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**THE ROLE OF SOIL ORGANIC MATTER IN THE SUSTAINABLE
MANAGEMENT OF THE GRASS GRUB *Costelytra zealandica* (WHITE) IN
CANTERBURY PASTURES**

A thesis

**submitted in partial fulfilment
of the requirements for the Degree**

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by

F.J. Villalobos-Hernández

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**Abstract of a thesis submitted in partial fulfilment of the requirements
for the Degree of Doctor of Philosophy.**

**THE ROLE OF SOIL ORGANIC MATTER IN THE SUSTAINABLE MANAGEMENT OF GRASS
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F.J. Villalobos-Hernández

Larvae of *Costelytra zealandica* (White) have historically been considered as important pests in New Zealand pastures. Control of this species has mainly been based on the green revolution paradigm. In recent years, this paradigm has collapsed leaving behind multiple environmental problems. Interactions among soil organic matter (SOM), the feeding activity of *C. zealandica* larvae and their amber disease caused by the bacterium *Serratia entomophila* Grimont et al., were studied in soils from three pastures with the same soil type but different SOM status. A fractionation scheme has been followed to determine the total C and N in labile (soil biomass, cold and hot water extractions) and stable (humic acids, fulvic acids and humins) SOM fractions. In the laboratory, larvae were placed individually in pots containing soil from an old and a young pasture. Pots were inoculated with four doses of *S. entomophila* and incubated under similar conditions. Larval feeding activity was measured using pieces of carrot root. At the end of the experiment larvae were assessed for infection, mortality, feeding activity and live weight gains. In March 1992, a field experiment was conducted in these pastures. Soil cores were taken, covered with a gauze sleeve, treated, placed back in situ and protected. The following treatments were tested: (a) 5 larvae plus a pathogenic strain of *S. entomophila*; (b) as for "(a)" but a non-pathogenic strain was added; (c) as for "(a)" but bacteria-free nutrient broth was added; (d) as for "(a)" but H₂O was added and; (e) as for "(d)" Soil was broken down and sieved. Records of insect survival, larval disease and live weight, as well as plant production were taken for each soil core. Soil from the different treatments was sampled to estimate *S. entomophila* numbers, total C and N in SOM fractions. Dry matter yields of living root, herbage and plant residues and the amount of total C and N in plant variables were also sampled for each treatment. In March 1993, a laboratory and a field

experiment were conducted to evaluate the effect of the application of whey on the growth of *S. entomophila*, larval health status, herbage, living roots-plant residues yields, and labile SOM fractions. In the laboratory, pots containing soil in which ryegrass seedlings had grown were treated and incubated under similar conditions. Comparisons of these variables were made after testing 4 bacterial doses, additions of whey or H₂O, and the presence or absence of larvae. In the field experiment four treatments were tested: (a) 5 larvae plus a whey application; (b) as for "(a)" but H₂O was applied; (c) as for "(a)" but no larvae were introduced and; (d) as for "(c)" but H₂O was applied. After 2 months, variables were evaluated and determinations of total C and N in labile SOM fractions were made.

Total C and N present in cold and hot water extractions combined, representing the labile SOM fraction, showed that in the young pastures there was 45% less C and 37% less N than in the old pasture ($P<0.05$). Both in young and old pastures approximately 10% of C and N was present in the labile SOM fractions. A 95% higher ($P<0.05$) population of *S. entomophila* was present in fresh soil from the older than from the younger pasture. Under laboratory conditions, 63% more ($P<0.05$) amber disease occurred in the soil with the highest SOM content. After 30 days, larval mortality was closely associated with amber disease and was 50% higher ($P<0.05$) in the older than in the younger pasture. Carrot consumption of healthy larvae after 15 days was 38% higher ($P<0.05$) in soil with lower SOM content than in soil with high SOM content. Larval growth was 23% higher ($P<0.05$) in the soil from the young than in the soil from the old pasture.

Under field conditions, the soil with lower SOM content induced a greater larval herbivory; the living roots contained more N and a lower probability of infection by amber disease was observed. Larval mortality attributed to entomopathogens was higher ($P<0.05$) in the soil with the highest SOM content. The application of *S. entomophila* had positive effect ($P<0.05$) by itself in inducing leaf and root growth regardless of insect herbivory. A reduction ($P<0.05$) of 29%, 36% and 43% in the amount of total N contained in living plant roots was observed in soils with decreasing levels of SOM attributed to larval herbivory. No correlation was found between the impact of third instars with the reduction of herbage and living roots growth. The impact on growth of herbage and living roots caused by *C. zealandica* seems to be more severe when physical injuries occur in sensitive parts of the root system at the top five cm of the soil profile. The application of *S. entomophila* inhibited ($P<0.05$) soil microbial biomass and reduced the

symptoms of other larval diseases. The insect promoted an increase ($P<0.05$) in the total C and total N present in soil microbial biomass. Negative correlations were observed between larval growth and most of the SOM fractions considered. Reductions in the amount of total N from nonhumic substances were promoted by the presence of larvae. The application of *S. entomophila* was associated with a reduction ($P<0.05$) in the amount of total C and N from hot water extractions. A decrease ($P<0.05$) from 19-34% in the amount of total C and total N present in plant residues may be attributed to the feeding activity of the larvae. This fraction of the SOM is an important source of C for larval growth ($P<0.05$).

The application of whey by itself showed no significant short-term effect on the health status of *C. zealandica* larvae under microcosm and mesocosm conditions but the whey input interacted positively ($P<0.05$) with its entomopathogens. Amber disease levels increased ($P<0.05$) by either combining the whey application with the medium dose of *S. entomophila* or by enhancing the indigenous background of entomopathogens present in soil from the old pasture. Positive ($P<0.05$) growth of soil-borne or artificially applied low doses of *S. entomophila* populations was observed as a response to whey inputs. The medium dose of *S. entomophila* combined with the pre-application of whey promoted a positive ($P<0.05$) interaction between the insect and the plant resulting in an increase in the herbage DM production and in its content of total C and N. The interaction whey-insect under low SOM conditions had an effect on reducing ($P<0.05$) the proportion of clover in the field. The whey application produced a positive effect ($P<0.05$) on the build up of the total C and N in the labile hot water extraction of SOM under field conditions. Survival of *S. entomophila* and *S. protamaculans* was enhanced ($P<0.05$) by the whey applications where larvae were included, in soil from the old pasture.

The SOM effects on plant damage caused by *C. zealandica* act in a multifactorial way and their global effect may be greater than that due to the addition of the individual components. Efforts should be made to increase the efficiency of the bacterial applications to the soil; to encourage the build up of SOM in order to reduce damage; to increase the presence of indigenous micro-organisms in the soil and to enhance the contribution that these insects make to SOM turnover. These actions are also recommended to prevent pasture damage. Conservation and increased soil fertility may be crucial as to prevent *C. zealandica* damage within the framework of sustainable agriculture.

Keywords: *Costelytra zealandica*; pastures, management, sustainability; soil organic matter; *Serratia entomophila*; biological control; plant damage; soil microbial biomass; cold water extraction, hot water extractions; humic acids, fulvic acids, humins.

A Erick, espíritu invencible que inunda mi corazón...

A Aldebarán, tenue luz del firmamento que ilumina mi camino...

A mi Madre y a mi Padre, los milagros más grandes de mi vida...

A mis Hermanos, ejercito diverso de potencialidades infinitas...

A mis sobrinos, una razón más para mejorarme a mi mismo...

A *Costelytra zealandica*, mi humilde escarabajo sagrado...

Al TODO, que es superior a la suma de las partes....

"I learned this, at least from my experiment, that if one advances confidently in the direction of his dreams, and endeavours to live the life which he has imagined, he will meet with a success unexpected in common hours. In proportion as he simplifies his life the laws of the Universe will appear less complex, and solitude will not be solitude, nor poverty, poverty, nor weakness weakness".

H.D. Thoreau

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"What is an alchemist?" He asked, finally.

"It's a man who understands nature and the world"

"The alchemist", P. Coelho

One spring day he went out of the castle with his father and together they were watching a farmer at his plowing. He noticed a bird descending to the ground and carrying off a small worm which had been turned up from the earth by the farmers's plough. He sat down in the shade of a tree and thought about it, whispering to himself:

"Alas! Do all living creatures kill each other?"

"The teaching of Buddha", B. D. Kyokai

Who the living would explain

He must enter death's domain.

C. Morgenstern

Throughout the living world and particularly in the soil, all organisms constantly enact the famous phrase of Lucretio's poem; "Like runners in a race, they hand on the torch of life"

"A God Within" R.J. Dubos

"...Tane occupied an important place in the panteon of deities. He was the life giver, the fertiliser, the sustainer, the God of the forest and indeed of the whole world of nature, and the active element in all earthly life, as well as the bringer of knowledge".

"Maori Myth and Legend", A.W. Reed

"He who would seize All Under Heaven in order to manipulate it, I have seen that he will certainly fail. All Under Heaven is like a sacred vessel; it cannot be manipulated. To manipulate is to destroy it; To grasp it is to lose it..."

Taoist principle

"Philosophy is not a theory but an activity"

"Tractatus Logico-Philosophicus", L. Wittgenstein

CHAPTER I

1) THE PROBLEM CAUSED BY THE LARVAE OF *Costelytra zealandica* (WHITE) IN NEW ZEALAND PASTURELAND: THE NEED FOR A SUSTAINABLE AGRICULTURE APPROACH

1.1 Introduction and review of literature

1.1.1 Importance of pastoral farming in New Zealand

Pastoral farming in New Zealand has been the major economic activity for 150 years. The economic dependence of New Zealand on pastoral agriculture is emphasised by the fact that grassland sown with improved grasses and clover now form 35% of New Zealand's land area. Pasture production is responsible for about 65% of New Zealand export earnings (Kerr, 1984). According to Cameron et al. (1989) this amounts to about NZ\$4,500 million annually.

Pastoral farming in New Zealand has traditionally been characterized by: (1) the introduction of high producing exotic pasture species into land cleared from native vegetation; (2) the application of mineral fertilisers, predominantly phosphatic; (3) the combination of white clover (*Trifolium repens* L.) for nitrogen fixation in pastures with grass species, particularly ryegrass (*Lolium perenne* L.); and (4) the efficient utilization of pasture *in situ* by the grazing animals (Kain, 1975). Recently new approaches are being sought which include; (5) the incorporation of modern methods of biological control as a substitute for chemical insecticides (Jackson et al., 1992); (6) introduction of genetically engineered crops more productive and resistant to pests (Wigley, 1985; Longworth, 1988) and, (7) consideration of development and conservation as complementary elements of an environmentally and economically sustainable agricultural system (Blakeley, 1990). However, to be successful, these approaches require a holistic understanding of the pasture agroecosystem.

1.1.2 Economic importance of *Costelytra zealandica* larvae

Grass grub, *Costelytra zealandica* (White) (Coleoptera: Melolonthidae), is considered as the major arthropod pests of New Zealand pastures. Extensive reviews on the biology of this insect have been made by Dumbleton (1942), Kelsey (1970), East (1972) and Kain (1975). The different stages of the life cycle of *C. zealandica* are presented in Plate 1. According to Chapman (1990) large-scale surveys in Canterbury have been carried out over several years using aerial photography to estimate the proportion of pastures showing plant damage, and soil sampling to estimate *C. zealandica* population densities. Damaged areas and density of *C. zealandica* larvae varied widely between pastures and years, but in the worse cases up to 50% of the pasture area showed plant damage and populations of 300-400 larvae per m² were found in the soil (Plate 2). This author states: "Assuming the annual dry matter requirements for a 45 kg ewe is 535 kg, then the maximum loss due to *C. zealandica* larvae could amount to about two ewes per ha. Similar studies have been carried out on dairy farms in the North Island where *C. zealandica* larvae accounts for losses up to 40-50 kg/ha of milkfat." Other more subtle effects may also occur through soil insects altering the composition and quality of pastures (Chapman, 1990). Garnham and Barlow (1993) estimated that the annual costs associated with a density between 74-228 larvae m² in Canterbury pastures were in the range of \$41-\$89M. These are, until now, the more tangible estimates of the losses caused by *C. zealandica* larvae.

1.1.3 The need for a sustainable agriculture

In the philosophy of science, a "paradigm" is defined as a central overall way to regarding phenomena, within which a scientist normally works. The paradigm will dictate what type of explanations will be found acceptable, but in periods of crisis, a science may exchange paradigms (Flew, 1984). In the last 25 years the paradigm of the green revolution has collapsed leaving behind a severe environmental impact, increasing reliance on the use of pesticides and fertilisers in agriculture. The realization that in agriculture more production does not necessarily mean greater returns (Pimentel et al., 1989; Reganold et al., 1990) has also been the driving force in the search for alternatives to high agricultural inputs.

Plate 1

Life cycle of *Costelytra zealandica* (White) (Coleoptera:Melolonthidae). Upper part from the left to the right hand side: egg, first instar larvae, second instar larvae, third instar larvae. Bottom part, from the left to the right hand side: pupae and adult beetle (From the photographic collection of the Department of Entomology & Animal Ecology, Lincoln University).

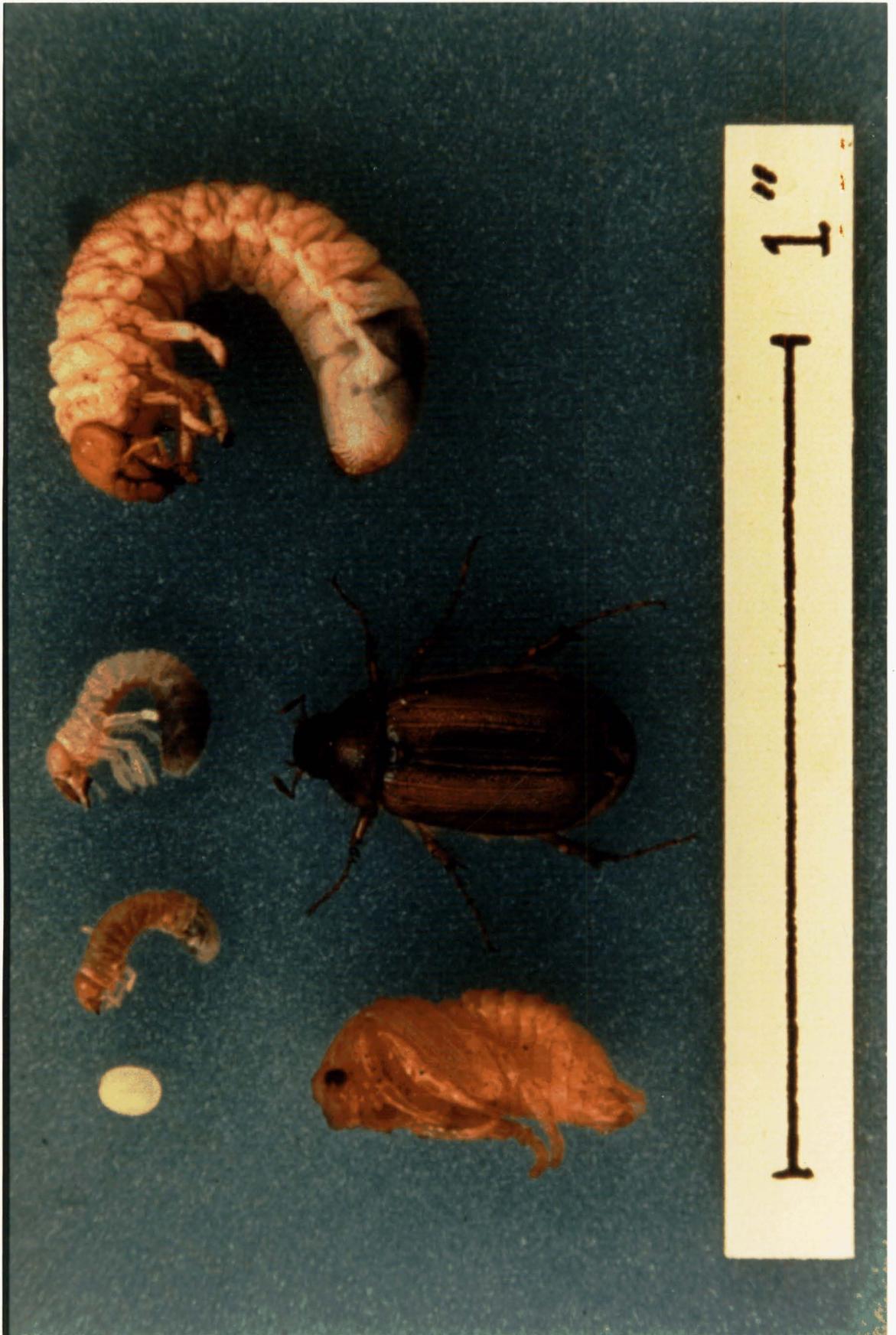


Plate 2

Obvious symptoms of plant damage caused by larvae of *C. zealandica* in New Zealand pastoral agriculture.

(From the photographic collection of the Department of Entomology & Animal Ecology, Lincoln University).



Additional arguments that justify the search for alternative tactics to chemical pesticides include: (1) concern about the effects of pesticide on food webs and particularly on wild life; (2) concern about the effects of pesticides on public health; (3) increasing costs of research for development and production of chemical pesticides; (4) pesticide induced resistance in pests; (5) increased consumer demand for agricultural products free of pesticide residues.

Alternative approaches to the use of high inputs of insecticides in New Zealand agriculture are biological control (Carter, 1989), introduction of genetically engineering crops (Meeusen and Warren, 1989), integrated pest management (Allen, 1988) and sustainable agriculture (Reganold, 1990; Blakeley, 1990).

According to Blakeley (1990), in the last 10 years the pastoral industry in New Zealand is being challenged to operate within a growing awareness of the vulnerability of its environment and with changing public concern. A removal of land development encouraging loans and government subsidies has occurred. The prevailing paradigm of sustainable development sees development and protection not as a countervailing forces but complementary elements of a healthy society (Blakeley, 1990). Within this paradigm, a good understanding is required of the way the environment works and the interactions among biological, physical, economic and cultural systems (Blakeley, 1990).

