Minimum		reading	59.4	(%RH)				
Maximum		reading	109.3	(%RH)				
Average	reading	103.6	(%RH)					

14/5/1998	82.36	8.96	17/5/1998	92.68	10.13
15/5/1998	93.09	10.17	18/5/1998	95.68	9.33
16/5/1998	96.98	11.74	19/5/1998	97	8.97

20/5/1998	95.65	10.21	2/7/1998	107.46	9.68
21/5/1998	99.39	10.93	3/7/1998	102.68	8.67
22/5/1998	97.87	13.48	4/7/1998	102.56	8.67
23/5/1998	99	13.80	5/7/1998	104.23	6.06
24/5/1998	106.33	13.51	6/7/1998	104.53	7.88
25/5/1998	102.43	13.26	7/7/1998	106.75	9.77
26/5/1998	105.76	11.87	8/7/1998	107.88	10.59
27/5/1998	105.62	10.27	9/7/1998	108.59	11.62
28/5/1998	101.58	10.80	10/7/1998	105.37	11.68
29/5/1998	101.11	12.40	11/7/1998	108.17	10.68
30/5/1998	101.93	13.35	12/7/1998	105.23	10.23
31/5/1998	90.54	9.00	13/7/1998	104.1	9.37
1/6/1998	97.48	10.34	14/7/1998	106.33	9.91
2/6/1998	105.05	11.37	15/7/1998	107.03	9.86
3/6/1998	86.97	6.85	16/7/1998	108.73	9.92
4/6/1998	102.93	8.61	17/7/1998	103.67	8.19
5/6/1998	99.95	7.10	18/7/1998	104.94	7.74
6/6/1998	102.25	8.78	19/7/1998	103.79	8.87
7/6/1998	104.37	11.39	20/7/1998	104.64	8.49
8/6/1998	107.61	13.91	21/7/1998	103.16	10.58
9/6/1998	106.47	13.25	22/7/1998	106.18	11.61
10/6/1998	102.23	9.29	23/7/1998	107.6	11.60
11/6/1998	99.28	8.81	24/7/1998	104.92	10.48
12/6/1998	103.08	10.39	25/7/1998	101.81	9.72
13/6/1998	104.34	12.90	26/7/1998	99.71	10.04
14/6/1998	104.49	11.85	27/7/1998	105.62	10.76
15/6/1998	94.81	8.83	28/7/1998	103.37	9.28
16/6/1998	99.48	9.71	29/7/1998	104.2	11.60
17/6/1998	102.93	11.73	30/7/1998	106.05	11.93
18/6/1998	103.82	9.45	31/7/1998	107.03	11.03
19/6/1998	104.21	7.41	1/8/1998	109.16	10.67
20/6/1998	104.92	7.14	2/8/1998	107.88	9.45
21/6/1998	105.62	9.20	3/8/1998	107.03	5.27
22/6/1998	102.08	10.36	4/8/1998	108.17	7.41
23/6/1998	105.33	9.14	5/8/1998	109.16	9.06
24/6/1998	101.09	10.25	6/8/1998	109.16	10.53
25/6/1998	108.88	11.76	7/8/1998	109.3	8.53
26/6/1998	103.28	11.16	8/8/1998	108.88	6.98
27/6/1998	101.27	8.12	9/8/1998	107.18	7.81
28/6/1998	105.07	8.29	10/8/1998	109.3	10.88
29/6/1998	104.94	9.19	11/8/1998	109.3	12.63
30/6/1998	102.56	9.78	12/8/1998	109.3	11.24
1/7/1998	109.16	11.96	13/8/1998	109.3	

8.2 A sub plot sample of field layout on 4 x 4 factorial design.



8.3 Percent soil water from Massey Campus.

15-days				
	W1	W2	W3	%
1	3.4	38.68	34.9	12
2	2.8	47.05	42.5	11.46
3	3.1	49.74	44.6	12.39
4	2.7	48.71	43.2	13.6
5	3.2	42.31	38.4	11.11
6	3.1	41.19	37.6	10.41
7	2.8	36.04	32.4	12.3
8	2.9	40.7	36.8	11.5
9	3.2	47.07	42.1	12.78
10	3	44.27	39.7	12.45
	30.2	435.76	392.2	120

1	2.6	61.83	45.64	37.62
2	2.8	58.64	43.72	36.46
3	2.7	52.87	38.61	39.71
4	3.1	58.83	42.55	41.27
5	3	58.75	43.27	38.44
6	2.8	55.67	40.39	40.65
7	2.9	47.49	35.2	38.05
8	2.8	51.09	38.46	35.42
9	3	42.91	32.74	34.2
10	3.2	56.85	41.8	38.99
	28.9	544.93	402.38	380.81
1	2.7	55.11	34.95	62.51
2	3.1	56.4	35.28	65.63

3	3	61.95	42.64	48.71
4	2.8	54.67	33.71	67.81
5	2.9	60.57	40.6	52.97
6	2.8	61.73	38.55	64.84
7	2.6	51.83	33.42	59.73
8	2.7	68.15	42.17	65.82
9	3	59.48	38.65	58.43
10	2.8	52.88	31.62	73.76
	28.4	582.77	371.59	620.21

1	3.3	67.38	39.54	76.82
2	3.2	71.74	41.72	77.93
3	2.9	56.49	32.63	80.26
4	2.8	46.72	28.67	69.77
5	3.1	75.18	45.39	70.44
6	2.7	72.41	41.72	78.65
7	2.8	62.67	38.25	68.89
8	3.1	48.13	28.62	76.45
9	3	56.44	33.55	74.93
10	3.2	61.01	34.76	83.17
	30.1	618.17	364.85	757.31

1	2.9	67.92	45.23	53.6
2	2.6	58.44	39.52	51.25
3	2.8	62.44	42.65	49.66
4	3	72.24	47.72	54.83
5	2.8	54053	36.74	52.42
6	2.9	63.5	43.67	48.64
7	2.7	49.59	33.85	50.53
8	3.1	61.06	41.76	49.92
9	3	57.62	38.64	53.25
10	2.8	58.69	38.33	57.3
	28.6	54604.5	408.11	521.4

30-days

	W1	W2	W3	%
1	2.7	61.77	38.54	64.82
2	2.8	55.71	34.82	65.24
3	2.7	57	35.26	66.77
4	2.6	67.21	42.13	63.45
5	3	60.84	37.37	68.29
6	2.9	64.82	40.58	64.33
7	2.8	63.93	38.65	70.52
8	2.7	54.55	35.32	58.95
9	3	61.65	38.67	64.42
10	2.8	66.44	41.79	63.22
	28	613.92	383.13	650.01

1	2.9	73.8	41.72	82.64
2	2.8	70.28	39.56	83.57
3	2.7	50.74	28.65	85.13
4	2.8	78.69	45.33	78.44
5	3	53.95	31.65	77.84
6	2.9	51.13	28.76	86.5
7	2.9	52.74	30.49	80.65
8	2.8	51.65	29.54	82.54
9	3	56.74	33.17	78.12
10	2.7	58.04	32.65	84.77
	28.5	597.76	341.52	820.2

1	3.1	71.13	42.2	73.99
2	3	58.3	34.51	75.5
3	3.1	65.77	38.62	76.44
4	2.9	71.23	42.16	74.04
5	2.8	76.42	45.44	72.65
6	2.8	56.12	34.27	69.43
7	3	61.47	37.65	68.74
8	2.7	59.98	35.43	75
9	3.1	69.6	40.79	76.44
10	3	75.32	43.68	77.77
	29.5	665.34	394.75	740

1	3.2	55.95	38.6	49.01
2	3	44.01	33.54	50.65
3	3	62.24	42.12	51.43
4	3.1	53.26	36.83	48.71
5	3.2	58.88	41.37	45.87
6	2.9	52.45	35.42	52.37
7	2.9	41.4	28.63	49.62
8	3.1	46.41	32.4	47.82
9	3.2	61.12	41.7	50.44
10	3	55.64	39.54	44.06
	30.6	531.36	370.15	489.98

1	3.1	55.6	41.7	36.01
2	2.9	52.06	38.64	37.55
3	2.8	46.88	34.23	40.25
4	3	56.66	43.51	32.46
5	2.8	61.76	45.33	38.63
6	3.1	51.68	37.61	40.77
7	2.9	47.28	35.47	36.26
8	2.7	42.97	32.61	34.64
9	2.8	54.14	41.2	33.7
10	3	45.43	35.7	29.76
	29.1	514.46	386	360.03

45-Days

	W1	W2	W3	%
1	2.8	60.18	45.3	35.01
2	2.7	57.37	42.65	36.85
3	3	50.63	38.21	35.27
4	2.9	48.91	37.61	32.56
5	2.7	56.02	41.44	37.64
6	2.7	46.8	35.27	35.4
7	2.8	55.47	42.16	33.82
8	3	58.86	43.35	38.44

9	3	39.23	29.9	34.68
10	2.9	49.35	38.54	30.33
	28.5	522.82	394.43	350
1	3.4	37.67	28.6	35.99
2	3.1	48.56	36.42	36.43
3	3.2	42.23	31.56	37.62
4	2.9	54.8	42.31	31.69
5	3	62.74	47.64	33.83
6	3.1	53.5	39.5	38.46
7	3.3	48.36	37.66	31.14
8	3.2	43.8	32.19	40.05
9	3	45.18	33.73	37.26
10	2.9	54.8	40.67	37.41
	31.1	491.64	370.28	359.88
1	3.3	42.59	32.4	35.02
2	3	47.92	35.97	36.25
3	3.1	57.94	42.63	38.73
4	3.1	53.18	38.71	40.63
5	2.9	47.43	36.5	32.53
6	3.2	50.51	39.41	30.65
7	3.1	54.55	41.26	34.83
8	2.9	50.34	38.64	32.74
9	3	42.22	31.75	36.42
10	3	43.32	33.45	32.41

	30.6	490	370.72	350.21
1	3.3	66.59	41.7	64.82
2	3	73.06	45.39	65.27
3	2.9	66.98	43.35	58.42
4	3.1	62.67	38.62	67.71
5	3.2	61.02	37.57	68.23
6	3	64.96	42.81	55.64
7	2.9	69.22	44.63	58.93
8	3	63.03	38.9	67.21
9	3	62.36	37.65	71.31
10	2.9	75.8	45.2	72.34
	30.3	665.69	415.82	649.88
1	2.9	54.14	39.5	40
2	2.8	48.62	34.92	42.65
3	2.8	49.98	35.62	43.75
4	3	61.6	45.34	38.4
5	2.7	65.4	48.26	37.62
6	2.7	51.33	37.81	38.51
7	2.9	63.76	45.35	43.37
8	2.6	62.28	44.7	41.76
9	2.7	65.74	47.66	40.21
10	3	52.2	39.75	33.88
	28.1	575.05	418.91	400.15

8.4 Data from feeding experiment at Massey University Campus. (Rep-replication, Treat – treatment, Wetwt –wet weight, Drywt – dry weight, lva – ragwort flea beetle larvae).

	Rep 1			Rep 2			Rep 3			Rep 4		
Treat	Wetwt	Drywt	lva	Wetwt	Drywt	lva	Wetwt	Drywt	lva	Wetwt	Drywt	lva
t01100	214.1	24.9938	0	220.31	30.7653	0	218.36	29.9457	0	198.66	27.4375	0
t02110	145.93	20.2247	1	150.65	21.3216	2	138.52	17.8864	2	148.71	20.9215	1
t03120	155.7	21.8645	2	140.31	19.5437	2	135.44	17.3471	3	138.44	18.8654	3
t04140	75.65	8.4226	3	120.32	14.8725	2	101.54	13.1257	3	115.72	14.0032	3
t05200	253.07	29.8156	0	223.78	30.4068	0	225.65	29.7034	0	252.87	32.7532	0
	194.48	23.998	0	224.54	31.6387	0	220.61	30.3782	0	196.44	28.1673	0
Total	447.55	53.8136	0	448.32	62.0455	0	446.26	60.0816	0	449.31	60.9205	0
Mean	223.775	26.9068		224.14	31.0228		223.13	30.0408		224.65	30.4605	
t06210	185.79	26.4066	1	150.63	25.7735	2	190.46	27.0743	1	165.47	23.4026	2
	151.96	25.9487	2	186.54	26.5073	1	174.55	25.0067	2	145.63	21.3247	2
Total	337.75	52.3553	3	337.17	52.2808	3	365.01	52.081	3	311.1	44.7273	4
Mean	168.875	26.1777	1.5	168.585	26.1401	1.5	182.505	26.0405	1.5	155.55	22.3637	2
t07220	120.97	16.0029	2	95.65	12.5438	3	115.68	13.8743	3	130.45	17.2019	2
	110.21	13.2804	1	128.64	17.329	2	101.73	13.0457	3	88.64	10.2983	3
Total	231.18	29.2833	3	224.29	29.8728	5	217.41	26.92	6	219.09	27.5002	5
Mean	115.59	14.6417	1.5	112.145	14.9364	2.5	108.705	13.46	3	109.545	13.75	2.5
t08240	101.27	13.0254	2	120.82	14.6752	3	108.73	13.8753	2	99.64	12.8371	3
	93.02	12.3188	4	101.55	13.237	3	95.32	12.11	3	93.73	12.3207	4
Total	194.29	25.3442	6	222.37	27.9122	6	204.05	25.9853	5	193.37	25.1578	7
Mean	97.145	12.6712	3	111.185	13.9561	3	102.025	12.9927	2.5	96.685	12.5789	3.5