According to Pimentel et al., (1989) the principles that underlie a low-input sustainable agriculture system are: (1) adapting the agricultural system to the environment of the region including soil, water, climate and biota present at the site and (2) optimizing the use of biological and chemical/physical resources in the agroecosystem. It is within this context that the present work has been conceived and an effort has been made to change the perception of the problem caused by larvae of *C. zealandica*.

1.1.4 Constraints in the management of soil insects

Soil insect pests are specially difficult to combat because: (1) they are a hidden target; (2) the main part of their life cycle takes place in a dynamic biological and physio-chemical environment; (3) their

damage on plants is quite difficult to evaluate; (4) there is difficulty in restoring the loss of biodiversity into the soil environment due to the strong modification of the landscape. The framework of the low-input sustainable agriculture using ecological management practices (Pimentel et al., 1989) seems an option for the management of the plant damage caused by soil insects and to avoid negative environmental impact.

Many insects have, at least temporarily, a stage in the soil; however, few successful attempts to manage soil-dwelling pests have been achieved using micro-organisms as an exclusive tactic (Klein, 1988). Many biological control agents used against soil insects have still some limitations. The use of milky-disease, *Bacillus popilliae* Dutky, against the Japanese beetle *Popillia japonica* Newman has been hampered by its narrow host range, slow build-up in the soil, and an inability to produce it *in vitro*. The bacterium *Bacillus thuringiensis* Berliner (*Bt*) has not been as successful in the soil environment as above-ground (Klein, 1988). The Colorado Potato Beetle *Leptinotarsa decemlineata* Say has recently developed resistance to *Bt* (M. Whalon et al., pers. comm.). The fungi *Beauveria* spp and *Metarhizium* spp are being used in pest management programmes in Europe, Asia and South America but they have not been very useful to control soil-inhabiting insects (Klein, 1988). Despite intensive research the use of *Beauveria brongniartii* (Sacc.) against *Melolontha* in Switzerland is still unreliable (Bigler, 1987). Baculovirus are also potential pathogens for the control of the scarab *Oryctes rhinoceros* (L.), a pest of coconut plants in South Pacific islands (Lacey and Harper, 1986). Although protozoa have been identified as pathogens of soil-dwelling melolonthid larvae, they do not appear to have much potential for direct use in pest management (Maddox, 1986; Hanula and Andreadis, 1992). The most promising use of entomogenous nematodes is against soil insects. In the soil, nematodes are protected from desiccation, UV radiation and temperature extremes (Gaugler, 1988). The nematode *Steinernema glaseri* (Steiner) was first isolated from *P. japonica* larvae and subsequently used in a control programme in the 1930's and 1940's. The success of this programme was reduced by a lack of an economical mass rearing technique and by a lack of knowledge of the importance of the symbiotic bacteria that are associated with the nematode and which are essential for their parasitism and reproduction in the field (Klein, 1988).

Knowledge about the insect pathogenic micro-organisms most often used for pest management is severely limited. An improved understanding of application methods, the movement of pathogens in the

soil environment and reasons for their success and failure once they are applied is required (Klein, 1988).

A major difficulty with biocontrol tactics against soil insects is the manipulation of the environment to reduce pest populations. Soil is a very complex medium (Hillel, 1980). Because of the spatial and temporal variability of soil, predictions, both in the field and laboratory experiments on the effect of a biocontrol agent are rarely successful. The complexity of critical interactions among the soil environment, entomopathogens and soil-dwelling insect pests is the main cause in the failure of biological control agents in the soil (Villani and Wright, 1990).

Wightman (1979a) recognized the importance of studying the environment of *C. zealandica* larvae in New Zealand. Because the complexity of the problem, this author divided the environment of this insect into factors of major and minor importance. However, he also states that "it can be misleading to consider the factors individually". There are considerable interactions between the abiotic and biotic components of the environment and even interactions with intrinsic traits of the larval biology. He suggested that, in view of the genetic plasticity of the insect, the possibility that larvae may adapt to particular soil types should not be overlooked. An indication of the physiological variation between regional biotopes was observed in larvae from Nelson Province that survived well in higher soil moisture, but not in dry conditions, by comparison with their counterparts from Canterbury. If micro-organisms and other entomopathogens are to be successfully utilized in biocontrol programmes more knowledge on soil biology and ecology is required.

1.1.5 Entomopathogens and the natural regulation of *C. zealandica* larval populations

Natural mechanisms that regulate *C. zealandica* larvae populations have been recognized in New Zealand pastures. Densities of *C. zealandica* larvae are usually low in new pasture or crops due to high mortality during cultivation (East and Willoughby, 1983; Jackson, 1990a). The sedentary nature of infestations and their tendency to increase steadily allows *C. zealandica* larval populations to be predicted with reasonable accuracy (East and Kain, 1982). The insect population reaches a peak 4-6 years after sowing. High levels of plant damage typically occur in 3-6 year-old pastures and farmers will often respond

to plant damage with cultivation thereby shortening the potential life of the pasture (Jackson et al., 1986). Observations suggest that post-outbreak populations decline to lower levels than predicted by these models. This occurred despite favourable weather for population growth and the recovery of the pasture from plant damage caused by *C. zealandica* larvae. Neither pasture composition, grazing management, depletion of the food supply, or predation appeared to be major causes of this mortality (East and Wigley, 1985). *C. zealandica* larval populations have been observed to decline naturally to a low level after reaching their peak and to remain at low levels for at least several generations (East and Willoughby, 1983; Jackson, 1990a).

In the last two decades, the importance of pathogens in the natural regulation of *C. zealandica* larval populations has been suggested. Organisms such as viruses (Dearing et al, 1980; Glare 1992a), rickettsia (Moore et al, 1974; Jackson and Glare 1992), bacteria (Fowler, 1974; Trought et al, 1982; East and Willoughby, 1983; Klein 1992), protozoa (Miln, 1979; Hanula and Andreadis, 1992) fungi (Glare, 1992b) and nematodes (Jackson and Trought, 1982) are involved. Examples of the role of pathogens in the control of *C. zealandica* larvae in New Zealand pastures are the following:

a) In the mid-North Island *C. zealandica* larvae and its protozoan pathogens *Nosema* spp and *Mattesia* sp cycle in abundance in a delayed density-dependent fashion (Miln, 1979) and where these micro-organisms occur eventually *C. zealandica* larval populations fall to tolerable levels. Diseased grubs are more susceptible to insecticides and the prolonged use of insecticides diminishes protozoan levels and their effects upon populations. Untreated *C. zealandica* larval populations are, on average, maintained at lower levels than insecticide treated populations as a result of the removal of the influence of protozoan pathogens following insecticide use (Miln, 1979; Miln and Carpenter, 1979; Thomson et al, 1985).

b) Screening for pathogens in Southern Hawkes Bay showed a higher level of infection by the protozoan *Nosema costelytrae* Hall and *Mattesia* sp pathogens and bacterial milky disease (*Bacillus* spp) in declining populations (average 41%) than in increasing populations (average 13%) (East and Wigley, 1985).

c) In Canterbury, the use of amber disease caused by the endemic bacterium *Serratia entomophila* Grimont et al. (Plate 3) as an indicator of population decline has been suggested (Jackson, 1984). Populations of *C. zealandica* larvae surveyed showed that high levels (>30% incidence) of amber disease in any one year caused the population to decline in the following season. *C. zealandica* larvae outbreaks have mainly been recorded in young pastures (< 4 years old) (Jackson, 1990a).

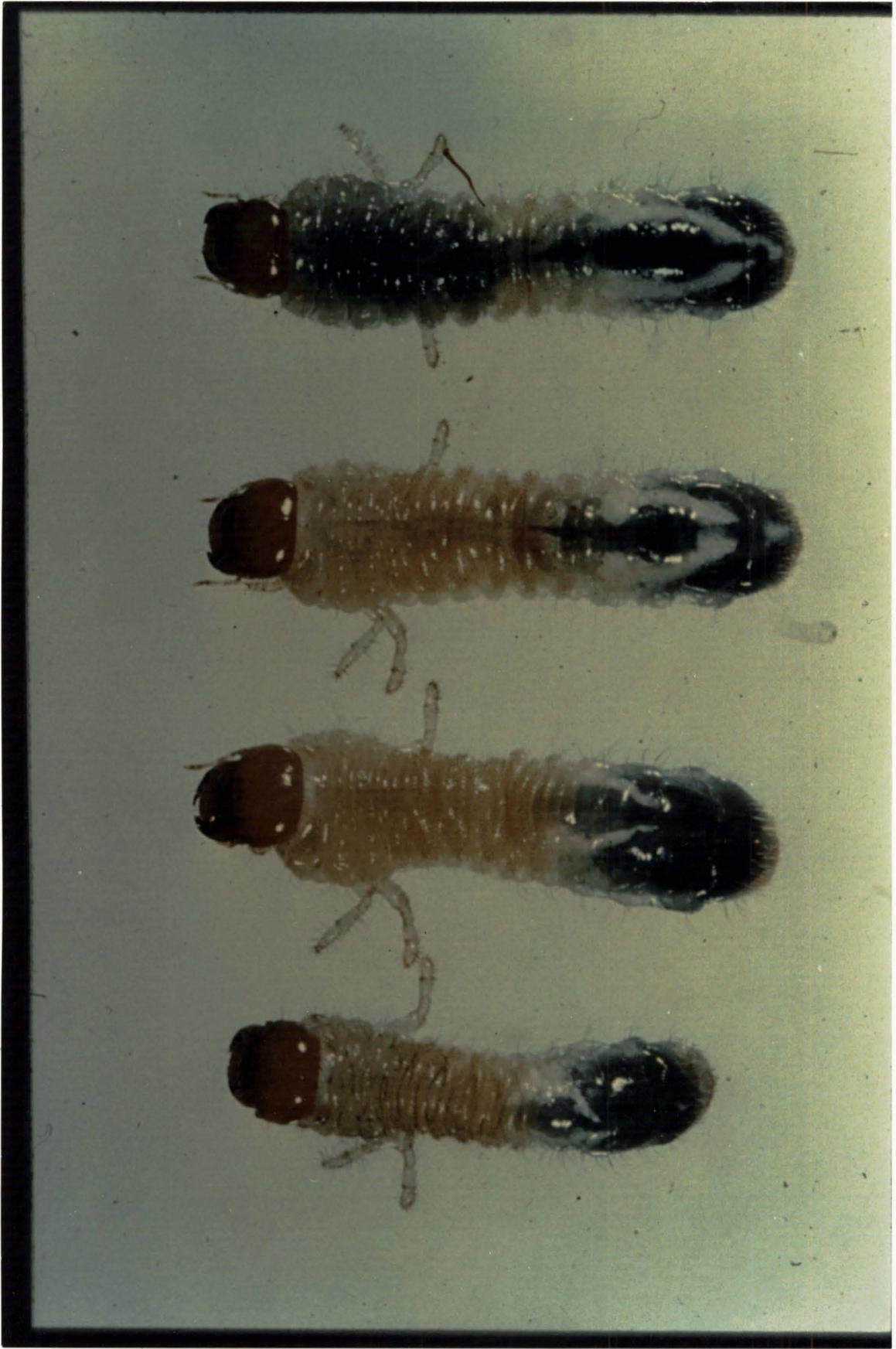
Outbreaks have been also observed in older pastures without a history of insecticide (East and Kain, 1982) and evidence suggests that pathogens are the major cause of *C. zealandica* larval population collapse (Cameron and Wigley, 1989).

1.1.6 The biological control of *C. zealandica* larvae by *S. entomophila*

Since the discovery of amber disease (formerly described as honey disease), research has led to the development of a microbial insecticide for control of *C. zealandica* larvae (INVADE, Monsanto, New Zealand). A survey carried out in 1981 in Canterbury indicated that up to 86% of some *C. zealandica* larval populations showed the symptoms of amber disease (Trought et al., 1982). Infected larvae cease feeding, develop an amber coloration and die. The development of these symptoms was associated with the presence of the bacterium *S. entomophila*. The bacterium appears to be specifically pathogenic to *C. zealandica* larvae and tests with closely related scarab larvae have produced no such symptoms of disease (Jackson et al, 1983). Insect-pathogenic strains were isolated from 11 of 19 sites throughout New Zealand (Stucki et al, 1984). The bacterium was artificially cultured and applied to field larval populations of *C. zealandica* and amber disease has been recorded in over 70% of the larvae in some artificially infected populations (Jackson and Pearson, 1985). Significant reductions of 30%-59% in the larval populations were obtained, and 47% of the remaining larvae in the treated plots were diseased, suggesting the long-term effect would be greater (Jackson et al, 1986). Applications of *S. entomophila* have resulted in a 30% increase in dry matter production in field (Jackson et al, 1986) and laboratory experiments (37% increase in white clover production) (Jackson, 1988). Different application methods were evaluated for their success in the establishment of *S. entomophila* into the pasture soil. Turf treatment deposited more bacteria into the target band (20-50 mm), and throughout the soil profile than surface treatments (jet stream and boom

Plate 3

Third instar larvae of *C. zealandica* showing different degrees of amber disease infection. From the left to the right hand side: three larvae presenting clear symptoms of amber disease and a larvae showing not apparent symptoms of disease (with permission of AgResearch, Lincoln).



spray). (O'Callaghan et al, 1987). The establishment of bacteria in soil was enhanced when higher volumes of water were used in the jet stream and boom spray. Once the bacterium is successfully established in the soil, *C. zealandica* larval populations remain low after one year of the application in treated plots (Jackson et al, 1989a). *S. entomophila* is more effective when applied in an inundative manner (Jackson, 1989). Based on the control achieved by *S. entomophila*, a model was developed to examine the effect of rotation on pasture damage produced by *C. zealandica* larvae (Jackson et al., 1989b). Plant damage can be minimized by: (a) maintaining pastures for as long as possible; (b) by reducing the period of time of pasture-crop rotations and (c) by concentrating cropping on as small area as possible of the farm (Jackson et al., 1989b). The use of *S. entomophila* is unlikely to be limited by intrinsic differences in soil type or in *C. zealandica* larval populations (Jackson et al, 1989c). Survival of *S. entomophila* in soil seems to be limited by environmental and biological factors such as soil moisture, competition with indigenous soil micro-organisms and larval density (O'Callaghan, 1989). A method for identification and recovery of *Serratia* isolates from field soils containing a diverse micro-flora has been developed (O'Callahan and Pearson, 1989). Field applications of *S. entomophila* persisted for long periods in soils with high numbers of larvae in which the bacteria could multiply (O'Callahan et al, 1989). The importance of quantity and quality of soil organic matter in the establishment of *S. entomophila* remains to be studied.

Research in molecular biology is looking for the pathogenic determinants of the amber disease for widening the potential use of the bacterium in the control of *C. zealandica* and other soil-dwelling scarab larvae (Jackson, 1990b; Glare and Jackson, 1990; Glare et al., 1992; Nuñez-Valdez, 1994). The optimization in the use of *S. entomophila* in the field in New Zealand agriculture is now being addressed. Therefore, ecological complexity of the soil environment and the behavioural traits observed in scarab larvae (Villani and Wright, 1990) should be considered.

1.1.7 Functions and definition of soil organic matter

Soil organic matter (SOM) is a major component in the soil system in pastoral agriculture. According to Stevenson (1982), humus or SOM is known to include a broad spectrum of organic constituents, many of which have their counterparts in biological tissues. The SOM has several functions

in agriculture (Allison, 1973; Stevenson, 1982) and its major functions are: (a) a nutritional function because it is a source of N, P and S that are available for plant uptake after mineralization and an energy source for nitrogen-fixing bacteria; (b) a biological function because it is a regulator of micro-flora and micro-fauna activities; and (c) a physical function because it has a positive effect on soil structure improving tilth, aeration and water holding capacity. Important additional functions of SOM are its effect on the chelation of soil micro-nutrients affecting the uptake by plants and micro-organisms as well as on the performance of herbicides and other agricultural chemicals due to their adsorption by soil organic matter (Stevenson, 1982).

An operational definition of SOM fractions has been considered in the present study, following the criteria of Goh (1980); Stevenson (1982) and MacCarthy et al. (1990). The biochemical composition of each of these fractions, in an ascending degree of stability, is as follows:

I) Soil microbial biomass. This fraction represents the C and N present in living microbial tissues. This is the living and most active fraction of SOM. Soil entomopathogens are part of this fraction.

II) Non-humic substances. This fraction consists of compounds belonging or similar to the well known classes of organic substances (carbohydrates, lipids, proteins and their allies). These compounds are extracted from the soil in the following fractions:

(1) Cold water extraction.- This fraction contains free biochemical groups as amino-acids, sugars or other monomeric labile substances which are readily mineralizable. These compounds may be quickly metabolized by micro-organisms, soil fauna and plants.

(2) Hot water extraction.- This fraction includes simple organic compounds, slightly decomposable carbohydrates and the compounds released after dead of soil micro-organisms. It reflects the level of organic matter supply to the soil and by this the ability to release N.

(3) Humic substances.- In this fraction are included a series of high molecular weight, brown to

black substances formed by secondary synthesis reaction. The synthesis of humic substances probably involves two main processes occurring simultaneously: (a) stabilization of recalcitrant plant material as lignin; (b) direct or indirect effect of microbial activity in chemical polymerization. The structures of humic substances are not known. These materials cannot be represented by discrete molecular or structural formulas. Even the term "structure" is difficult to define in the context of humic substances. These materials are comprised of complex mixtures which, to date, separation into pure fractions is impossible. Humic substances are generally classified on the basis of their solubility in water as a function of Ph as follows:

(1) Humic acids.- This is the dark-coloured organic material which can be extracted from soil by various reagents and which is insoluble in diluted acid.

(2) Fulvic acids.- This is the coloured material which remains in solution after removal of humic acid by fractionation¹

(3) Humins.- It is the alkali insoluble fraction of soil organic matter or humus. This fraction contains the most stable fraction of C and N in the soil.