t09400	190.24	26.9051	0	177.68	24.5023	0	136.85	17.2008	0	134.53	16.6201	0
	132.28	16.4449	0	186.53	26.7013	0	188.65	26.33	0	181.27	24.9347	0
	116.72	15.1662	0	120.54	15.8806	0	180.91	24.8821	0	190.66	26.9112	0
	187.65	26.5642	0	133.82	16.7045	0	129.64	15.8762	0	145.55	16.6378	0
Total	626.89	85.0804	0	618.57	83.7887	0	636.05	84.2891	0	652.01	85.1038	0
Mean	156.723	21.2701		154.642	20.9478		159.012	21.0723		163.003	21.276	
t10410	168.09	21.5672	1	120.49	15.8783	2	181.75	24.9357	1	101.35	12.9012	3
	175.88	23.8764	1	165.73	23.2248	1	98.74	12.4311	3	146.54	20.8766	2
	166.2	23.4539	1	145.39	20.8731	2	160.36	21.8754	2	177.19	25.0023	1
	100.87	12.8812	2	169.04	23.3201	1	169.45	22.6431	2	165.83	22.2477	1
Total	611.04	81.7787	5	600.65	83.2963	6	610.3	81.8853	8	590.91	81.0278	7
Mean	152.76	20.4447	1.25	150.163	20.8241	1.5	152.575	20.4713	2	147.728	20.257	1.75
t11420	108.58	13.8762	2	135.72	16.8341	2	102.45	11.9023	3	145.87	20.2088	1
	134.48	16.7326	1	127.09	15.5402	3	140.87	19.0783	2	120.26	14.5378	2
	136.46	16.4955	1	100.65	11.7034	3	99.36	11.7005	3	122.61	13.8756	2
	99.28	11.6423	2	105.33	12.6735	2	125.81	15.3022	2	101.82	11.7032	3
Total	478.8	58.7466	6	468.79	56.7512	10	468.49	57.9833	10	490.56	60.3254	8
Mean	119.7	14.6867	1.5	117.198	14.1878	2.5	117.123	14.4958	2.5	122.64	15.0814	2
t12440	86.63	9.7541	3	129.64	14.2211	2	95.48	10.9931	3	131.55	14.6083	2
	106.09	12.896	2	115.83	13.882	3	130.71	14.5736	2	122.44	13.9906	2
	85.74	9.7321	3	91.04	10.8723	4	100.66	12.4631	3	80.47	8.6115	3
	139.54	15.4491	1	80.67	8.6341	4	90.47	10.7743	4	92.13	10.9974	3
Total	418	47.8313	9	417.18	47.6095	13	417.32	48.8041	12	426.59	48.2078	10
Mean	104.5	11.9575	2.25	104.295	11.9024	3.025	104.33	12.201	3	106.648	12.052	2.5
t13800	170.69	22.4439	0	181.53	30.6744	0	126.21	14.2136	0	182.63	30.7642	0
	179.08	29.6216	0	177.92	29.2371	0	135.47	17.8542	0	100.27	12.7933	0
	100.22	12.7921	0	155.34	21.861	0	120.54	13.8358	0	163.54	21.4002	0
	163.45	21.4047	0	180.25	30.5716	0	179.65	29.4732	0	153.77	21.0035	0
	133.98	17.195	0	145.71	20.4423	0	171.35	28.9431	0	125.48	14.2095	0
	125.04	14.207	0	120.66	13.8754	0	.	.	.	177.8	28.8973	0
	.	.	.	135.44	17.3421	0	.	.	.	130.62	17.2264	0
	.	.	.	118.64	13.4762	0	.	.	.	.	.	.
Total	872.46	117.664	0	1215.49	177.480	0	733.22	104.319	0	1034.11	146.294	0
Mean	145.41	19.617		151.936	22.185		146.644	20.864		147.73	20.8992	
t14810	126.15	16.0092	1	140.34	18.4321	1	125.38	15.4764	1	145.27	18.9732	1
	102.67	12.8964	1	110.73	13.7364	2	65.87	5.6422	3	105.37	13.5571	3
	127.83	15.1991	1	75.92	9.4781	3	79.37	9.4195	3	75.15	9.4093	3
	79.87	9.4209	2	125.77	15.8547	1	135.77	16.8432	1	130.15	16.0579	2
	76.1	9.561	3	130.42	16.1544	1	127.31	15.0095	2	81.43	9.6105	3
	69.88	5.8712	3	70.25	6.8722	3	80.78	9.5422	3	98.06	12.2271	3
	124.84	15.8405	1	80.75	9.5431	3	.	.	.	125.88	15.9708	1
	.	.	.	86.83	10.2274	3	.	.	.	118.99	14.3791	2
Total	707.34	84.7983	12	821.01	100.298	17	614.48	71.933	13	880.3	110.185	18
Mean	101.049	12.114	1.5	102.626	12.5373	2.125	102.413	11.9888	2.167	110.038	13.7731	2.25
t15820	123.87	16.1538	1	120.54	16.0043	2	89.52	8.8723	3	135.44	16.9932	1
	74.88	9.4991	3	132.38	16.8542	2	75.05	9.0034	3	116.71	15.0003	2
	115.83	14.557	2	90.11	8.8934	3	123.65	16.0872	1	132.25	16.8027	1
	117.71	15.1399	2	75.42	9.5013	4	118.17	15.2004	2	72.66	7.3519	3
	89.12	8.8703	3	115.22	14.3581	2	70.82	7.2577	3	81.55	7.6893	3
	66.7	5.2821	3	65.77	6.0021	4	90.24	8.9002	3	115.73	14.5478	2
	69.96	5.7512	3	.	.	.	115.29	14.3581	2	.	.	.
	115.43	14.5547	2	.	.	.	120.33	15.8788	2	.	.	.
Total	773.5	89.8081	19	599.44	71.6134	17	803.07	95.5581	19	654.34	78.3852	12
Mean	96.6875	11.226	2.38	99.9067	11.9356	2.8	100.384	11.9448	2.375	109.057	13.0642	2
t16840	67.45	8.0527	3	101.65	12.0097	2	65.54	8.0077	4	100.82	12.0199	4