1.1.8 Interactions between *C. zealandica* larvae and SOM

Despite the fact that living roots are regarded as the primary source of nutrition for soil-dwelling melolonthid larvae, SOM remains an important component in the insect diet under field conditions (Smith and Hadley, 1926; Davidson and Roberts, 1968a). Organic materials (mainly living and dead root particles) constitutes about 67% by weight of the total material consumed by *P. japonica* larvae which may survive in soil without living roots upon which to feed. However, the presence of roots is considered as extremely important for the development of *P. japonica* larvae (Smith and Hadley, 1926). Moreover, Smith and

¹The term fractionation is defined in this study as the sequential estimation of C and N present in cold and hot water extractions, fulvic and humic acids and humins (Goh pers. comm.). See Figure 2.1 and Appendix I for details.

Hadley (1926) have observed that batches of *P.japonica* larvae have died for no apparent reason other than starvation when live plant material was not added to the soil. Soil-dwelling scarab larvae develop best when an available source of organic material is provided in rearing cages under laboratory conditions (Bedford, 1980; Wightman, 1972a; Villalobos, 1985).

The non-specific nature of food selection by *C. zealandica* larvae has been pointed out by Sutherland (1971) who stated: "Not only do the grubs feed on a variety of plant species but they also grow and develop satisfactorily on a diet of sheep dung or humus. Indeed, Miller (pers. comm.) has reared two generations of this insect in humus devoid of living plant matter". Similar observations have been made with larvae of *Macrodactylus mexicanus* (Burm.), a suspected root-feeder that completes its life cycle in bare soil under laboratory conditions. Furthermore, an abundant population of this insect was found as early third instars actively feeding in the soil of a bare fallow cornfield (Villalobos, 1992). It is not clear if *C. zealandica* larvae discriminate between the biotic or abiotic components of SOM and live plant material (Radcliffe, 1970). Damage on grass production has been reduced when a source of SOM has been added to the soil. Radcliffe (1970) observed a reduction in the plant damage caused by *C. zealandica* larvae on pastures when cow dung was applied to the soil. Mulching with hay was also recommended by Hallock (1936) to protect strawberry plants from damage caused by the rutelid *Autoserica castanea* Arrow in New Jersey.

1.1.9 Interactions between entomopathogens and SOM

Entomopathogens can have profound effects on the dynamics of their invertebrate host populations that may be regulated to low and relatively constant densities if sufficient numbers of pathogens are transmitted from pathogen reservoirs to habitats where transmission can occur (Hochberg, 1989). Although the number of bacteria and fungi in the soil from maize fields in Iowa was not higher in manured compared with non-manured soils (Somasundaram, et al., 1987), the microbial biomass was usually higher in soils from organic farming systems (Reganold et al., 1987). Infection of larvae of the scarabaeid *Holotrichia serrata* Fab by *B. popilliae* increased in a range between 18-45% when organic manure was applied to the soil (Rao and Veeresh, 1988). Evidence suggests that carbon and nitrogen sources are

necessary for germination of the conidia of entomopathogenic fungi (Jackson et al., 1991). In Canterbury, preliminary samples showed that in old pastures, the incidence of milky disease produced by *Bacillus* spp is higher than in young pastures and arable crops at Winchmore Irrigation Research Station. Furthermore, a protozoan infection (probably *Mattesia* sp) is more frequently found at Winchmore than in other conventional farming soils of Canterbury (T.A. Jackson pers. comm.).

1.2 Contribution of soil-dwelling melolonthid larvae to soil fertility

Larvae of soil-dwelling melolonthids, unlike most of their Scarabaeoidea relatives, are regarded as serious pests in areas of herbaceous vegetation all around the world (Hurpin, 1962; Miln, 1964; Fleming, 1972; Lim et al., 1980; Tashiro, 1987; Allen, 1987; Keller and Zimmermann, 1989; Chapman, 1990; Jackson 1992; Garnham and Barlow, 1993). This trend has been followed in developing countries where also significant reductions in crop production have been attributed to these insects (Jepson, 1956; Amaya and Bustamante, 1975; Alvarado, 1983; Veeresh, 1984; Rodríguez-del-Bosque, 1988; Gassen and Jackson, 1992; Villalobos, 1992).

Concern about the problems caused by soil insects has concentrated on the control, rather than on the consideration of the possible beneficial role these insects may play in the soil. As a result of this, little information is available to quantify and ponder on detrimental and beneficial effects. With the existing information, is not possible to compare, in quantitative terms, the beneficial role of melolonthid larvae with that of other soil macro-invertebrates. Concerning melolonthid larvae, there is a lack of integration of recent findings from economic entomology and soil biology and very often this information is contradictory. Evidence suggests that soil macrofauna may potentially have either beneficial or detrimental effects on soil functions and crop yields (Hole, 1981; Lal, 1988).

Economic entomologists have emphasized their efforts to test pest control strategies and to obtain immediate practical returns. On the other hand soil biologists have excluded these insects from the list of the beneficial soil macrofaunal groups (earthworms, myriapods, ants and termites) having a potential for amelioration and restoration of fertility (Anderson, 1988; Lal, 1988; Curry and Good, 1992; Sims, 1992).

In this review, some ideas about the possible beneficial contribution of melolonthid larvae to soil fertility are presented. Environmental conditions may determine if soil faunal effects are positive or negative. Therefore, sub-economic densities of soil-dwelling melolonthid larvae and balanced soil conditions may encourage their potential positive effects. Such ideas may help to explain obscure ecological patterns and unexpected experimental results, in trials based on the assumption that the activity of these insects in the soil is exclusively detrimental.

1.2.1 Ecological functions of melolonthid larvae

The direct and indirect activities that melolonthid larvae perform in the soil take place at different hierarchical levels. These functions are closely interconnected and are impossible to separate completely one from the other. As the soil is a dynamic system, the modifications of the soil environment caused by faunal alterations may be considered as beneficial or detrimental according to the global conditions. The functions of melolonthid larvae on soil fertility may be classified as physical, chemical and biological.

1.2.1.1 Physical functions

Many of the positive traits that have been attributed to the mechanical action of earthworms (Lavelle, 1983; Lee, 1991) may also be applied to melolonthid larvae. Melolonthid larvae belong to the soil macrofauna and have the ability to move freely around the soil environment. These insects may be useful in assisting aeration, drainage and root penetration. Moreover, through their feeding and burrowing activity, they may either transport organic and inorganic materials down to deeper layers or bring them to the soil surface. They may also help to mix mineral and organic material into the soil profile (Yaacob, 1967; Pottinger, 1976).

Movement of melolonthid larvae is affected by several interrelated factors that act in a hierarchical way. Soil moisture, soil temperature, soil texture and structure, root biomass, root attractants, CO₂ concentration, quality and amount of organic matter may affect the speed, distance and direction of larval migrations in the soil (Ritcher, 1958; Tashiro et al., 1969; Kelsey, 1970; Sutherland, 1972; Ridsdill-

Smith, 1975; Boyd, 1975; Galbreath, 1988).

Soil moisture and temperature are closely related and have a primary influence in melolonthid larvae migrations. According to Kelsey (1970) where moisture is not critical, the position of *C. zealandica* larvae in the soil profile is influenced by availability of food. This author observed that at soil moisture lower than 10% second instars of *C. zealandica* were located 15-18 cm deep in the soil, but in response to 2.5 mm of rain they moved into the top 2.5 cm within 18 hours. At moisture levels above 16%, the distribution of *C. zealandica* larvae appears independent of moisture. With the first and second instars of *A. majalis*, 4% of soil moisture was found to be the critical value that triggered movement in a gradient of soil dryness (Tashiro et al., 1969). In addition, the temperature range of 11.7-27.2°C was optimum for these insects. Movement into soils within this range occurred from both higher and lower temperatures.

Under field conditions, vertical movement of larvae has been considered as being more common than horizontal movement. According to Kelsey (1951) larvae of *C. zealandica* move to deeper soil layers depending of the stage of development, availability of food and soil moisture. Vertical movements have been mainly studied in third instar larvae. First instars seldom inhabit the upper 5 cm of the soil and feed in the vicinity of where the eggs were laid. Second instars are normally located in the upper 5 cm of the soil; however, the movements may be greater in second instar larvae of hemivoltine populations (Stewart and Stockdill., 1972). Under irrigated pasture East (1972) noted that approximately 40% of the second instar population inhabited the top 2.5 cm, where third instar larvae are often present. In a higher rainfall area in Otago, irrespective of larval maturity, the majority of larvae with exception of the prepupae were present in the top 5 cm (Stewart and Stockdill., 1972). According to Galbreath (1970), third instars are able to detect small changes in moisture level and move down to avoid hydric stress but become unresponsive to moisture at the prepupal stage. Moreover, second instar seem to be more responsive to moisture gradients than third instars.

Movement of soil-dwelling melolonthid larvae to deeper layers in the soil occurs during the cool or dry seasons. *C. zealandica* larvae descend in the soil in response to climate to hibernate and ascend with the rising temperature during spring to feed before pupation (Miller, 1945 in Kain, 1975). However

contradictory observations suggest that *C. zealandica* behaves as do most New Zealand melolonthids, being present in the upper layers and actively feeding even in frozen soil (Given, 1952). Larval movement up to and across grass surface by *C. zealandica* larvae has been reported at Dorie in Canterbury (Pottinger pers. comm. in Barratt, 1982). Tashiro (1987) mentions that larvae of *Cotinis nitida* L. crawl to the surface at night to feed on the dead and decaying organic matter in golf courses. A similar observation has been made for the Tasmanian grass grub (*Aphodius tasmaniae* Hope) in New Zealand pastures (Scott, 1984).

Food sources have also a remarkable effect on melolonthid larval migrations. In a laboratory study of feeding behaviour, third instars of *Sericesthis nigrolineata* (Boisd.) moved less in soil in the presence of dead roots than in soil alone, and less in soil in the presence of living roots than with dead roots (Ridsdill-Smith, 1975). Third instars of *C. zealandica*, under laboratory conditions and in the absence of plant tissue, are able to move over 6.4 m/month (Kelsey, 1970). However, under field conditions lateral movement does not exceed 61 cm in one generation (Fenemore, 1965). Tashiro et al., (1969) reported an average rate of 30 cm per day in third instars of *Amphimallon majalis* (Raz.). The economically important melolonthid larvae in Britain which include *Melolontha melolontha* (L.), *Phyllopertha horticola* (L.) and *Amphimallon solstitialis* (L.) seem to have restricted lateral movement. (Anon., 1971 in Kain, 1975). Miln (1964) estimated that the average distance travelled by *P. horticola* from egg to pupae is less than 31 cm. The possible existence of larval arrestants in plant roots has been suggested to explain the limited movement of *C. zealandica* larvae under field conditions (Kain, 1975). Under laboratory conditions, *C. zealandica* larvae shows a trend to move towards an artificial source of CO₂ (Galbreath, 1988).

Repellency of entomopathogens by soil-dwelling melolonthids may also affect larval movement. Villani et al., (1991) have recently found that *Metharhizium anisopliae* (Metch.) mycelial particles repelled *P. japonica* larvae. According to these authors, the presence of the pathogen was detected and the insects moved to avoid contact with it.

Interspecific and age-dependent differences in patterns of horizontal and vertical distribution among Melolonthid larvae are also suspected. In a tropical grassland (Villalobos, 1991), a diverse (five main species were found) larval community of melolonthid larvae coexisted. Most species were highly

aggregated during egg, first, second and early third instars. However larval distribution in the soil became more randomly scattered as the dry season progressed. Late third instars of *Hoplia squamifera* Burm. were an exception as they aggregate during the dry season. During first instar, only *Cyclocephala immaculata* (Oliv.) was represented at a depth of 20-30 cm. Second and early third instar *Phyllophaga ravidata* (Blanch.), *Phyllophaga trichodes* (Bates) and *H. squamifera* could be found below 20 cm. Late third instars occurred during the dry season and a high proportion of larvae of all species migrate to deeper horizons (10-30 cm). Villani and Nyrop (1991) found that significantly different patterns of movement occurred between *P. japonica* and *A. majalis* larvae in a soil-turfgrass microcosm. Differences were also significant among age-groups. The developmental stage of the larvae had a large effect on *P. japonica* behaviour and a measurable but lesser effect on *A. majalis*. Ritcher (1958) observed differences in the depth which *Phyllophaga* spp pupae inhabit and suggested that the species even though closely related taxonomically are distinct entities biologically.

Therefore, movement of soil melolonthids may be explained primarily in terms of soil physical conditions (moisture, temperature and structure) and secondarily by nutritional, behavioural and intrinsic factors.

1.2.1.2 Beneficial effects of melolonthid larvae in soil erosion

Some authors suggest that plant damage of melolonthid larvae is associated with soil erosion problems (Smith and Hadley, 1926; Tashiro et al., 1969). However, their presence in a stable soil environment, may have the opposite effect. Evidence suggests that soil macro-invertebrates are involved in the formation of structural aggregates in the soil (Lal, 1991). This effect is related to the ability of the soil macrofauna to produce humic and fulvic acids (Tisdall and Oades, 1982 in Lal, 1991). Moreover, through the ability of melolonthid larvae to stimulate soil microbial activity (Yaacob, 1967) they also contribute to minimising soil erosion. Fungal hyphae and polysaccharides of microbial origin play an important role in soil aggregation (Harris et al., 1960 in Lal, 1991; Haynes et al., 1991). In addition, with the physical transformations that the macrofauna produce into the soil matrix the structure of the soil improves (Lee, 1991).

1.2.2 Chemical functions of melolonthid larvae

Melolonthid larvae may contribute in a significant way to the nutrient cycles, particularly N, S and P. There are five main pathways in which they may participate: (a) immobilization and prevention of nutrient leaching; (b) exportation of nutrients above and below-ground; (c) horizontal redistribution of soil nutrients; (d) synergistic catalysis of mineralization and (e) humification.

1.2.2.1 Immobilization of nutrients and prevention of nutrient leaching

Elements are immobilized in the insect body and this is a biological mechanism which counteracts leaching. Yaacob (1967) suggests that *C. zealandica* larvae are selective feeders on the N rich organic matter in the soil and this selective feeding habit accounts for the fact that casts contain higher total and mineral N than the adjacent soil. Based on Yaacob's calculations, the amount of N mobilized by *C. zealandica* is about 1.2% of the larval live weight. Considering a population density of 300-1000 larvae m⁻² and an average live weight of 100 mg for a third instar, the amount of N locked up in the insect bodies may account for 3.6-11.9 kg N ha⁻¹. Survival of *C. zealandica* from egg to adult has been estimated as 17% under field conditions (Plunket and Kain, 1979). In a tropical grassland the survival from egg to adult under field conditions was calculated to be between 5 and 8.5% for the five main species (Villalobos, 1991). Therefore, most of this N is slowly released back to the soil.

1.2.2.2 Exportation of nutrients above and below-ground

Nutrients are exported from the soil through predation of larval populations or through adults emerging from the soil and being incorporated in the above ground trophic web. Additionally, nutrients are exported to deeper layers of the soil through vertical migration and the release of material by metabolic activity or death.

Predation of *C. zealandica* larvae and adults by starlings (*Sturnus vulgaris* L.) has been studied by East and Pottinger (1972) and was suggested as a biological control agent. These authors showed that

reductions of 40-60% in grub populations during May and July may be attributed to starling predation. Although predation of adults appears to have a negligible effect on *C. zealandica* populations this is a clear example of the flux of matter and energy from soil to the above ground system in which melolonthids participate. Taking into account the above data of Yaacob (1967) and the conditions observed by East and Pottinger (1972), it is possible to make a theoretical estimation of the magnitude of N transfer from *C. zealandica* to *S. vulgaris*. Under Winchmore conditions (a larval density of 400-900 larvae m⁻² and a predation rate of 50% by a population of 150 starlings) the amount of N that a starling would obtain from this resource between March and July would range from 1.59 to 3.57 mg N per bird. However, this estimation assumes an even distribution of the resource and a 100% assimilation rate, conditions that are uncommon in nature. According to Cairns (1980) larvae of the melolonthid *Rhopaea verreauxi* Blanch accumulate higher levels of N, P, S, Na, K and, at some stages Mg, than those of the surrounding soil. Except for N, the standing crop of the chemical elements measured were quite small. This author concluded that it was unlikely that this insect could act as a sink for nutrients in grazed pastures. Despite the fact that this ecological process has received little attention, it is suspected that its magnitude in fragile modified ecosystems may be significant in the long-term.

1.2.2.3 Horizontal redistribution of soil nutrients.

Nutrient redistribution through the action of melolonthid larvae may be subtle and its ecological significance is unknown. In pastures, grazing cattle may cause the concentration of nutrients in particular areas of the soil under dung and urine patches (Williams and Haynes, 1990; Nyguyen and Goh, 1993). *C. zealandica* larvae are one the few abundant species in New Zealand soils that may help to redistribute nutrients. Because of the restricted movement of the life stages of *R. verreauxi* in Australian pastures, they were not considered as significant agents in the movement of nutrients inwards or outwards of the soil system. However the long-term effect that these insects may have in nutrient redistribution should also be considered, especially in areas of low soil fauna diversity. Overall, the invertebrate fauna of New Zealand, both in indigenous tussock grasslands and improved pastures, is "fairly meagre" in diversity though not necessarily in numbers (Harrison and White, 1969 in Mark, 1993). The flux of minerals from upper to lower layers in the soil and vice-versa is closely associated with the movements, metabolic activities and

death of melolonthid larvae. Understanding the real contribution of melolonthid larvae in nutrient redistribution will help to optimize the levels of fertiliser inputs in pastures.