	103.79	12.2882	2	65.43	8.1573	6	83.37	9.5761	4	115.63	13.876	4
	111.61	13.399	2	88.17	9.7562	4	120.65	14.0254	2	103.76	12.2788	3
	103.67	12.2771	3	115.73	13.8764	3	110.91	13.2475	3	65.74	8.1663	5
	94.71	10.0451	8	102.22	12.2559	3	83.22	9.5755	4	85.25	9.6783	5
	83.21	9.5759	6	70.1	8.9733	5	113.47	13.4527	3	94.16	10.0079	4
	62.51	8.4678	6	95.46	10.1253	5	.	.	.	120.14	14.0007	3
	.	.	.	100.73	12.0087	4	.	.	.	77.62	9.1268	4
Total	626.95	74.1058	30	739.49	87.1628	32	577.16	67.8849	20	763.12	89.1547	32
Mean	89.5643	10.5865	4.3	92.4363	10.8954	4	96.1933	11.3143	3.3	95.39	11.1443	4
t01100	234.43	31.1315	0	280.45	38.6544	0	287.65	40.5412	0	285.64	40.6718	0
t02110	202.23	28.0771	1	185.43	25.4677	1	195.44	27.6419	1	198.64	27.9861	1
t03120	144.68	19.7202	3	150.46	20.3645	2	154.38	21.0087	2	140.75	19.6425	3
t04140	126.2	16.5567	3	149.57	20.6612	3	116.43	15.4781	2	123.21	16.2068	3
t05200	303.75	44.7681	0	250.64	30.7125	0	287.37	40.5541	0	258.77	31.3415	0
	230.87	27.7272	0	284.55	40.3617	0	261.88	30.1764	0	265.31	32.8743	0
Total	534.62	72.4953	0	535.19	71.0742	0	549.25	70.7305	0	524.08	64.2158	0
Mean	267.31	36.2477		267.595	35.5371		274.625	35.3653		262.04	32.1079	
t06210	228.48	29.3767	1	197.64	28.4166	1	248.93	31.6623	1	225.43	29.0647	1
	268.49	32.7954	1	201.45	29.0054	1	217.64	28.8763	1	215.75	28.9763	1
Total	496.97	62.1721	2	399.09	57.422	2	466.57	60.5386	2	441.18	58.041	2
Mean	248.485	31.0861	1	199.545	28.711	1	233.285	30.2693	1	220.59	29.0205	1
t07220	152.6	20.2078	2	150.64	20.0645	2	160.54	22.0785	2	198.26	23.8086	1
	198.22	23.8064	2	179.38	22.4516	2	171.33	23.1736	1	197.35	23.7821	2
Total	350.82	44.0142	4	330.02	42.5161	4	331.87	45.2521	3	395.61	47.5907	3
Mean	175.41	22.0071	2	165.01	21.2581	2	165.935	22.6261	1.5	197.805	23.7954	1.5
t08240	207.71	28.0892	1	168.71	19.445	2	154.61	23.2374	2	162.72	18.9763	2
	144.62	17.7887	3	134.85	14.7012	2	187.65	26.1746	1	165.55	19.0082	2
Total	352.33	45.8779	4	303.56	34.1462	4	342.26	49.412	3	328.27	37.9845	4
Mean	176.165	22.939	2	151.78	17.0731	2	171.13	24.706	1.5	164.135	18.9923	2
t09400	222.92	32.0769	0	235.45	33.9615	0	245.97	32.1762	0	249.92	32.9768	0
	182.28	23.082	0	223.78	32.884	0	202.25	24.8519	0	251.64	32.2157	0
	193.65	23.9155	0	238.65	34.0126	0	189.62	23.9812	0	203.65	30.8713	0
	249.47	32.9728	0	187.66	24.3417	0	264.51	38.6573	0	197.44	24.6544	0
Total	848.32	112.047	0	885.54	125.199	0	902.35	119.666	0	902.65	120.718	0
Mean	212.08	28.0118		221.385	31.3		225.588	29.9167		225.663	30.1796	
t10410	122.88	14.9252	2	170.41	20.9553	1	123.81	15.0125	1	165.16	19.5216	1
	168.75	20.0253	1	168.75	20.0211	2	146.37	17.8413	1	171.47	20.5407	1
	165.15	19.5325	1	165.38	19.6001	2	170.58	21.1588	1	125.81	16.2154	1
	165.06	19.5303	1	140.33	16.422	2	167.34	20.0067	1	154.36	18.8643	1
Total	621.84	74.0133	5	644.87	76.9985	7	608.1	74.0193	4	616.8	75.142	4
Mean	155.46	18.5033	1.25	161.218	19.2496	1.75	152.025	18.5048	1	154.2	18.7855	1
t11420	176.46	23.2332	2	138.54	17.7216	2	249.61	32.9817	1	130.68	16.0543	2
	138.61	17.7322	2	148.36	21.3145	2	138.54	23.1264	2	170.55	21.8712	1
	111.09	13.8842	3	135.43	16.8894	2	118.25	14.4133	3	141.21	20.6443	2
	146.9	20.3314	2	173.62	22.9701	1	120.61	11.6179	2	127.64	15.8761	3
Total	573.06	75.181	9	595.95	78.8956	7	627.01	82.1393	8	570.08	74.4459	8
Mean	1443.26	18.7953	2.3	148.988	19.7239	1.75	156.753	20.5348	2	142.52	18.6115	2
t12440	72.18	8.0608	4	156.46	19.6541	2	86.15	11.4325	3	74.38	9.2601	3
	145.75	20.4978	1	70.12	8.0063	4	145.31	17.6318	2	156.16	19.6533	2
	174.35	22.0415	1	171.55	20.5411	1	184.96	25.1464	1	166.43	20.0087	2
	103.46	13.801	2	98.47	13.6421	3	125.71	14.8871	3	88.21	11.0015	3
Total	495.74	64.4011	8	496.6	61.8436	10	542.13	69.0978	9	485.18	59.9236	10
Mean	123.935	16.1003	2	124.15	15.4609	2.5	135.533	17.2745	2.25	121.295	14.9809	2.5

t13800	109.02	14.6622	0	205.64	34.0615	0	231.02	37.8331	0	203.45	34.0071	0
	142.59	19.7394	0	231.55	37.854	0	250.3	39.5463	0	159.23	20.4588	0
	171.45	22.6945	0	167.08	21.6954	0	175.46	23.1126	0	178.64	23.5495	0
	205.79	34.2204	0	151.64	19.7824	0	168.44	21.9645	0	230.71	36.932	0
	231.27	37.8447	0	183.66	24.5784	0	132.78	17.6411	0	206.11	35.4541	0
	164.18	20.6919	0	147.52	18.9965	0	202.73	36.4126	0	187.24	28.6374	0
	149.21	20.0071	0	135.44	18.4112	0	184.64	24.4865	0	132.66	18.1127	0
	150.3	19.6324	0	170.87	22.443	0	170.36	22.0041	0	.	.	.
Total	1323.81	189.492	0	1393.4	197.822	0	1515.73	223.000	0	1298.04	197.151	0
Mean	194.349	26.9994		201.415	27.5127		219.339	30.9373		211.613	30.2285	
t14810	161.98	23.0198	1	165.44	24.2195	1	98.75	13.6451	2	88.64	10.7785	3
	96.48	13.276	2	123.21	17.1584	2	165.64	24.2201	1	125.61	16.9981	2
	121.23	16.5278	1	125.66	17.8419	2	128.43	18.6327	2	160.75	23.0018	2
	86.53	10.6295	1	96.88	13.5411	3	94.82	12.8543	2	158.48	22.8761	1
	64.88	8.4069	2	115.43	15.841	1	135.61	19.6101	2	125.31	16.9987	2
	94.52	12.8321	3	106.95	14.7118	2	148.88	21.0017	1	135.14	18.43	1
	44.79	6.8321	3	94.51	13.0611	2	116.87	18.0125	2	.	.	.
	123.45	16.8902	1	98.54	14.2167	2	123.41	18.3112	2	.	.	.
Total	793.86	108.414	14	926.62	130.591	15	1012.41	146.287	14	793.93	109.083	11
Mean	99.2325	13.5518	1.75	115.827	16.3239	1.9	126.551	18.2859	1.75	132.321	18.1805	1.83
t15820	117.15	14.5882	2	115.2	13.8971	1	145.61	17.6145	1	128.14	16.9017	2
	70.42	8.3964	3	85.43	11.0051	2	121.15	15.897	2	115.53	14.3881	2
	97.71	13.294	1	121.47	15.661	1	136.44	17.4321	2	121.77	16.1782	2
	88.85	11.0995	5	112.64	12.9544	1	199.86	23.644	1	216.81	27.8893	1
	125.54	16.6071	2	98.51	11.8875	2	80.4	10.1764	3	154.16	19.0018	2
	89.97	11.4342	3	89.63	11.6577	2	123.61	16.4142	1	123.67	16.5176	2
	.	.	.	72.11	9.0065	3	87.15	11.0015	3	86.45	13.6543	3
	.	.	.	80.64	10.0764	2	120.64	15.6171	2	165.78	18.1216	2
Total	589.64	75.4194	16	775.63	96.1457	14	1014.86	127.796	15	1112.31	142.652	16
Mean	98.2733	12.5699	2.7	96.9538	12.0812	1.75	126.857	15.9746	1.9	139.038	17.8316	2
t16840	203.86	27.3236	1	128.31	15.9055	1	182.36	24.8712	1	233.46	30.5817	1
	135.42	16.1847	1	80.85	10.2114	2	140.66	16.8964	2	130.36	16.0081	3
	140.46	16.431	2	140.12	16.4227	1	110.58	12.0126	3	142.54	16.8312	2
	128.35	15.855	2	78.61	8.7715	3	121.73	14.6741	2	101.67	11.7543	3
	98.37	9.3406	3	73.54	7.9368	3	95.41	9.0122	4	92.11	9.0066	3
	108.4	11.3849	3	107.77	12.5514	2	128.35	15.1261	3	156.43	19.3387	2
	80.85	8.8622	4	73.25	8.1266	4	100.16	12.0016	3	88.58	10.6431	4
	82.5	9.0462	3	70.66	8.1051	4	89.8	8.9641	3	.	.	.
Total	978.21	114.428	19	753.11	88.031	20	969.05	113.558	21	945.15	114.163	18
Mean	122.276	14.3035	2.4	94.1388	11.0039	2.5	121.131	14.1948	2.6	135.021	16.3091	2.6
t01100	259.33	31.7458	0	248.77	30.2365	0	264.33	31.9863	0	261.68	31.844	0
t02110	222.86	31.5752	1	216.81	29.5752	1	235.41	30.2587	1	218.79	29.6783	1
t03120	186.65	26.9961	2	187.55	27.0032	2	195.3	27.3458	2	218.79	27.0042	2
t04140	139.91	17.6277	4	140.22	17.8431	3	129.37	16.489	4	130.11	16.5371	3
t05200	345.12	45.6411	0	240.95	29.8736	0	245.62	29.9873	0	239.82	29.7641	0
	336.78	44.8725	0	251.53	30.9932	0	238.63	29.6744	0	252.67	31.0005	0
Total	681.9	90.5136	0	492.48	60.8668	0	484.25	59.6617	0	492.49	60.7646	0
Mean	340.95	45.2568		246.24	30.4334		242.125	29.8306		246.245	30.3823	
t06210	230.87	28.5926	1	231.79	28.6058	1	244.2	29.8903	1	228.94	27.9854	2
	212.68	23.4524	1	200.63	22.5471	2	198.67	22.2306	2	224.35	26.0254	2
Total	443.55	52.045	2	432.42	51.1529	3	442.87	52.1209	3	453.29	54.0108	4
Mean	221.775	26.0225	1	216.21	25.5765	1.5	221.435	26.0605	1.5	226.645	27.0054	2
t07220	240.51	29.784	2	211.92	22.9435	1	205.93	21.734	2	230.65	28.59	1
	208.09	22.9282	1	204.72	21.6783	2	208.99	22.9304	2	201.67	20.9865	2
Total	448.6	52.7122	3	416.64	44.6218	3	414.92	44.6644	4	432.32	49.5765	3
Mean	224.3	26.3561	1.5	208.32	22.3109	1.5	207.46	22.3322	2	216.16	24.7883	1.5