1.2.2.4 Synergistic catalysis of SOM mineralization

Melolonthid larvae participate in SOM mineralization. Theoretically mineralization of organic compounds may be either accelerated or delayed. Synergistic degradation occurs through ingestion and comminution of complex organic compounds, which renders them more accessible to microbial attack. Delay may take place if the larvae overgraze the microbial populations involved in the decomposition process, discouraging microbial regeneration and renewal. As these insects have the ability to feed on SOM as an alternative to plant roots, this must have an effect on the mineralization of organically fixed N, S, and P, which is a source of nutrients for plants. The significance of *C. zealandica* larvae in soil organic matter mineralization in introduced pastures has been pointed out by Yaacob (1967). In this respect the contribution of *C. zealandica* to improve soil fertility in New Zealand pastures is similar to that of earthworms (Yaacob, 1967; Pottinger, 1976).

1.2.2.5 Effect of melolonthid larvae on humification

Nothing is known about the effect that the stimulation of microbial activity caused by melolonthid larvae may have in the humification process. Neither it is known if there is an increase of the cation exchange capacity as a result of direct or indirect activities of these insects. Yaacob (1967) identified the importance of assessing the contribution of larvae of *C. zealandica* in forming clay colloid complexes and humic materials. In theory, formation of stable organic compounds in the soil which increases cation exchange capacity may be a factor linked to an increase of the carrying capacity of pasture plants to compensate larval herbivory².

1.2.3 Biological functions of melolonthid larvae

²Larval herbivory is defined in this study as the modification caused by soil-dwelling melolonthid larvae in the root system either by severing or ingesting living root plant material.

Soil-dwelling melolonthid larvae are involved in many biological functions in the soil. They affect producers (primarily plant roots), soil micro-organisms, microfauna, mesofauna and other macrofaunal groups. These effects are mainly related with the trophic flux of abiotic and biotic soil components.

1.2.3.1 Trophic interactions with plant roots

The mainstream of entomological research on melolonthid larvae has historically considered that the economically important species are obligate root-feeders (Hurpin, 1962; Miln, 1964; Lim et al., 1980; Tashiro, 1987; Allen, 1987; Keller and Zimmermann, 1989; Jackson, 1992; Garnham and Barlow, 1993). However the complex feeding behaviour of this group remains a matter of discussion. The relative importance of living roots and other organic matter in the soil in the nutrition of melolonthid larvae has not been clearly defined and there is no evidence to prove that any of the economic species is an obligate root-feeder (Richter, 1958; Davidson and Roberts, 1968b). Observations suggest that melolonthid larvae feed on a range of substrates that vary from living and dead roots of plants (Smith and Hadley, 1926) to other fractions of the complex pool defined as "humus" (Davidson and Roberts, 1968a; Miller in Sutherland, 1972; King, 1977).

The way in which melolonthid larvae utilize the tissues of living roots for their nutrition remains an unsolved aspect of their biology. Bauchop and Clarke (1975) failed to find cellulose-utilizing bacteria and cellulolytic activity in the gut of third instar *C. zealandica* larvae. These authors concluded that larvae did not make appreciable use of the structural carbohydrates in the roots of pasture plants, but utilised the small quantities of starch and soluble sugar present. Furthermore they state: "Such digestion may explain the high throughput of dietary root material and the highly destructive effect of the larvae on pasture plants". After dissections of the foregut of *P. japonica* larvae, Smith and Hadley (1926) found that the material eaten was composed of small soil particles, fresh plant tissue and small pieces of plants which were partially decomposed.

A different degree of genetic determinism of the root herbivory may also be recognized among populations of melolonthid larvae. Larvae of *Phyllophaga vetula* (Horn), *Phyllophaga blanchardi* (Arrow)

and *M. mexicanus* were reared in bare soil under identical laboratory conditions. Larvae of both species of *Phyllophaga* were reared successfully from egg to third instar. However, a massive mortality of third instar larvae occurred even when replacements of fresh soil were made every two weeks. In contrast to both *Phyllophaga* species, which failed to complete their life cycle, larvae of *M. mexicanus* completed their whole life cycle under similar conditions. (Villalobos unpubl. obs.). In New Jersey, 50 plots without vegetation were examined and no larvae of *P. japonica* were found but in 50 plots with vegetation, an average of seven larvae were found close to plant roots (Smith and Hadley, 1926). Presence of the larval stage in the soil of a particular crop is an unreliable indication of insect plant damage. Insect presence in the crop has been commonly linked to plant damage since the beginning of the century (Hayes, 1930).

Richter (1958) and Morón (1983) have suggested different degrees in the evolution of rhizophagy and saprophagy among subfamilies of Scarabaeoidea. Nevertheless, this pattern should be taken with reservation because local adaptations may occur among populations in the same subfamily or genus. Most of soil Scarabaeidae Aphodiinae are an ecologically and morphologically uniform saprophagous-coprophagous group of insects. However, larvae of *A. tasmaniae* have become an important pest of pastures of Australasia (Carne, 1956; Maelzer, 1960; Pottinger, 1968). Furthermore, although many of the presumed rhizophagous *Phyllophaga* spp larvae collected by Hayes (1930) were present in soils of crops, most larvae of *P. submucida* were collected under manure in pastures in Kansas. Halfpeter and Edmonds (1982) and Cambefort (1991) suggest that in Scarabaeinae the saprophagous habits are a primitive character of the family. This evolutionary trait may also be shared by their Melolonthidae relatives in which a trend to increase the degree of rhizophagy would be the modern character (Crowson, 1981; M.A. Morón pers. comm.).

1.2.3.2 Trophic interactions with micro-organisms

The possibility that larvae of Melolonthidae may graze on soil micro-organisms should not be excluded. Theoretically, soil algae could be a potential source of food. The presence of a fermentation chamber containing an abundant and diverse microflora suggests the existence of an internal rumen in their hindgut (Bauchop and Clarke, 1975). A similarly rich microflora was also observed in the midgut

of larvae of *Holotrichia serrata* (F.) (Gupta and Rana, 1988) but the nature of this relationship is uncertain.

It is unknown to what extent these microbes are a direct or indirect source of nutrients for the insect, or if they establish a particular kind of symbiotic relationship. As these insects are commonly found close to the living roots of plants, the grazing activity they may have on microbial populations present in the rich rhizosphere should be strongly considered.

1.2.3.3 Trophic interactions with other soil microfauna, mesofauna and macrofauna

No information is available about the potential for melolonthid larvae to feed on protozoa, mites, springtails or even nematodes. Many species of mites are associated with melolonthid larvae and adults (Crocker et al., 1992). The relationship between these mesofaunal groups is presumed to be either phoretic, parasitic or mutualistic. However little experimental evidence about these interactions exists. Competition among grubs, mites, collembolans and earthworms in the soil is an aspect that has been scarcely explored. Feeding preferences among soil invertebrates may overlap at some stages. Occasional predation of earthworms by third instar larvae of *C. zealandica* in field and laboratory conditions has been observed in Canterbury pastures (Villalobos unpubl. obs.).

Melolonthids are the source of matter and energy for many species in the soil. Rickettsia, bacteria, protozoa, fungi, nematodes, mites, wasps, carabids, spiders, frogs, lizards, birds, moles and other mammals have been known to be natural enemies of melolonthid larvae and adults since the beginning of the century (Davis, 1919).

1.2.3.4 Dispersion of mycorrhizae

Melolonthid grubs may have an important role in the transmission and distribution of microorganisms involved in soil fertility. There is an important amount of external mycelium of vesicular-arbuscular mycorrhizal (VAM) fungi in soils of grasslands (Tisdall and Oades, 1979). Such a network comprises a potentially large source base for soil food webs (Rabatin and Stinner, 1988). Evidence suggests that many species of macroarthropods affect the density or distribution of external hyphae by grazing or

other mechanisms. VAM fungal spores have been found in the gut of scarab beetles (Rabatin and Stinner, 1988). Although no reports are available, melolonthid larvae are in close contact with the rhizosphere and are likely to ingest VAM mycelium and spores. Whether soil-dwelling melolonthid larvae may ingest, digest or disperse fungi requires further research.

1.2.3.5 Grazing of plant root pathogens

Root-feeding by melolonthid larvae may cause colonization of roots by fungal mycelium. This event may be regarded in two opposite directions: (a) pathogen entry into roots via lesions may result after insect attack (Brown and Gange, 1990) and (b) the action of plant pathogens could be reduced either as a result of insect grazing on plant pathogenic micro-organisms, or a better colonization of plant roots by beneficial micro-organisms, such as mycorrhizal fungi that could compete with soil-borne plant pathogens (Rabatin and Stinner, 1988; Chung et al., 1988). A low larval density and a high diversity of microbial communities in the soil may lead the direction of this process towards beneficial net effects.

1.2.3.6 Root pruning and regeneration

Low densities of melolonthid larvae may stimulate vegetative growth. In a recent review, Brown and Gange (1990) strongly suggest that when parts of the root system are removed by feeding, the replacement of roots is very rapid. The ability to produce adventitious roots is common in plants and root proliferation in response to moderate feeding appears to be the rule. According to these authors: "When lateral root proliferation occurs in response to levels of root herbivory below those affecting foliar production, root attack may be of possible benefit for a plant". Feeding by melolonthid larvae on decaying parts of plant roots could also improve soil fertility. The nutritive value of this fraction of the SOM may be increased once microbial colonization and decomposition activity take place.

1.3 General hypothesis

Pastures that have built up a high content of SOM may have a higher carrying capacity for soil

invertebrate populations, more efficient insect natural regulation mechanisms and a higher plant ability to compensate root herbivory by *C. zealandica* larvae. Beneficial effects of larval activity in soil dynamics may also be greater under high soil conditions.

SOM has a positive effect on soil microbial biomass. Non-humic substances are readily available source of nutrients for plants and soil micro-organisms and humic substances are a potential source of nutrients for soil biota after microbial attack. They also have a positive effect on soil structure. On the other hand, by improving soil structure and water holding capacity, SOM may also act as reservoir of entomopathogens of *C. zealandica* larvae. An increase in the cation exchange capacity in the soil may also increase the retention and dynamics of the soil nutrient turnover.

If organic matter has a positive effect in the control of *C. zealandica* larvae, it is important to recognize a source of SOM with potential to prevent or reduce problems caused by this insect in Canterbury. Organic wastes are a resource that could be used as soil amendments; to enhance levels of soil-borne entomopathogens; or to optimise biocontrol tactics against *C. zealandica* larvae in Canterbury.

Possible mechanisms involved in the reduction of plant damage caused by *C. zealandica* observed under high SOM conditions are: (a) SOM is an alternative direct or indirect source of food for the insects; (b) SOM encourages the presence of *C. zealandica* natural enemies; (c) SOM offers better nutritional conditions for plants so that they can compensate larval herbivory (d) *C. zealandica* larval spatial distribution is less likely to produce plant damage under high SOM conditions. It is recognized that there may be considerable difficulty in isolating the independent contribution of each one of these factors as all of them are likely to occur either independently or simultaneously.

1.4 Research approaches

The three research approaches commonly used in soil biology listed by Crossley et al., (1989) were used to explore the isolation of effects of SOM on larval feeding behaviour, bacterial infection and survival

and plant compensation of larval herbivory:

(a) Non-intrusive. The amount of total C and N present in different fractions of SOM were evaluated in soils from pastures with different ages and short-term fluctuations of bacterial populations were recorded in sites with similar environmental conditions except by their agronomic history and SOM content.

(b) Intrusive. Field applications of a bio-insecticide (INVADE, Monsanto NZ Ltd) and organic amendment (cheese whey) were made as manipulations.

(c) Artificial. Field mesocosms and laboratory gnotobiotic microcosms experiments were carried out using soil of pastures with different content of SOM.

Each approach has advantages and disadvantages. The main value of the non-intrusive approach relies in the description of patterns that actually occur in nature. However they have the limitation of not being able to explain ecological processes. On the other hand, laboratory microcosms experiments have the advantage of a tight control of biota, homogeneity, adequate replication and repeatability. These experiments are therefore ideal to explore processes but extrapolation from microcosm to field is difficult because field situations are more complex biotically and climatically. In the middle of both approaches are the intrusive types that allow selected elements of the soil biota to be manipulated in order to reveal processes and to interpret patterns. Although they are more realistic in maintaining the diversity of field micro-communities, both the intrusive approach and field mesocosm studies also have the limitation of time, space and resources that are needed. Despite the disadvantages in each of these approaches, their complementarity may help to grasp a more realistic conclusion in the interactions studied in the present work.

1.5 Research objectives

Major objectives of this study were to:

- (1) Determine the abundance and distribution of total C and N in SOM from three pastures with similar agronomic conditions except for their content of SOM.**
- (2) Compare the levels of indigenous populations of *S. entomophila* in soils from these pastures.**
- (3) Investigate the relationships between SOM, amber disease infection caused by *S. entomophila* and mortality caused by soil entomopathogens in *C. zealandica* larvae.**
- (4) Assess the performance of *S. entomophila* as a biological control agent under different SOM conditions.**
- (5) Relate *C. zealandica* root feeding activity and growth with different SOM conditions.**
- (6) Correlate plant damage caused by third instar *C. zealandica* on living roots and herbage growth and production during autumn/winter under different SOM conditions.**
- (7) Correlate larval growth with plant variables present in these three pastures.**
- (8) Evaluate the effect of *C. zealandica* early third instars on SOM dynamics.**
- (9) Assess the consequences of *S. entomophila* inundative applications in SOM turnover.**
- (10) Evaluate the role that the application of cheese whey may have in the growth and health status of *C. zealandica* larvae.**
- (11) Assess the effect of whey on the growth and infectivity of *S. entomophila* on *C. zealandica* larvae.**

(12) Measure the effect of applications of whey on growth and performance of above and below-ground parts of plants and larval herbivory caused by *C. zealandica*.

(13) Determine the effect of the application of whey in the build up of the cold and hot water extractions of SOM.

CHAPTER II

2) A MICROCOSM APPROACH FOR EXAMINING THE INTERACTIONS AMONG SOIL ORGANIC MATTER, AMBER DISEASE AND THE FEEDING ACTIVITY OF *Costelytra zealandica* (WHITE) LARVAE.

2.1 Introduction

Interactions among insects, entomopathogens and plants in soils are complex and dynamic. Soil organic matter (SOM) is a primary source of carbon (C), and nitrogen (N) and other elements for soil biota. Previous studies have shown that artificially added organic matter may reduce the damage caused by soil melolonthid larvae (Hallock, 1936; Davidson and Roberts, 1968b; Radcliffe, 1970; Miller in Sutherland, 1971; King, 1977). Moreover, SOM is an important source of C and N which is required for soil micro-organisms involved in biological control programmes (Jackson et al., 1991). Furthermore, SOM increases the probability of infections of soil-dwelling melolonthid larvae by soil entomopathogens (Rao and Veeresh, 1988) and may promote the formation of entomopathogen reservoirs. Reservoirs of entomopathogens have been suggested as being critical in the natural regulation of soil insect populations (Hochberg, 1989). In addition, the multiple beneficial role of SOM on plant performance and other aspects of soil fertility has been widely documented (Stevenson, 1982; MacCarthy et al., 1990).

The effect of SOM on the reduction of plant damage by soil scarab larvae is not well understood. No previous attempts have been made to integrate and quantify the extent to which larval root herbivory, insect entomopathogens and soil fertility are involved in this process. The present study attempts to assess the magnitude of these factors. Interactions among SOM, the feeding behaviour of *C. zealandica* larvae and their amber disease caused by *S. entomophila* (Jackson et al., 1993) are examined. A microcosm approach (Crossley et al., 1989) was used to explore the independent effects of these factors. This approach was useful as it isolates these factors from the environmental variability and complexity in which they occur in nature.

Major objectives of this study were to:

- (1) Measure the total C and N content of different fractions of the SOM of two pastures with different agronomic histories.
- (2) Compare the levels of soil-borne indigenous populations of *S. entomophila* in soils from these pastures.
- (3) Examine the interactions among SOM, larval mortality and amber disease.
- (4) Relate *C. zealandica* root feeding activity and growth with the SOM content in both pastures.

2.2 Materials and methods

2.2.1 Description of the sites

Soil was collected in March 1991 from two experimental sites located at the Winchmore Irrigation Research Station, Canterbury, New Zealand. Two improved pastures of 4 and 38 years were chosen for this study. The 38-year-old pasture (S14) had a dominant grass vegetation consisting mainly of perennial ryegrass (*Lolium perenne* L.). The 4-year-old pasture (A6) had mainly white clover (*Trifolium repens* L.)³. Site A6 received alternate cycles of 3 or 4 years cropping (wheat, barley, pea and oat) followed by 3 or 4 years in pasture. Both sites are close to each other (ca. 500 m) and had the same soil type. According to Nguyen and Goh (1992) these pastures are established in a Lismore stony silt loam soil (Udic Ustochrept). Both pastures received regular fertilizer applications and are exposed to similar climatic conditions and irrigation regimes. The assumption was made that SOM fractions at these sites were quantitatively different as a result of pasture age. It was also assumed that these differences may affect soil fertility, larval root-feeding behaviour and level of larval disease.

³See Chapter Appendix II for a quantitative estimation of plant composition in these sites.

2.2.2 Soil collection

Four random samples of soil (225 cm²) from each site were taken from the surface to 10 cm depth, placed in plastic bags and transported to the laboratory. These soil samples were stored at 5°C in darkness. Before determinations were made, the soil from these samples was crushed, sieved through a 4 mm mesh screen, bulked and air-dried for 3 days. The dried soil was then sieved through a 2 mm mesh screen.