t08240	219.78	24.8998	2	211.92	23.5643	2	198.54	20.9764	3	201.68	20.9873	3
	204.06	22.5564	2	190.67	20.3365	3	197.22	20.9783	3	187.25	19.9653	3
Total	423.84	47.4562	4	402.59	43.9008	5	395.76	41.9547	6	388.93	40.9526	6
Mean	211.92	23.7281	2	201.295	21.9504	2.5	197.88	20.9774	3	194.465	20.4763	3
t09400	320.76	41.4937	0	318.16	41.0083	0	285.44	36.0073	0	320.57	41.4206	0
	320	41.389	0	320.81	41.4207	0	316.55	40.6782	0	280.54	35.7368	0
	345.16	43.5398	0	279.78	35.6704	0	317.37	40.6895	0	344.23	43.4211	0
	286.71	36.0626	0	302.67	36.7743	0	330.42	42.5433	0	275.43	35.1293	0
Total	1272.63	162.485	0	1221.42	154.873	0	1249.78	159.918	0	1220.77	155.707	0
Mean	318.158	40.6213		305.355	38.7184		312.445	39.9796		305.193	38.927	
t10410	321.87	42.6792	1	264.53	34.1265	2	282.42	35.7542	2	290.71	36.567	1
	250.03	27.266	2	249.54	27.1264	2	261.02	34.0051	2	283.54	35.8833	1
	294.27	36.6838	1	288.73	35.6722	1	240.17	26.5433	3	252.83	27.0577	2
	263.5	34.0023	2	256.88	27.6839	2	224.54	24.7329	3	246.63	26.8346	2
Total	1129.67	140.631	6	1059.68	124.609	7	1008.15	121.035	10	1073.71	126.342	6
Mean	282.418	35.1578	1.5	264.92	31.1523	1.75	252.038	30.2589	2.5	268.428	31.5857	1.5
t11420	208.2	28.9493	2	227.58	30.2726	2	224.3	29.8753	2	200.63	28.8311	2
	227.67	30.7999	1	186.43	25.3412	3	268.54	31.6673	2	280.54	33.3683	1
	273.74	32.7429	1	272.61	32.6453	3	272.54	32.4327	1	208.55	28.9542	2
	200.72	26.8325	2	210.78	27.8774	2	228.94	30.8543	2	220.34	29.4367	2
Total	910.33	119.324	6	897.4	116.136	10	994.32	124.829	7	910.06	120.590	7
Mean	227.583	29.8312	1.5	224.35	29.0341	2.5	248.58	31.2074	1.75	227.515	30.1476	1.75
t12440	234.81	29.4835	2	225.02	27.2726	2	235.67	29.5634	1	198.62	24.1265	3
	239.86	28.3518	2	211.54	25.5687	3	214.66	25.7085	3	201.54	24.5673	3
	211.26	25.5645	3	200.12	24.3357	3	210.24	25.5081	3	239.88	28.3564	1
	214.15	25.6905	3	190.53	23.8764	3	228.94	27.6273	2	233.72	29.4811	1
Total	900.08	109.090	10	827.21	101.053	11	889.51	108.407	9	873.76	106.531	8
Mean	225.02	27.2726	2.5	206.803	25.2634	2.75	222.378	27.1018	2.25	218.44	26.6328	2
t13800	291.36	36.2018	0	290.94	36.0023	0	266.78	32.3535	0	290.57	36.0072	0
	286.07	35.8791	0	305.44	37.0541	0	246.87	30.1476	0	301.66	36.8433	0
	276.88	33.3325	0	256.45	31.4482	0	278.54	33.5682	0	284.55	35.658	0
	265.8	32.1567	0	284.32	35.6573	0	301.81	36.9871	0	255.12	31.5026	0
	307.35	37.2058	0	301.63	36.8427	0	315.44	37.6592	0	287.64	35.8745	0
	318.16	37.9843	0	275.44	33.4378	0	287.62	35.8743	0	307.68	37.3582	0
	.	.	.	284.37	35.668	0	265.77	32.1554	0	.	.	.
	.	.	.	264.33	32.2654	0	275.23	33.2374	0	.	.	.
Total	1745.62	212.760	0	2262.92	278.375	0	2238.06	271.982	0	1727.22	213.243	0
Mean	290.937	35.46		282.865	34.797		279.758	33.9978		287.87	35.5406	
t14810	172.35	31.1337	3	210.35	30.9873	2	183.75	27.6534	2	172.62	30.5437	2
	167.69	22.8656	2	188.54	28.7543	3	209.88	31.5568	2	175.43	30.9774	2
	209.86	35.2075	2	189.87	29.1238	3	167.48	22.872	3	167.31	22.8705	3
	204.75	31.2272	2	205.77	30.1673	2	186.37	28.2373	2	205.74	31.3684	1
	202.4	30.76	2	158.45	21.6873	3	201.53	29.8976	2	201.66	29.9054	2
	204.54	30.0252	2	190.11	28.7954	2	165.44	22.4681	3	189.34	28.8965	2
	167.44	22.8716	3	172.12	30.8975	2	.	.	.	165.41	22.6834	3
	189.94	28.8997	2	195.43	28.9701	2	.	.	.	.	.	.
Total	1518.97	232.990	18	1510.64	229.383	19	1114.45	162.685	14	1277.51	197.245	15
Mean	189.871	29.1238	2.25	188.83	28.6729	2.4	185.742	27.1142	2.3	182.501	28.1779	2.1
t15820	186.01	27.855	2	189.34	27.9763	2	197.83	25.6071	2	202.45	26.8753	1
	228.13	27.1331	1	150.11	22.4371	3	189.48	27.553	2	227.13	27.0062	1
	197.44	24.241	3	145.67	21.4702	3	195.22	25.6184	2	153.64	22.7082	3
	189.65	27.5587	3	186.08	27.8564	2	138.42	20.6785	4	177.63	23.7758	2
	152.95	22.6488	3	195.44	25.621	2	189.77	29.1206	2	157.96	22.5622	3
	177.82	23.7762	2	182.47	26.9077	2	145.86	21.4789	3	144.32	21.4706	3
	193.39	25.4321	2	151.64	22.643	3	170.28	23.3451	3	.	.	.
	.	.	.	.	.	.	146.54	21.5728	3	.	.	.
Total	1325.39	178.644	16	1200.75	174.911	17	1373.4	194.974	21	1063.13	144.398	13

Mean	189.341	25.5207	2.3	171.536	24.9874	2.4	171.675	24.3718	2.6	177.188	24.0664	2.2
t16840	130.17	19.9499	4	157.88	22.4135	3	136.54	20.6708	3	206.75	28.1082	1
	175.18	23.8973	3	165.35	23.2357	3	127.43	17.5082	3	197.54	26.1985	2
	207.63	28.8705	2	121.44	19.9872	4	102.64	14.3072	4	165.34	23.234	3
	206.52	28.0923	2	147.88	21.4327	3	200.85	26.2178	2	209.43	28.9076	1
	128.01	19.6431	4	128.65	17.8864	4	197.63	26.2008	2	145.72	21.1024	3
	147.78	21.351	3	138.42	20.9884	3	129.67	17.7305	3	129.03	17.7219	3
	129.4	17.7228	4	205.81	27.9843	1	130.86	19.9503	3	121.54	19.99	3
	139.03	20.9703	3	202.33	27.6548	2	.	.	.	98.67	13.2588	4
Total	1263.72	180.497	25	1267.76	181.583	23	1025.62	142.585	20	1274.02	178.521	20
Mean	157.965	22.5622	3.1	158.47	22.6979	2.9	146.517	20.3694	2.9	159.253	22.3152	2.9

8.5 SAS programme for analysis of RFB larvae feeding experiment at Massey Campus.

```
options ls=78 ps=63 nodate;
data fdexp1;
   do time=1 to 3;
   do density=1 to 4;
   do larvae=1 to 4;
   do block=1 to 4;
     input pltwt @@;output;
   end; end;end;end;
cards;
214.10 220.31 218.36 198.66
145.93 150.65 138.52 148.71
155.70 140.31 135.44 138.44
 75.65 120.32 101.54 115.72
223.78 224.14 223.13 224.65
168.88 168.59 182.51 155.55
115.59 112.45 108.71 109.55
 97.15 111.19 102.03 96.69
156.72 154.64 159.01 163.00
152.76 150.16 152.58 147.73
119.70 117.20 117.12 122.64
104.50 104.30 104.33 106.65
145.41 151.94 146.64 147.73
101.05 102.63 102.41 110.04
 96.69 99.91 100.38 109.06
 89.56 92.44 96.19 95.39
234.43 280.45 287.65 285.64
202.23 185.43 195.44 198.64
144.68 150.46 154.38 140.75
126.20 149.57 116.43 123.21
267.31 267.60 274.63 262.04
248.49 199.55 233.29 220.59
175.41 165.01 165.94 197.81
176.17 151.78 171.13 164.14
212,08 221.39 225.59 225.66
155.46 161.22 152.03 154.20
143.27 148.99 156.75 142.52
123.94 124.15 135.52 121.30
194.35 201.42 219.34 211.61
 99.23 115.83 126.55 132.32
```

```
98.27 96.95 126.86 139.04
122.28 94.14 121.13 135.02
259.33 248.77 264.33 261.68
222.86 216.81 235.41 218.79
186.65 187.55 195.30 218.79
139.91 140.22 129.37 130.11
340.95 246.24 242.13 246.25
221.78 216.21 221.43 226.65
224.30 208.32 207.46 216.16
211.92 201.30 197.88 194.47
318.16 305.36 312.45 305.19
282.42 264.92 252.04 268.43
227.58 224.35 248.58 227.52
225.02 206.80 222.38 218.44
290.94 282.87 279.76 287.87
189.87 188.83 185.74 182.50
189.34 171.54 171.68 177.19
157.97 158.47 146.52 159.25
;
run;
title 'Analysis of RFB larvae feeding data - Factorial Design';
proc format;
    value dft 1='15days' 2='30days' 3='45days';
    value densi 1='a plant' 2='2 plants' 3='4 plants' 4='8 plants';
    value lvl 1='nolarvae' 2='10larvae' 3='20larvae' 4='40larvae';
run;
proc print ;
run ;
proc glm data=fdexp1;
    format time dft. density densi. larvae lvl.;
    class block larvae density time;
    model pltwt=block time larvae density time*larvae time*density density*larvae/ss1;
    means time larvae density time*larvae time*density density*larvae/ tukey;
run ;
proc means data=fdexp1 noprint;
    class time density larvae;
    var pltwt;
output out=mfdexp1 mean=mpltwt;
run;
options ps=30;
proc plot data=mfdexp1;
    format time dft. density densi. larvae lvl.;
        plot mpltwt*density=time;
        plot mpltwt*time=density;
run;
proc means data=fdexp1 noprint;
    class time density larvae;
    var pltwt;
output out=mfdexp1 mean=mpltwt;
run;
options ps=30;
proc plot data=mfdexp1;
    format time dft. density densi. larvae lvl.;
        plot mpltwt*larvae=time;
        plot mpltwt*time=larvae;
run;
```

```
/*set the graphic environment*/
goptions reset=global gunit=pct border
ftext=swissb htitle=4 htext=3;
/*create the data set soil water*/
data swater;
    input day $ water @@;
cards;
A 12 A 38 A 62 A 76 A 52
B 65 B 82 B 74 B 49 B 36
C 35 C 36 C 35 C 65 C 40
;
run;
/*define title*/
/*Comparison of Soil Water by % Survival*/
/*define symbol characteristics*/
symbol interpol=boxt10
     value=square
     height=4;
axis1 value=('12.25%' '9.98%' '11.68%')
    offset=(5,5)
    length=50;
/*generate plot*/
proc gplot data=swater;
   plot water*day /haxis=axis1
              vaxis=15 to 85 by 10
              frame;
run;
quit;
/*set the graphic environment*/
goptions reset=global gunit=pct border
ftext=swissb htitle=4 htext=3;
/*create the data set soil water*/
data swater3;
    input sw lvasur pltwt duration $;
cards;
48.0 12.25 1.85 15-days
61.2  9.98 3.95 30-days
42.2 11.68 1.51 45-days
;
run;
/*define title*/
/*'Soil Water effect on % Survival and Plant Growth*/
proc g3d  data=swater3;
   scatter lvasur*pltwt=sw / shape=duration;
run;
quit;
```

8.6 Data for RFB eggs collection.

RFB eggs collection
(for Life table)

date	egg laid	RFB adult	unhatched eggs
90498	754	388	

100498	750	388
110498	770	386
120498	701	385
130498	627	383
140498	802	383
150498	951	380
160498	903	380
170498	660	379
180498	745	378
190498	652	375
200498	662	375
210498	665	375
220498	754	379
230498	750	372
240498	770	372
250498	697	370
260498	765	368
270498	745	368
280498	721	365
290498	432	364
300498	589	364
10598	542	356
20598	702	356
	17109	1973