2.2.3 Enumerations of *S. entomophila* in soil before the microcosm experiment

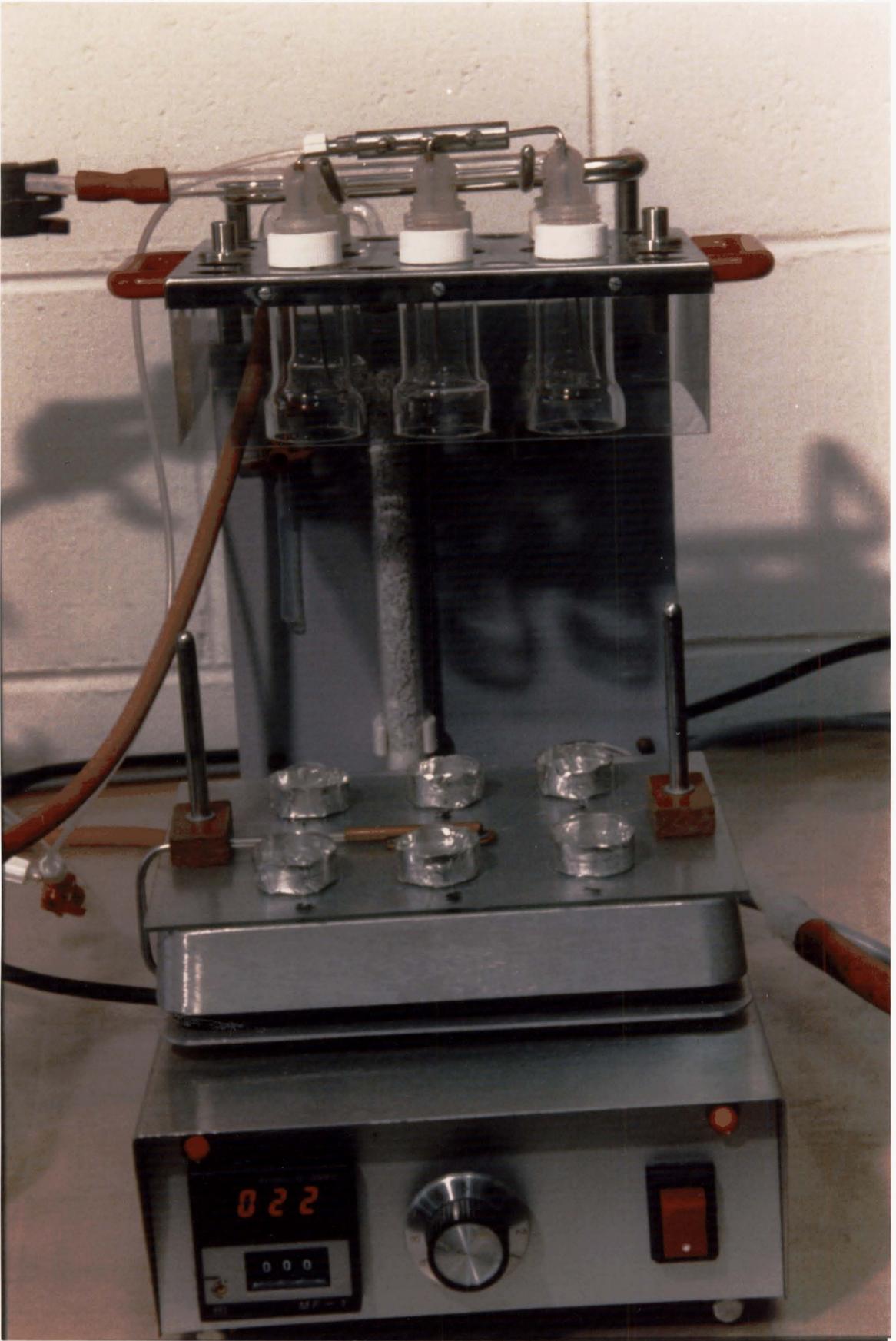
A duplicated estimation of *S. entomophila* numbers was made by the dilution plate method as described by O'Callaghan and Jackson (1993a). Enumerations were made in sub-samples of soil collected before and after the soil was air-dried.

2.2.4 Analyses of total C and N in SOM fractions

Before the microcosm experiment described below was set up, a further subsample was used to determine total C and N from both soils by the Dumas combustion method using a continuous flow C-N analyzer, connected to an isotope ratio mass spectrometer (ANCA-MS, Roboprep, Europa, United Kingdom (Grewal et al., 1991; K.M. Goh pers. comm.)). At the end of the microcosm experiment, a duplicated subsample of bulked soil per treatment was used to determine the total C and N from different SOM fractions. Soil microbial biomass C and N were extracted by the fumigation-extraction method of Jenkinson and Powlson (1976) as modified by Vance et al., (1987); Ocio et al., (1991) and L. Nguyen (pers. comm.). Soil microbial biomass C was measured according to Walkey and Black (1934) and not by the Dumas method, because ashes derived from K₂SO₄ interfered with the combustion of C. Soil microbial biomass N was measured by Dumas combustion after evaporating 2 ml aliquots in a N inert atmosphere evaporator developed at the Soil Science Department, Lincoln University (R.J. MacPherson pers. comm., Plate 4). A relationship of biomass C and N was established using 10 soil samples that gave a mean C/N value of 7. This value was then used for the estimation of biomass N in samples which could not be determined in the mass spectrometer due to salt interference.

Plate 4

MacPherson's Apparatus. A Nitrogen inert atmosphere evaporator developed in the Department of Soil Science by R.J. MacPherson (unpubl.).



A procedure for sequential fractionation of SOM (Figure 2.1) was used and total C and N in most SOM fractions were determined by Dumas combustion (Grewal et al., 1991). The sequential fractionation involved: (a) cold water extraction (Haynes et al., 1991); (b) hot water extraction (Korschens et al., 1990 modified by K.M. Goh pers. comm.); (c) humic substances extracted as humic acids, fulvic acids and humins (Goh and Molloy 1978; Goh, 1991). A detailed description of the methods followed and biochemical compositions of each of the SOM fractions can be found in Appendix I and Section 1.1.7 (Chapter I) respectively. The SOM fractions were operationally defined according to the criteria of Goh (1980), Stevenson (1982); MacCarthy et al. (1990). The percentage of organic matter was calculated according to Foth (1979) as $\%SOM = 1.724 \times \%C$.

2.2.5 Description of the microcosm experiment

A microcosm laboratory experiment was carried out to explore the relationships among SOM, amber disease and the larval feeding activity of *C. zealandica*. Early third instars of *C. zealandica* were collected from Te Anau in early December 1991. Because of the lower bacterial background present in the air-dried soil, a subsample of this was used for the microcosm experiment. Before the experiment, soil moisture was determined and adjusted to 25%. Larvae displaying no apparent symptoms of disease and actively feeding on small diced pieces (15 mg) of fresh carrot root were selected. Larvae were placed individually in the 1 cm cube cavities of polythene ice-cube trays, covered with a plastic bag and moistened tissue paper. Larvae were then incubated in a controlled environment chamber at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ under a 12:12 light:dark photoperiod. Feeding assessments were carried out every second day during the following two weeks. Larvae were weighed on an electronic balance (type 1412 Sartorius) the same day the experiment was set up. At this time larvae were placed individually into plastic pots (4.5 cm diameter x 6 cm height) containing 50 g of soil (dry weight) that had been sieved through a 2 mm mesh screen. In the centre of each pot a uniform cylindrical piece of carrot root (3 mm diameter x 13 mm height) was introduced to determine the amount of carrot root ingestion by larvae (Plate 5).

The experiment consisted of four treatments:

Plate 5

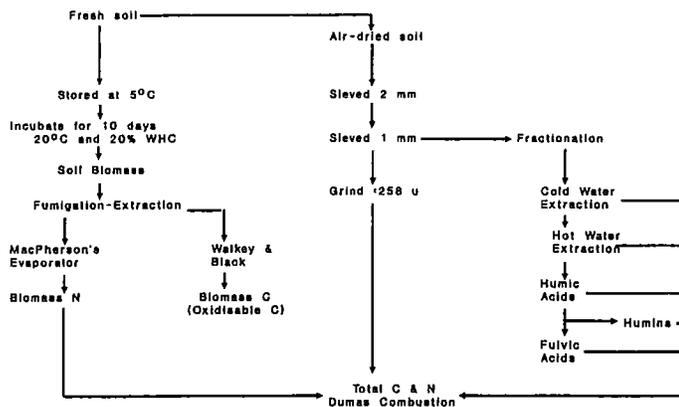
Experimental units used during the microcosm experiment described in section 2.2.5 (Chapter 2). The larvae is showing clear symptoms of amber disease and the piece of carrot root was removed from the centre of the pot to show the inhibition of the larval feeding activity after 20 days.



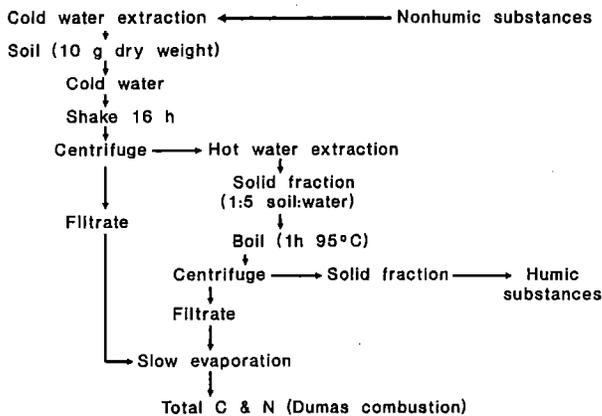
Figure 2.1

A diagrammatic representation of the fractionation scheme for SOM in pasture soils. (a) overall fractionation; (b) fractionation of nonhumic substances (cold and hot water extractions); (c) fractionation of humic substances (humic acids, fulvic acids and humins).

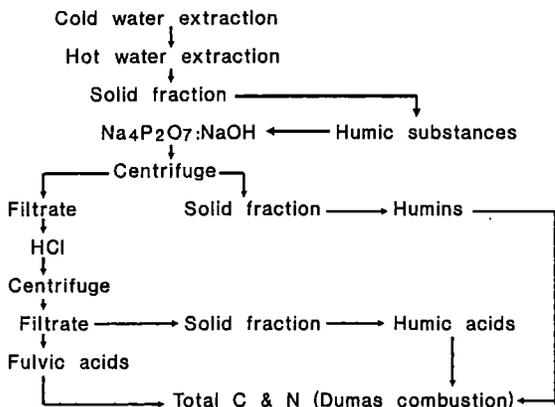
(a)



(b)



(c)



(1) Low dose = addition of two ml 7×10^6 cell ml^{-1} of a pathogenic strain of *S. entomophila* (BC4B) resuspended in nutrient broth which consisted of raw sugar, yeast extract diet, urea, Na_2HPO_4 , KCl and NH_4NO_3 .

(2) Medium Dose = similar to "(1)" but two ml 7×10^8 cells ml^{-1} of BC4B were inoculated.

(3) High dose = similar to "(1)" but two ml 7×10^{10} cells ml^{-1} of BC4B were inoculated.

(4) Control (Nil dose) = similar to (1) but two ml of nutrient broth free of *S. entomophila* were added.

The *S. entomophila* strain BC4B is currently being used as a commercial bio-insecticide in New Zealand (Jackson et al., 1992). In addition to the treatments described above, a single pot per treatment was included as a blank (without a larva) and placed in incubation under the same conditions. The arrangement of the pots in the incubator followed a randomized complete block design. Sets of ten pots per treatment were incubated under the conditions described above, allowing the entrance of air and light and maintaining soil moisture at 25%. Each pot was inspected on two occasions, at 15 and 30 days.

During each inspection, the appearance of amber disease symptoms, larval mortality, dry weight of remaining carrot, and larval live-weight were recorded. Larval infection by amber disease was determined by visual assessments following the description of disease symptoms described by Jackson et al., (1993). In this work larval carrot consumption was defined as the percentage of the carrot cylinder removed by insect feeding. This consumption was determined by visual comparison of the volume of the remaining carrot in treated pots after 15 days (Plate 6) with a newly cut carrot cylinder that had the same dimensions as those used at the beginning of the experiment. Carrot decomposition was expressed in percentage and is defined as the loss in dry weight (24 h at 105°C) after 15 days of incubation in the blank pots in comparison to the initial dry-weight of the carrot root cylinder. In these pots the remaining carrot cylinders were washed with distilled water before drying. An extra fresh carrot cylinder was provided to

Plate 6

Differences in the amount of carrot root remaining at the end (after 30 days) of the microcosm experiment described in Section 2.2.5 (Chapter II). OLD = in soil from a 38-year-old pasture; YOUNG = 4-year-old pasture; BC4B = pots treated with a pathogenic strain of *S. entomophila*; CONTROL = pots treated with *S. entomophila*-free nutrient broth; BLANK = pots incubated without larvae; LOW, MEDIUM and HIGH represent the different doses that have been used (for details see Section 2.2.5).



OLD LOW
BC4B



YOUNG LOW
BC4B



OLD MEDIUM
BC4B



YOUNG MEDIUM
BC4B



OLD HIGH
BC4B



YOUNG HIGH
BC4B



OLD CONTROL



YOUNG CONTROL



OLD BLANK



YOUNG BLANK

all larvae before the second 15 days period that lasted this experiment. Larval growth is defined as the live-weight gain after 15 days of incubation.

2.2.6 Statistical analysis

Most statistical analysis were carried out following the criteria of Little and Hill (1978) and Devore and Peck (1986) using the software computer programme MINITAB release 8.0 (PWS Publishers). To compare levels of amber disease and mortality after 15 and 30 days of the experiment, an analysis of variance (ANOVA) was carried out using a generalized linear model with a binomial error term (D. Saville pers. comm.). This model recognizes the 4*2 factorial structure with the 4th level factor broken into three orthogonal contrasts (D. Saville, pers. comm.). ANOVA and least significant differences (LSD) analysis were performed to compare the dry weight of remaining carrot and larval dry weight gain. \log_{10} transformed data were used to compare the levels of total C and N in different SOM fractions and bacterial numbers in the soil. Arcsin transformations of the results were also carried out to compare larval carrot consumption. Contingency tables were used to compare the differences in levels of amber disease and larval mortality among treatments. Correlations and Student's t-tests were used to compare larval live weight gains between soils.

2.3 Results and Discussion

2.3.1 Total C and N in SOM fractions

The agronomic history of the sites studied are reflected in the content of SOM. The percentage of organic matter in the older pasture (S14) was almost twice that of the younger pasture (A6) and this difference was significant ($P < 0.05$) (Table 2.1). The amount of total C and N present in all SOM fractions was higher in soil from S14 than from A6 pasture (Table 2.2, Figure 2.2a and 2.2b). As expected, changes in the amount of organic matter followed the normal trend shown in studies of soil chronosequence in other soils of New Zealand (Goh et al., 1976; Nguyen and Goh, 1990). Grewal et al. (1991) found a range of 5.2-10.8% organic matter for soils with minimum tillage, long term-pastures and permanent grasslands

Table 2.1.

Percentages of total C, total N and organic matter in two soils from Winchmore.

	Pasture		LSD _(P<0.05)
	S14 ¹	A6 ²	
Total C (%)	4.40	2.56	0.22
Total N (%)	0.42	0.24	0.02
Organic Matter³ (%)	7.59	4.41	0.38

¹S14 = 38-year-old pasture; ²A6 = 4-year-old pasture.

³= The percentage of organic matter was calculated as %SOM= % of total C x 1.724 (Foth, 1979).

Table 2.2.
Amount of total C and N in SOM fractions of two pasture soils.

Fraction	Soil							
	S14 ¹				A6 ²			
	mgC g ⁻¹	%C	mgN g ⁻¹	%N	mgC g ⁻¹	%C	mgN g ⁻¹	%N
Soil microbial biomass ³	1.62	4	0.16	4	0.62	2	0.06	3
Cold water extraction	0.60	1	0.09	2	0.44	2	0.09	4
Hot water extraction	3.94	9	0.39	9	2.07	8	0.21	9
Humic acids	3.92	9	0.41	10	2.34	9	0.22	9
Fulvic acids	13.25	30	0.90	22	10.02	39	0.43	18
Humins	20.25	46	1.87	44	13.06	51	1.16	49
Total fractionation⁴	40.85	93	3.67	87	27.51	108	2.06	87
Dumas combustion	43.96	100	4.20	100	25.57	100	2.36	100
LSD_(P<0.05)	3.32		0.25		3.32		0.25	

¹S14 = 38-year-old pasture; ²A6 = 4-year-old pasture.

³Corrected from dichromat-oxidizable C values to total C values according to Grewal et al. (1991).

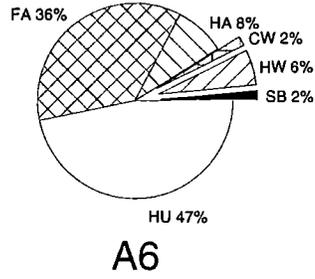
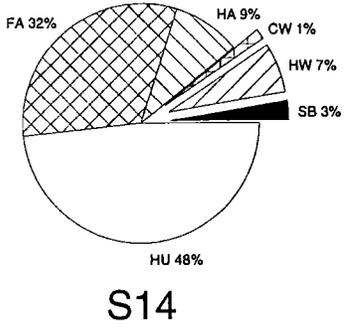
⁴Fractionation values exclude soil microbial biomass results.

Percentages were calculated on the basis of the value obtained by Dumas combustion of total dry soil samples.

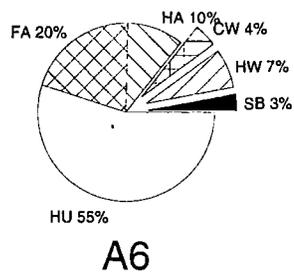
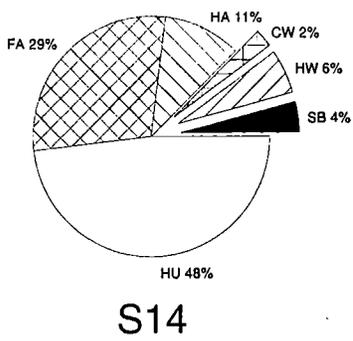
Figure 2.2

Percentages of C and N in different SOM fractions from a 38-year-old pasture (S14) and a 4-year-old pasture (A6). Labile SOM fractions are represented by protruding slices. Figures for each element are proportional. SB=soil microbial biomass; CW=cold water extraction; HW=hot water extraction; HA=humic acids; FA=fulvic acids; HU=humins.

(a)



(b)



in New Zealand soils. These authors also reported a range of 4.2-4.3% organic matter content in cultivated soils and short-term grasslands. Results from Table 2.1 suggest that the organic matter content in soils from pastures S14 and A6 of Winchmore are representative of each of those agronomic conditions.

The only non-significant ($P>0.05$) difference between sites was observed in the amount of N from cold water extraction. In most SOM fractions C and N were significantly higher in the soil from S14 than from A6. An explanation of the similarity in the amount of N in cold water extraction from both soils is that probably N is being quickly metabolized by the higher soil microbial biomass present in the older pasture (Table 2.2). Total C and N from the labile fractions (soil microbial biomass, cold and hot water extractions) varied from 12-16% (Figure 2.2a and 2.2b). There were no significant differences ($P>0.05$) in either C or N detected in hot water extraction and humic acids from both sites. Soil microbial biomass is closely related with SOM content and agronomic history (Reganold et al. 1987). Arable soils contain about 2% of their organic C in the biomass whereas uncultivated soils have about 3% (Jenkinson and Powlson 1976). Bolton et al. (1985) found a higher soil microbial biomass in organic farming systems in comparison with conventional systems. Carbon and N values of soil microbial biomass in S14 and A6 pastures were within the range reported by these authors for several sites. Similar results for soil microbial biomass C have also been reported in other soils from Winchmore (Haynes et al. 1991). A constant proportion (less than 20%) of total C and N is, in a short-term, in available form for soil organisms irrespective of the agronomic history of the site. However, the absolute quantity of potential food sources for plants, micro-organisms and insects is higher in soil from S14 than in soil from A6 pasture.

Both C and N were more abundant in fulvic acids than in humic acids in the soil of both pastures. The highest proportion (46-51%) of C and N was found in humins. Total C and N present in cold and hot water extractions combined showed that in the younger pasture there was 45% less C and 37% less N with a potential short-term biological use than in the older pasture (Figure 2.2a and 2.2b). In most cases, the amount of total C and N recorded during the fractionation was lower than those obtained for total unfractionated dry soil samples, however, these differences were non-significant ($P>0.05$) (Table 2.2). Up to 87%-93% of the total C and N in soil recorded by Dumas combustion in A6 and S14 pastures respectively was recovered through the fractionation (Table 2.2). Goh et al. (1976) found that in a

chronosequence of soils from New Zealand, the order of increasing total weights of the stable organic fractions was humin>fulvic acid>humic acid. Present results (Table 2.2) showed that the amount of total C and N in these fractions in Winchmore soils followed the same order. Although SOM stable fractions (humic acids, fulvic acids and humins) contained most of the C and N, these elements are slowly released into the soil (Goh, 1980). Therefore, an estimation of C and N from the most labile fractions is likely to be more useful to explain the pattern of damage caused by the insect than a measure of total C and N in humic fractions. Jackson (1990a) suggested that the damage caused by *C. zealandica* is more often associated with younger rather than older pastures. There is no available evidence that *C. zealandica* larvae may be able to use, in the short-term, stable organic compounds as a direct or indirect source of food. In fact, most early third instars placed in non-renewed soil devoid of living roots under microcosm laboratory conditions died within two months (Villalobos pers. obs.).

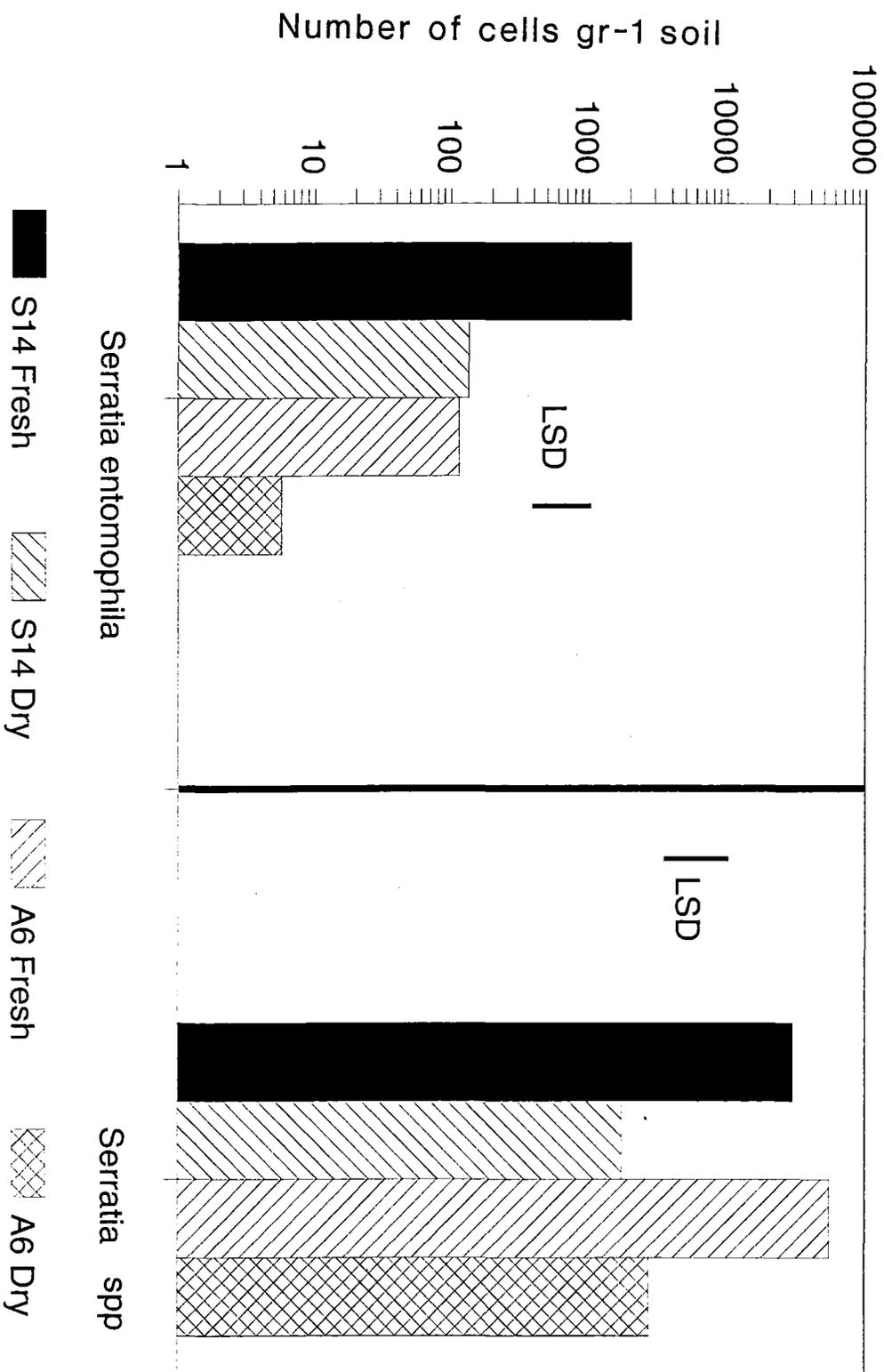
2.3.2 Levels of *S. entomophila* in the background soil

Data in Figure 2.3 showed that, even though the total number of *Serratia* spp. cells in fresh soil from A6 was higher than from S14 pasture, a significantly ($P<0.05$) higher (95%) population of *S. entomophila* was present in fresh soil from S14 than from A6 pasture. Total numbers of *Serratia* spp. cells in fresh soil from A6 was 46% higher than from S14. The higher content of soil nutrients and organic compounds in the soil from S14 than in A6 pasture may have contributed to the increased survival and growth of *S. entomophila*. Evidence available suggests that sources of C and N are required for the production of the soil microbial biomass (Jackson et al., 1991).

The air-drying process produced a significant impact in reducing the numbers of *Serratia* spp. and *S. entomophila* in both soils ($P<0.01$). More than 90% of those bacteria that were recorded in fresh soils were killed after this treatment. A significantly ($P<0.05$) higher background level of *S. entomophila* was recorded in soil from S14 than from A6 pasture. In the air-dried soil, at the beginning of the experiment, the levels of *S. entomophila* were below 200 cells g⁻¹ dry soil and levels of *Serratia* spp from both soils varied between 1.5x10³-3.0x10³ cells g⁻¹ dry soil in S14 and A6 pasture respectively. Although drying the soil before the experiment significantly ($P<0.05$) reduced bacterial populations some larvae in the control

Figure 2.3

Enumerations of *S. entomophila* and *Serratia* spp in soils from S14 (38-years-old pasture) and A6 (4-years-old pasture) sites in fresh soil and after 3 days of air-drying. LSD=least significant difference ($P<0.05$).



plots showed symptoms of amber disease. The dramatic decrease on *S. entomophila* populations after air-drying the soil confirms previous observations (O'Callaghan, 1989). Probably the background population of *S. entomophila*, especially in the soil from S14 pasture, slowly reached the threshold level for infection after the addition of nutrient broth. Nutrient amendment allowed the growth of *S. entomophila* populations after 24 h (O'Callaghan, 1989). In addition, a massive release of organic compounds have been observed to occur after soil sterilization (Griffiths and Birch, 1961; Jenkinson and Powlson, 1976) and the bacteria may benefit from the nutrient flush released after drying. In addition, *S. entomophila* can grow quickly under conditions of low competition with other indigenous soil micro-organisms (O'Callaghan et al., 1989).

2.3.3 Amber disease and larval mortality under microcosm conditions

From 40-70% more amber disease was found in larvae present in S14 than in A6 soil in the control groups after 15 and 30 days respectively ($P<0.001$) (Figure 2.4a and Table 2.3). Differences in amber disease between treatments (control vs treated) were also significant ($P<0.05$) after 15 days. Because high levels of amber disease occurred in the control group in S14 soil (>80%) differences were not significant ($P>0.05$) after 30 days between control and treated groups. For amber disease, there was a significant interaction between treatments and soils at 30 days.

Mortality was very closely associated with amber disease. After 30 days, mortality was 60% higher in the control groups in S14 than in A6 soil ($P<0.05$) (Figure 2.4b). Because high mortality (>75%) occurred in the control group containing soil from S14 pasture, differences in mortality between treatments were non-significant ($P>0.05$) at 15 or 30 days. Both for amber disease and mortality differences were non-significant ($P>0.05$) among treatments in S14 soil (Table 2.4). However, in A6 soil, a significant difference ($P<0.05$) was observed when comparing amber disease in the control with medium and high dose treatments both at 15 and 30 days. Mortality of larvae present in soil from A6 pasture in the low dose treatment after 30 days was the lowest but 60% of larvae showed amber disease symptoms. In the control treatment containing soil from A6 pasture no amber disease was observed at 15 days and 70% of the larvae showed no visible symptoms of amber disease at the end of the experiment (Table 2.4 and Figure 2.3a). Amber disease level and larval mortality were significantly different ($P<0.05$) between dates. After

Table 2.3.

Chi² values and significance for comparisons between sites, treatments and their interactions for amber disease and mortality of *C. zealandica* third instar larvae after 15 and 30 days.

Parameter	Observation	Comparison	Chi ² (d.f.=18)	Significance
Amber disease	15 days	A6 vs S14 (a)	10.0	**
		Control vs Treated (b)	9.8	**
		Interaction a*b	2.1	ns
	30 days	A6 vs S14 (a)	7.6	**
		Control vs Treated (b)	3.6	ns
		Interaction a*b	4.4	*
Mortality	15 days	A6 vs S14 (a)	0.1	ns
		Control vs Treated (b)	1.9	ns
		Interaction a*b	0.6	ns
	30 days	A6 vs S14 (a)	26.8	**
		Control vs Treated (b)	1.8	ns
		Interaction a*b	0.0	ns

* = $P < 0.05$; ** = $P < 0.01$; ns = non-significant difference ($P > 0.05$).

S14 = 38-years-old pasture; A6 = 4-years-old pasture.

Treated = average of low, medium and high doses of a pathogenic strain of *S. entomophila*. Control = *S. entomophila*-free nutrient broth.

Table 2.4.
Proportion (%) of amber disease and mortality after 15 and 30 days¹ under laboratory microcosm conditions.

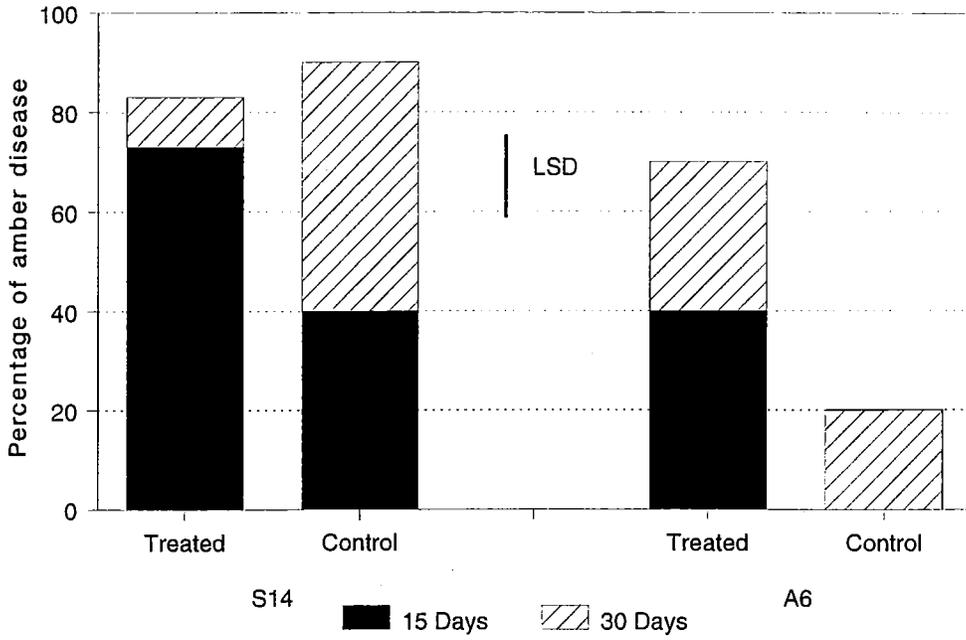
Site	Treatment	Amber disease (%)		Mortality (%)	
		15 days	30 days	15 days	30 days
S14	Low	70 ^{ab}	80 ^{ab}	40 ^{gh}	100 ^e
	Medium	80 ^{ab}	90 ^a	20 ^{gh}	90 ^e
	High	70 ^{ab}	80 ^{ab}	40 ^{gh}	100 ^e
	Control	40 ^{bc}	90 ^a	10 ^h	90 ^e
A6	Low	20 ^{cd}	60 ^{abc}	20 ^{gh}	20 ^{gh}
	Medium	50 ^{abc}	80 ^{ab}	40 ^{gh}	60 ^{efg}
	High	50 ^{abc}	70 ^{ab}	20 ^{gh}	70 ^{ef}
	Control	0 ^d	20 ^{cd}	20 ^{gh}	30 ^{gh}

¹Values showing the same letter are non-significantly different (Chi² $P < 0.05$; d.f.=18).

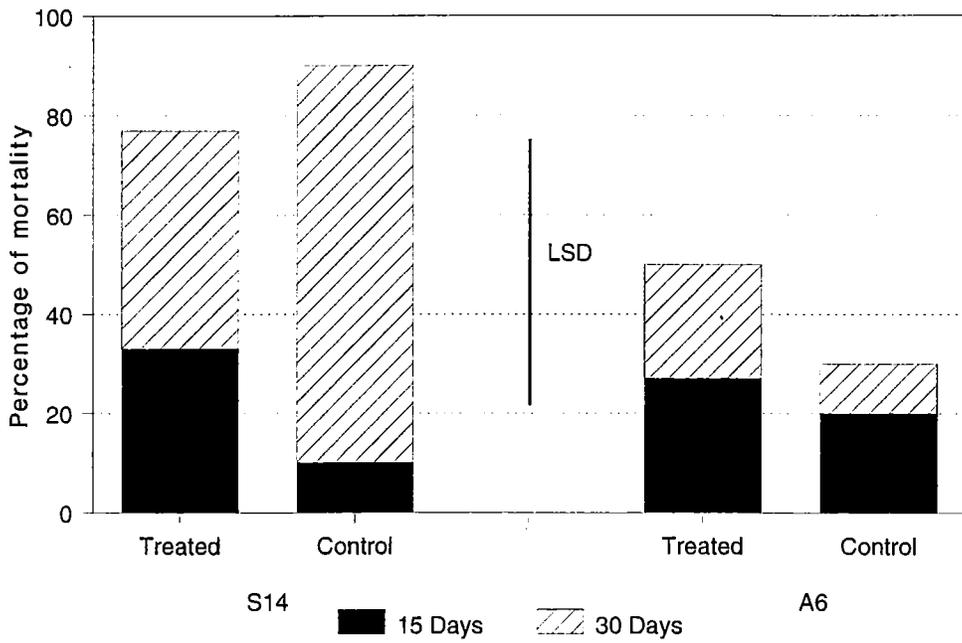
Figure 2.4

(a) Percentage of amber disease of third instars *C. zealandica* observed in control and treated groups from two pastures (S14=38-years-old pasture and A6=4-years-old pasture). The group labelled as "treated" represent the mean values from low, medium and high doses of *S. entomophila*. (b) Percentage of *C. zealandica* larval mortality. LSD=least significant difference ($P<0.05$).

(a)



(b)



15 days a minimum in larval survival of 60% was observed in high and medium dose treatments. However, after 30 days, mortality increased significantly ($P<0.05$) particularly in soil from S14 pasture (Table 2.4).

The significant interaction observed for amber disease between sites and treatments ($P<0.05$) after 30 days (Table 2.3) suggests that amber disease is more likely to occur in soil from the old pasture. Non-significant ($P>0.05$) differences were observed among most of the treatments in which the pathogenic strain was used. A significant ($P<0.05$) linear trend was observed between dose and mortality only after 30 days. This information may contribute to the optimization of biological control tactics in the future and foreshadow the need to evaluate the SOM status before field applications of *S. entomophila* are made.