(for movement field experiment)

30598	664	356	
40598	473	354	
50598	543	354	
60598	447	354	
70598	512	351	
80598	440	350	
90598	398	350	
100598	575	348	
110598	666	348	
120598	654	348	
130598	542	346	
140598	535	346	
150598	626	346	
160598	612	342	
170598	610	342	
180598	447	340	
190598	543	340	
	9287		1256

8.7 Data for RFB newly hatched larvae.

RFB 1st Instar larvae hatching
(for factorial design & life table)

date	larvae alive	larvae death	total
110598	534	104	915

120598	794	130	924
130598	773	142	915
140598	645	130	775
150598	888	148	1036
160598	1383	315	1698
170598	501	57	558
180598	801	102	903
190598	1159	288	1447
200598	1347	369	1716
210598	1065	298	1363
220598	381	62	443
230598	477	86	563
240598	264	53	317
250598	495	72	567
260598	709	105	814
270598	384	75	459
	12600	2536	

(for nested design)

280598	651	148	799
290598	555	163	718
300598	582	154	736
310598	477	69	546
10698	780	184	964
	3045	718	

Appendices 9 Formula of STELLA models

9.1 Formula of STELLA – RFB (ragwort flea beetle) model

adult(t) = adult(t - dt) + (hatch - adtdeath - alive) * dt

INIT adult = 161

hatch = pupa*0.5

adtdeath = adult*(fungi2+others)

alive = adult*0.918

day1lva(t) = day1lva(t - dt) + (egghatched - growth - d1lvadeath) * dt

INIT day1lva = 7146

egghatched = egg*0.885

growth = day1lva*0.832

d1lvadeath = day1lva*(egg_unhatched+dehydration)

egg(t) = egg(t - dt) + (laying - egghatched - death) * dt

INIT egg = (3.67*10)*220

laying = (alive*3.67)*220

egghatched = egg*0.885

death = egg*(fungi+unhatched)

lva1(t) = lva1(t - dt) + (growth - lva1growth - lva1death) * dt

INIT lva1 = 5945

growth = day1lva*0.832

lva1growth = (lva1*0.465)+f_per_d_by_lva1

lva1death = lva1*unknown1

lva2(t) = lva2(t - dt) + (lva1growth - lva2growth - lva2_death) * dt

INIT lva2 = 2764

lva1growth = (lva1*0.465)+f_per_d_by_lva1

lva2growth = (lva2*0.321)+f_per_d_by_lva2

lva2_death = lva2*unknown2

lva3(t) = lva3(t - dt) + (lva2growth - lva3growth - lva3_death) * dt

INIT lva3 = 887

lva2growth = (lva2*0.321)+f_per_d_by_lva2

lva3growth = (lva3*0.363)+f_per_d_by_lva3

lva3_death = lva3*unknown3

pupa(t) = pupa(t - dt) + (lva3growth - pupa_death - hatch) * dt

INIT pupa = 322

lva3growth = (lva3*0.363)+f_per_d_by_lva3

pupa_death = pupa*unknown4

hatch = pupa*0.5

dehydration = 0.05

egg_unhatched = 0.118

fungi = 0.05

fungi2 = 0.01

others = 0.0725

unhatched = 0.065

unknown1 = 0.535

unknown2 = 0.679

unknown3 = 0.637

unknown4 = 0.5

9.2 Formula of STELLA – ragwort model

flowering1(t) = flowering1(t - dt) + (growth2) * dt

INIT flowering1 = 8.03*0.75

growth2 = rosette1*0.14

flowering2(t) = flowering2(t - dt) + (growth3 - death7) * dt

INIT flowering2 = 5.2*0.75

growth3 = (multirosette1*0.57)+(rosette2*0.16)

death7 = (flowering2*0.925)*(f_per_d_by_lva1+f_per_d_by_lva2+f_per_d_by_lva3)

flowering3(t) = flowering3(t - dt) + (growth4 - death8) * dt

INIT flowering3 = 7.3*0.75

growth4 = (multirosette2*0.71)

death8 = (flowering3*0.94)*(f_per_d_by_lva1+f_per_d_by_lva2+f_per_d_by_lva3)

multirosette1(t) = multirosette1(t - dt) + (change5 - change3 - death4) * dt

INIT multirosette1 = 4.2

change5 = rosette1*0.07

change3 = (multirosette1*0.19)+(rosette1*0.12)+(rosette2*0.15)

death4 = multirosette1*(0.24+f_per_d_by_lva1+f_per_d_by_lva2+f_per_d_by_lva3)

multirosette2(t) = multirosette2(t - dt) + (change3 - death5 - multirosette3) * dt

INIT multirosette2 = 10.3

change3 = (multirosette1*0.19)+(rosette1*0.12)+(rosette2*0.15)

death5 = multirosette2*(0.17+f_per_d_by_lva1+f_per_d_by_lva2+f_per_d_by_lva3)

multirosette3 = multirosette2*0.12

rosette1(t) = rosette1(t - dt) + (growth1 - death2 - growth2) * dt

INIT rosette1 = 57.33

growth1 = seedling*.52*0.75

death2 = rosette1*(0.37+f_per_d_by_lva1+f_per_d_by_lva2+f_per_d_by_lva3)

growth2 = rosette1*0.14

rosette2(t) = rosette2(t - dt) + (change4 - death3 - rosette3) * dt

INIT rosette2 = 17.2

change4 = rosette1*0.30

death3 = rosette2*(0.43+f_per_d_by_lva1+f_per_d_by_lva2+f_per_d_by_lva3)

rosette3 = rosette2*0.12

seedling(t) = seedling(t - dt) + (germination - growth1 - death1) * dt

9.3 Formula of STELLA – RFB, ragwort combined model

Appendix 9.1 + appendix 9.2 and the following formula:

INIT seedling = 147

germination = ((flowering1*0.038)+(flowering2*0.075)+(flowering3*0.06))*(17570)*(75/100)*(0.5)

growth1 = seedling*.52*0.75

death1 = seedling*(0.48+f_per_d_by_lva1+f_per_d_by_lva2+f_per_d_by_lva3)

f_per_d_by_lva1 = f_per_lva1*631

f_per_d_by_lva2 = f_per_lva2*255

f_per_d_by_lva3 = f_per_lva3*71

f_per_lva1 = lva1*0.02043

f_per_lva2 = lva2*0.027

f_per_lva3 = lva3*0.073