Entomopathogens, and particularly *Serratia* spp, accounted for a mortality between 45-95% of *C. zealandica* larvae after 30 days in soil from A6 and S14 pastures respectively (Figure 2.4a and 2.4b). High levels of amber disease and mortality in the control plot of S14 soil masked the differences between test and control groups for this soil. Greater pathogen reservoirs may be available in soils with a higher SOM content where a better formation of micro-aggregates may occur. A possible explanation for the formation of reservoirs for entomopathogens in the soil may be through the interaction of organic and inorganic soil constituents. Goh (1980) suggested that organic matter may be inaccessible to microbes or their enzymes by being entrapped within clays and inorganic colloids. Both organic (waxes, lignin, melanin, humic acids) and inorganic substances (silica, sesquioxides, manganese oxide) may form coatings over some organic compounds rendering them inaccessible (Stout et al., 1981) Such intricate micro-sites have been diagrammatically shown by Coleman et al. (1988) which may similarly be micro-habitats for bacteria such as *S. entomophila*. The importance of reservoirs in the natural regulation mechanisms of insects has been modelled by (Hochberg, 1989).

Feeding assessments carried out at the beginning of the experiment showed that the larvae used were actively feeding. However, healthy larvae may carry *S. entomophila* cells (Jackson et al., 1993). The significant ($P<0.05$) higher number of larvae infected in the control treatment from S14 soil than in its counterpart from A6 soil suggest that the disease was transmitted through soil reservoirs rather than by background levels of *S. entomophila* in larval bodies. O'Callaghan (1989) mentioned that survival of *S.*

entomophila in micro-colonies within soil micro-aggregates, perhaps in a protective matrix, may allow some cells to survive even under dryness and starvation stress. This may be specially significant for *Serratia* spp because these species do not form endospores and thus tolerate environmental stress in the soil (O'Callaghan et al., 1989).

Whether by nutritional or physical means, it seems that SOM has a positive effect on *S. entomophila* pathogenic activity on *C. zealandica* larvae. This has also been observed for other interactions between melolonthid larvae and entomopathogens in the soil. The infection of *Holotrichia serrata* Fabr. larvae by *Bacillus popilliae* Dutky, producing milky disease, increased between 18-45% after applications of organic inputs to the soil in India (Rao and Veeresh, 1988). Preliminary samples from several soils at Winchmore revealed a higher incidence of milky disease in *C. zealandica* larvae from minimum-tillage soils than in soil from younger pastures and crops. Furthermore, symptoms of protozoan disease of *C. zealandica* larvae (probably caused by *Mattesia* spp) were more frequently found in organic pastures than in conventional pastures at Winchmore (T.A. Jackson pers. comm.).

2.3.4 Effect of SOM on larval carrot consumption under microcosm conditions

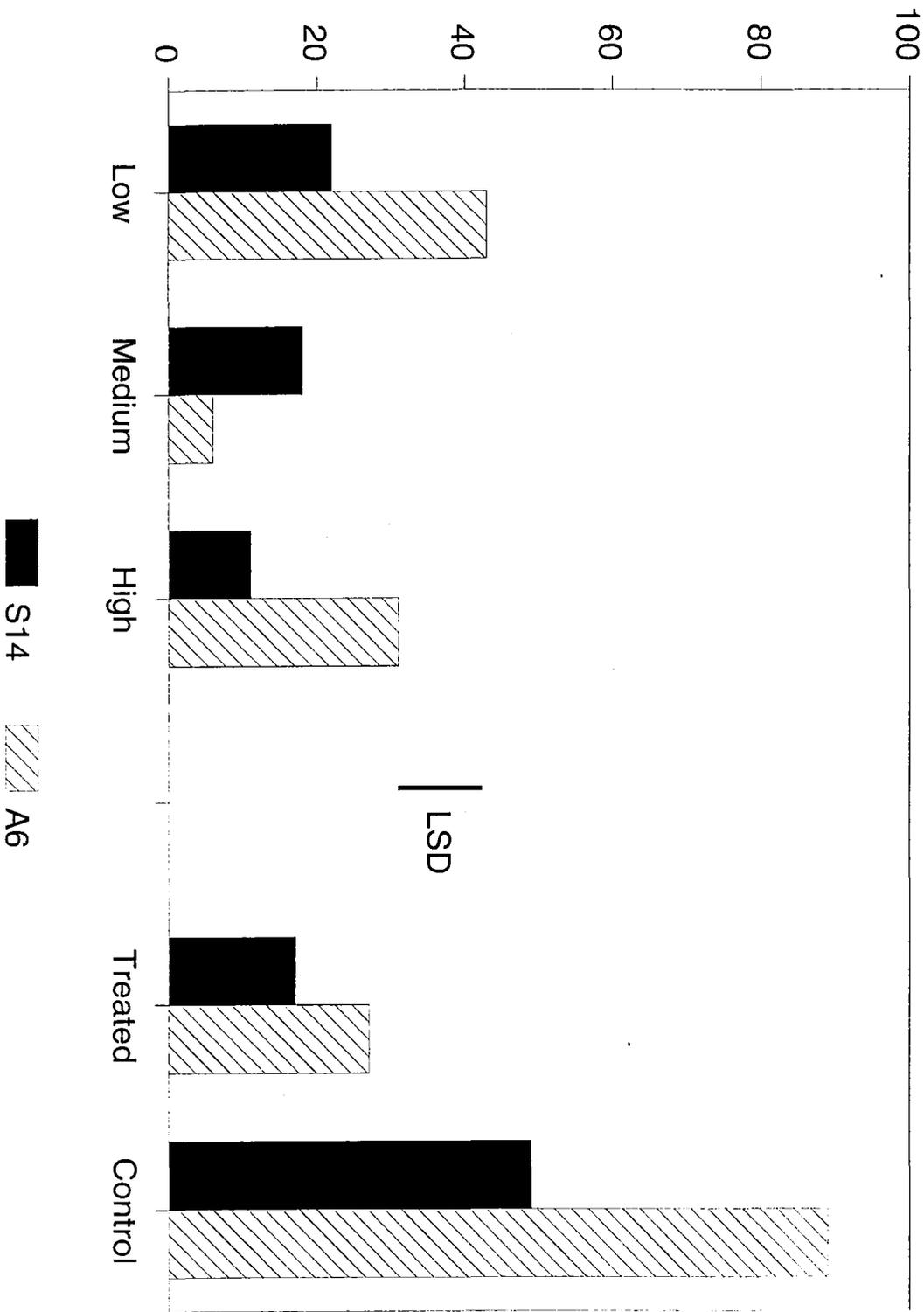
Mean values of the percentage of larval carrot consumption from low, medium and high dose treatments were averaged and represented under the subheading "treated" in Figure 2.5 and subsequent Figures). After 15 days, in the control treatments, 40% more carrot was consumed by *C. zealandica* larvae present in A6 soil than in S14 soil ($P < 0.01$) (Figure 2.5). Except for the medium dose, where differences were non-significant ($P > 0.05$), in all treated groups significantly lower carrot consumption occurred in soil from S14 than in soil from A6.

As amber disease inhibits larval feeding (Jackson et al., 1993), the higher larval infection and mortality that occurred in S14 soil in comparison to that observed in A6 soil also reduced carrot consumption (Figure 2.4a and 2.4b). However, carrot ingestion by healthy larvae after 15 days was $50 \pm 17\%$ ($n=6$) for larvae in S14 soil and $88 \pm 3\%$ ($n=9$) for larvae in A6 soil (Student's *t* test $P < 0.05$). Only in soil

Figure 2.5

Percentage of larval carrot consumption by third instars of *C. zealandica* in soil from two pastures (S14=38-years-old pasture and A6=4-years-old pasture) subjected to a low, medium and high dose of *S. entomophila*. The group labelled as "treated" represent the mean values from low, medium and high doses of *S. entomophila*. LSD=least significant difference ($P<0.05$).

Percentage of larval carrot consumption



from S14 pasture a linear trend in the reduction of larval carrot consumption was observed with increasing doses in the experimental groups. The medium dose in the soil from A6 pasture significantly ($P<0.05$) reduced carrot consumption.

The lower consumption of carrot in the soil from the S14 pasture than in the soil from the A6 pasture suggests that SOM may be an alternative source of food for the third instar of *C. zealandica* (Miller, pers. comm. in Sutherland, 1972). Therefore a difference of 2% in total C and 0.2% in total N may account for the reduction of 40% in frequency and/or intensity of larval consumption of carrot. Nevertheless, the simulation of plant root by the carrot cylinder assumes that the rhizosphere is irrelevant in the root-feeding behaviour of larvae. Moreover, in this experiment, the root regenerative growth was also excluded as a compensatory response to larval herbivory. In addition, possible interactions between larval density and plant susceptibility under soil moisture and temperature stress suggest that insect damage is far more complicated. On the other hand, under natural conditions root grazing by soil insects at low densities has been suggested to have a pruning effect on plants (Brown and Gange, 1990). Notwithstanding, the limitations of extrapolating these results to the field, the simulation of plant roots by the carrot cylinder was a useful tool as it allowed to the testing of the hypothesis that the intensity of larval herbivory is lower in a soil environment surrounded by a high quantity of organic compounds (Plate 6).

2.3.5 Larval growth under microcosm conditions

Larval growth in the control treatment containing soil from A6 pasture was higher (23%) than in its counterpart from S14 pasture (Figure 2.6). In the control group containing soil from A6, only 10% of the carrot remained after 15 days of incubation, whereas 33% remained in the control group of S14 pasture. There were no significant ($P>0.05$) differences in the remaining carrot among treatments in S14 soil. Despite an average of 17% larval carrot consumption being measured in the treated groups of S14 soil, larval growth was very low. Even though 10% more larval carrot consumption was observed in their counterparts in A6 soil, a decrease in larval weight was recorded.

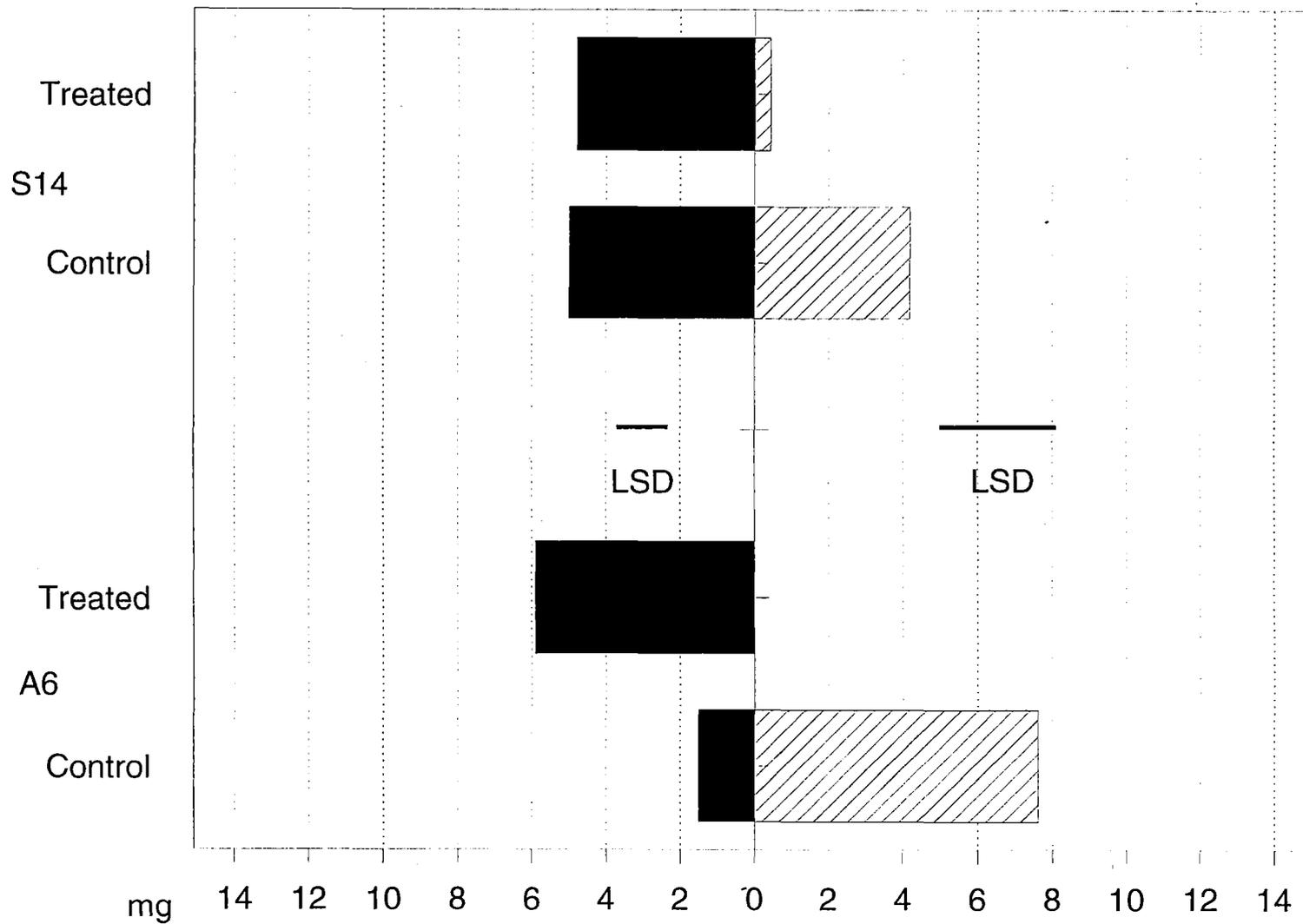
It has been reported that weight of amber disease infected larvae was lower than the weight of the

Figure 2.6

Amount of unconsumed carrot and larval dry weight gain after 15 days of incubation with third instars of *C. zealandica* in two pasture soils (S14=38-years-old pasture and A6=4-years-old pasture) with different bacterial dose. LSD=least significant difference ($P<0.05$).

Dry weight of remaining carrot

Larval dry weight gain



healthy larvae (Trought et al., 1982). Results from the present experiment showed that under laboratory conditions, amber disease seemed to inhibit the nutritional effect of plant tissues on larval biomass. This inhibition was evident in the experimental group of both A6 and S14 soils regardless of the consumption of carrot attributed to diseased larvae (Figure 2.6).

The unspecific nature of food selection by *C. zealandica* larvae has been previously suggested by Sutherland (1971). This author suggested that apart from feeding on roots of several plant species *C. zealandica* larvae also grow and develop satisfactorily on a diet consisting of sheep dung or humus. In fact, Sutherland (1971) cited that D. Miller (pers. comm.) has reared two generations of *C. zealandica* in humus deprived of living plant material. Radcliffe (1970) has observed that when SOM in the form of cow dung was added to a soil a reduction in plant damage occurred.

C. zealandica larvae experienced the greatest significant ($P<0.05$) live-weight gains in soils with the lowest SOM content and where the highest significant ($P<0.05$) carrot ingestion was recorded (Figure 2.6). This result suggests that larval carrot consumption contributed to larval nutrition, specially in the healthy larvae from the control group in A6 soil. A positive relationship between body weight in *C. zealandica* larvae, pupae and adults, and fecundity-development has been suggested by Farrell (1972; 1973). Therefore, that a higher larval herbivory in *C. zealandica* larvae may also be associated with an increase in survival, fecundity and development. These relationships may partially explain the observations of several workers (Kelsey, 1970; Kain, 1975; Jackson, 1990a; Chapman, 1990) that *C. zealandica* outbreaks occurred after the introduction of improved pastures in New Zealand. Pastures with a high content of SOM may therefore encourage natural regulation mechanisms in the long-term and develop a higher carrying capacity for *C. zealandica* larvae.

In Figure 2.6, the maximum value of the scale in the X axis (15 mg) represents the mean dry weight of the carrot cylinder at the beginning of the experiment. The bars on the left represent the dry weight of the unconsumed carrot cylinder after 15 days of the microcosm experiment. The bars on the right hand side of Figure 2.6 represent the larval dry-weight gains after 15 days of incubation. This Figure

was made with the assumption that the dry matter content of *C. zealandica* larvae is 28% as has been reported for other soil-dwelling melolonthids (Hutchinson and King, 1975). Approximately 56%-42% of the carrot consumed was assimilated into larval tissue in the control group from A6 and S14 soil respectively. These results suggest that third instars of *C. zealandica* were able to assimilate part of the structural compounds of the carrot root under the conditions of the microcosm experiment and that the greater the amount of carrot consumed the higher the assimilation efficiency. Bauchop and Clark (1975) suggest that *C. zealandica* are unable to degrade in a significant way plant structural carbohydrates. More research is required to confirm the accuracy of the assimilation efficiency in *C. zealandica* larvae and to identify the components of the carrot root that are promoting larval growth.

2.3.6 Carrot decomposition under microcosm conditions

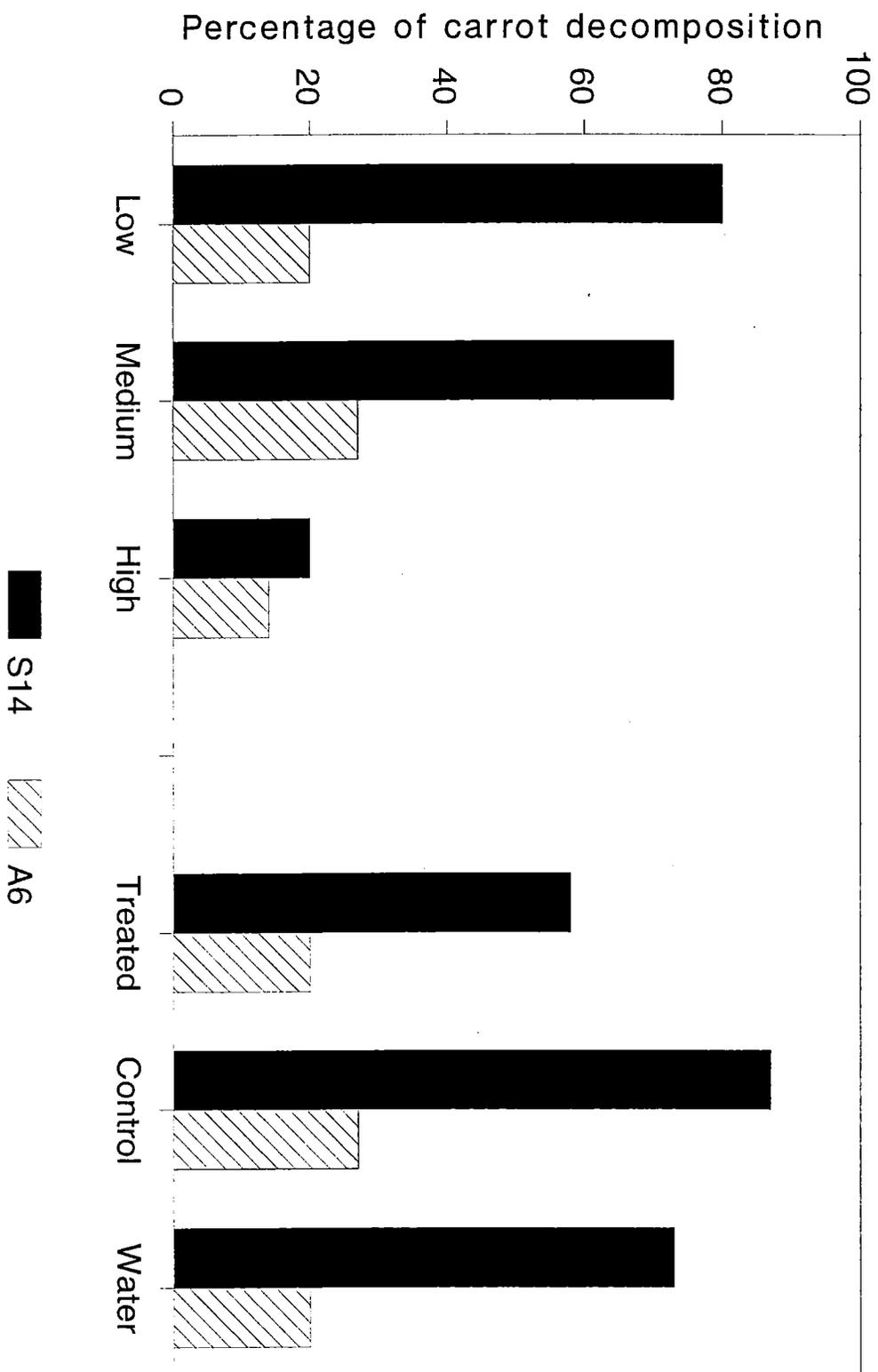
In most treatments carrot decomposition was higher in S14 than in A6 soil (Figure 2.7). Assuming that the presence of the larvae have no effects on the rate of carrot decomposition, results from the blank pots revealed that 60% more carrot was decomposed in the control group from S14 soil than in soil from A6 pasture (Figure 2.7 and Plate 6). The highest dose of *S. entomophila* reduced considerably the carrot decomposition process, an effect that was particularly evident in A6 soil (Plate 6). An average of 30% carrot decomposition occurred when the bacteria was applied. The addition of the nutrient broth accelerated carrot decomposition to 7% and 14% in A6 and S14 soils respectively in comparison to the pots where only distilled water was applied.

The method of measuring larval feeding activity was reliable because carrot decomposition and larval carrot consumption were easy to distinguish (Plate 6). Carrot decomposition in the blank pots was manifested by a dark coloration in the cylinder and a slight change in shape and size was observed. Larval carrot consumption modified conspicuously the shape of the carrot cylinder. Even with the higher decomposition observed in S14 soil, the difference in larval carrot consumption was clearly distinguishable when compared with that observed in A6 soil.

Interactions among soil micro-organisms may help to explain some results observed in the present microcosm experiment. The significant reduction of larval carrot consumption (Figure 2.5) and the 100%

Figure 2.7

Percentage of carrot decomposition recorded under microcosm conditions in low, medium, and high doses of bacteria in the absence of *C. zealandica* larvae after 15 days. Treated groups were inoculated with *S. entomophila* and the control groups were treated with bacteria-free nutrient broth and distilled water. The group labelled as "treated" represents the mean values from low, medium and high doses of bacteria. LSD=least significant difference ($P<0.05$).



mortality after 30 days in the medium dose treatment (Figure 2.4b) in the soil from A6 pasture may be due to interactions among different species of pathogens and clearly deserve more research. The higher carrot decomposition in soil from S14 seems to be caused by a higher and more diverse microbial biomass than in soil from A6 pasture (Figure 2.7). High doses of *S.entomophila* reduced carrot decomposition, an effect that was particularly evident in the soil from S14 (Figure 2.7). Competitive interactions among *S. entomophila* and the microbial populations involved in carrot decomposition in the soil may be the factor responsible for this result.

2.4 Conclusions

Results from the present microcosm experiment suggest that SOM has a buffering effect on plant damage caused by soil-dwelling scarab larvae.

Agronomic history determined differences in the content of C and N in the pasture soil studied. A significantly higher content of organic matter was found in the soil from the older than from the younger pasture. Similar results were obtained for C and N in all SOM fractions, except for N in the cold water extraction. Total C and N present in cold and hot water extractions combined, representing the labile SOM fraction, showed that in A6 there was 45% less C and 37% less N than in S14. These fractions are considered to have a potential short-term biological fate. In both soils approximately 10% of C and N was present in the labile SOM fractions. Stable SOM fractions, as represented by humic fractions (humic acids, fulvic acids and humins) seem to exert less influence on feeding behaviour and amber disease infection.

Both pasture soils harboured a different background of entomopathogens. A 95% higher population of *S. entomophila* was present in fresh soil from the older than from the younger pasture. Under microcosm experimental conditions it was observed that 63% more amber disease occurred in the soil with higher SOM content than in the soil with lower SOM content. After 30 days the larval mortality observed was closely associated with amber disease and was 50% higher in the older than in the younger pasture. Therefore, the soil environment in the older pasture is more likely to produce amber disease in early third

instars of *C. zealandica* and to cause larval mortality than the soil environment from the younger pasture.

Both pasture soils may induce differences in rhizophagy and performance of larvae of *C. zealandica*. Under microcosm conditions, a difference of 42% of SOM content, observed between these two soils, may account for a reduction of 40% in frequency and/or intensity of insect root grazing. In the control groups, larval carrot consumption, considering only healthy insects, after 15 days was 38% higher in soil from the younger than from the older pasture. Larval growth was 23% higher in the soil with lower SOM content.

The results obtained suggest that all these SOM effects act in a multifactorial way in nature and their overall effect may be greater than that due to the addition of the individual components. The microcosm approach used offers an excellent model to explore complex interactions and provides some insights in the understanding of the natural regulation mechanisms of soil scarab larvae. Hopefully, this information will be useful to optimize biological control tactics.

3) INTERACTIONS AMONG SOIL ORGANIC MATTER, AMBER DISEASE, LARVAL FEEDING AND PLANT DAMAGE CAUSED BY *Costelytra zealandica* (White): A MESOCOSM APPROACH.

3.1 Introduction

Plant damage¹ caused by the soil-dwelling larvae of *Costelytra zealandica* (White) has been historically of great concern to farmers in New Zealand pastureland. According to Chapman (1990) damaged areas and larval numbers vary widely between pastures and years, but in the worst cases up to 50% of the pasture area may show damage and populations of 300-400 larvae m⁻² may be found in the soil. Plant damage is mainly caused by third instars and often becomes obvious through the autumn, winter and early spring (Flay and Garrett, 1942). Extensive research has been done to find ways of controlling this insect and multiple strategies have been tried (Cameron and Wigley, 1989; Chapman, 1990). Despite these efforts, there is still a need for cheap, simple and effective alternatives of insect pest management (IPM) (Blakeley, 1990). Preventative solutions, which take into account ecological principles and that are compatible with the conservation of soil fertility, are currently being sought.

The problem caused by *C. zealandica* has been recognized in New Zealand pastures since the early days of European settlements (Kirk 1896). Since then, some assumptions have been made about the pattern of plant damage caused by this insect. Larval numbers are usually correlated with damage (Townsend et al., 1993) and larval densities are considered as the prime criterion in the decision to determine whether control practices are required (Barratt et al., 1990; Townsend et al., 1993). *C. zealandica* larval root feeding behaviour has been shown to be detrimental for root growth and hence herbage growth (East, 1972; Kain, 1975).

A field mesocosm approach (Crossley et al., 1989) has been used in this study to explore

¹Damage is defined in this work as the measurable loss of yield and quality of plants caused by the effect of insect feeding or other activity.

experimentally the damage caused by *C. zealandica* in Canterbury pastures and its relationship with the soil organic matter (SOM) content. This approach has the advantage of being more realistic than the microcosm approach, however, it has the limitation of allowing a less restricted control of variables. Both approaches are, however, complementary and their combination may help to increase the validity of conclusions in the decision-making process of IPM.

Within the paradigm of sustainable agriculture, SOM plays a crucial role because it is one of the main factors involved in soil fertility and soil conservation (Anderson et al., 1989; Pimentel et al., 1989; Reganold et al., 1990; Lal, 1991). Previous studies have shown the importance of SOM in reducing plant damage caused by *C. zealandica* larvae (Radcliffe, 1970; Sutherland, 1971). A reduction of plant damage with increasing soil fertility has also been observed (Davidson, 1969) in other soil-dwelling Melolonthid larvae. Evidence suggest that SOM may encourage the action of entomopathogens in controlling these insects (Rao and Veeresh, 1988).

Interactions among SOM, plant damage caused by *C. zealandica* larvae and its regulation by one of its indigenous natural enemies, the bacterium *Serratia entomophila* (Grimont et al) have not been previously studied in a holistic way.

The aims of the research presented in this chapter were to:

- (1) Investigate the relationships between SOM, amber disease infection caused by *S. entomophila* and mortality caused by soil entomopathogens in *C. zealandica* larvae.
- (2) Compare *S. entomophila* populations and performance as a biological control agent under different SOM conditions.
- (3) Correlate damage caused by third instar larvae of *C. zealandica* on living roots and herbage growth and production under different SOM conditions.

(4) Correlate larval growth with plant variables recorded in these three pastures.

3.2 Materials and methods

3.2.1 Description of sites

In addition to pastures S14 and A6 described in Section 2.2.1 (Chapter II), a further 2-year-old pasture (site A1) was included for this experiment. All sites are located at the Winchmore Irrigation Research Station in Canterbury. Site A1 has been in previous annual cycles of wheat, pea, oat, grass and barley in consecutive years. White clover (*Trifolium repens* L.) was the dominant pasture plant species, however, a high abundance of weeds such as dandelion (*Taraxacum officinale* L.) and nodding thistle (probably *Cirsium arvense*) were also present (J. Talbot pers. comm.). The assumption was made that the five previous years under cultivation had a significant effect on SOM depletion at site A1. Cultivation is known to reduce the amount of C and N from nonhumic substances (Stout et al., 1981). Crop rotations at Winchmore usually consist of four consecutive years under crop followed by four consecutive years under pasture (P. Cunningham pers. comm.).

3.2.2 Collection and marking of *C. zealandica* larvae

Larvae used for this experiment were collected from a pasture at Lincoln in March 1992. Feeding assessments were made in the same way as described for the microcosm experiment (Section 2.2.3 in Chapter II). Once selected, larvae showing no symptoms of disease were marked with a mixture of carmine red powder and super bond glue (Super Bond BOSTIK). A spot (ca. 1 mm diameter) was placed on the posterior part of the head capsule and allowed to dry. A further feeding assessment was made for the following two days. Any effects on the larvae and the persistence of the mark on healthy insects were assessed under laboratory conditions in pots containing soil before the experiment was set up. The mark proved to be innocuous to third instars and it was not worn off. Larvae were marked and weighed the day before the experiment began in each pasture.

3.2.3 Field mesocosm experiment

A field experiment was conducted from late March 1992 to early June 1992 in the Winchmore pasture described above. Twenty five soil cores of 10 cm diameter and 20 cm depth were taken in each pasture, covered with a gauze (2 mm mesh size) sleeve (12 cm diameter and 30 cm height), treated as below and placed back *in situ* (Plate 7). The assumption was made that this manipulation had no effect on the plant-soil system. The soil corer used for this operation was developed by Kain and Young (1975) specifically for sampling *C. zealandica* populations (Plate 8). A detailed description of it can be found in East (1972). These soil cores were used as the experimental units of a latin square design and they offered a mesocosm soil environment close to field natural conditions. At each site, five replicates, (1 m apart between cores as shown in Plate 9), were used for each of the following five treatments:

(a) five marked early third instars *C. zealandica* (L) + 10 ml of a pathogenic strain of *S. entomophila* (BC4B) at a concentration of 4.7×10^{10} cells ml⁻¹ resuspended in nutrient broth. (treatment abbreviated as 5L+BC4B onwards). Nutrient broth consisted of raw sugar, yeast extract diet, Urea, Na₂HPO₄, KCl and NH₄NO₃.

(b) As for "(a)" but a non-pathogenic strain (A20) of *S. entomophila* was used instead (treatment 5L+A20)

(c) As for "(a)" but 10 ml of nutrient broth free of *S. entomophila* were added (treatment 5L+NB).

(d) As for "(a)" but 10 ml of distilled water were added (treatment 5L+H₂O) and

(e) As for "(d)" but no larvae were introduced (treatment 0L+H₂O).

Larvae were introduced into the experimental units and placed into five small cavities (ca. 5 cm depth) which were previously made around the soil surface. The applications were then poured slowly all around the surface of the experimental unit using a 10 ml volumetric cylinder. A label was introduced

Plate 7

Soil cores used as experimental units in the field mesocosm experiment described in Sections 3.2.3 and 5.2.2.1.



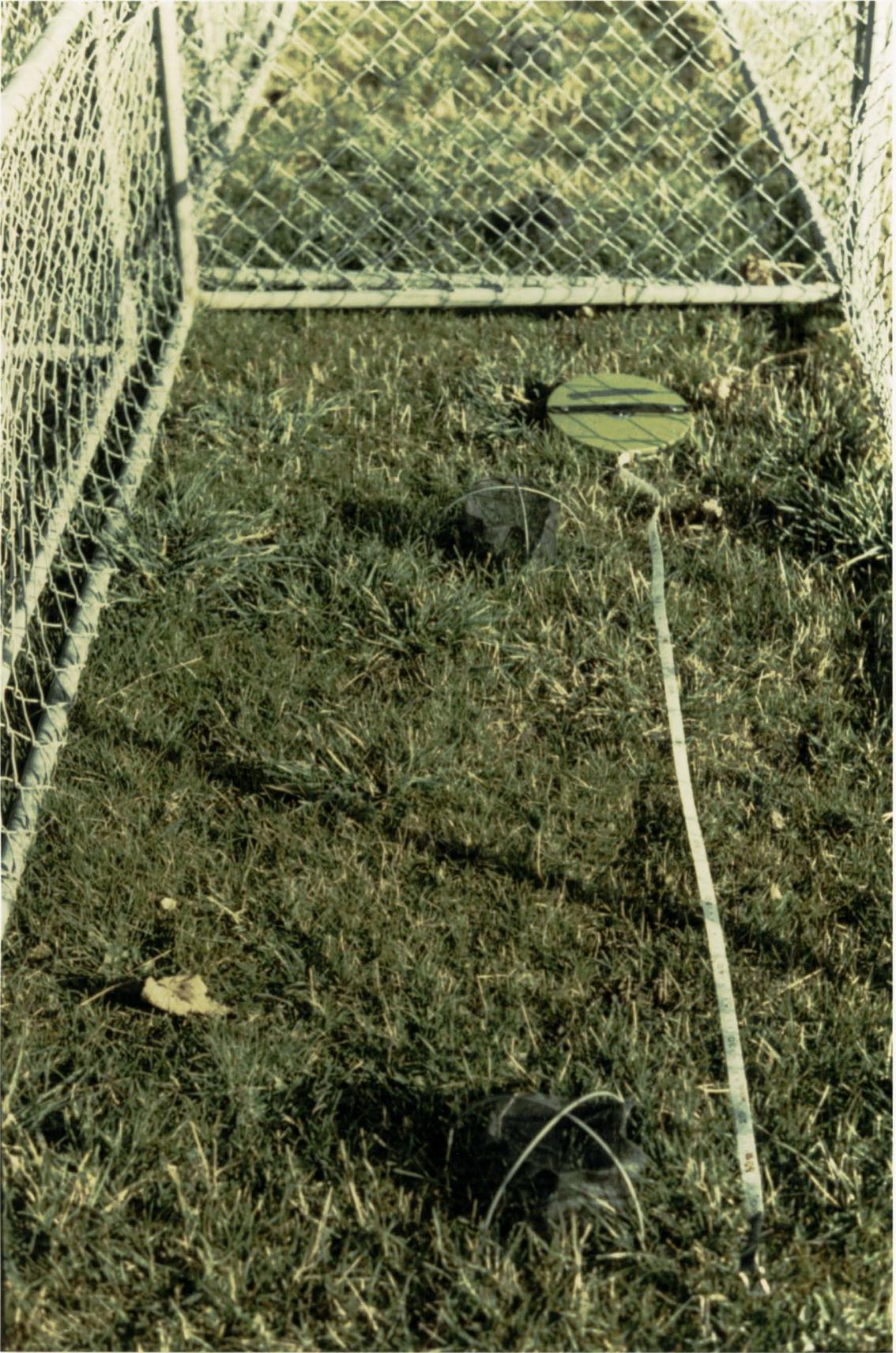
Plate 8

Soil corer used to prepare the experimental units during the field mesocosm experiment described in Sections 3.2.3 and 5.2.2.1. This soil corer was originally developed by Kain and Young (1975) specifically for sampling *C. zealandica* populations.



Plate 9

Layout of the experimental units used during the field mesocosm experiment described in Sections 3.2.3 and 5.2.2.1. Experimental units were arranged in a latin square design and place 1 m apart one from the other.



to each experimental unit indicating the core position and treatment to which it was exposed (Plate 7). After the treatments were completed, the gauze sleeves were closed at the top by attaching a gauze cover using staples. This cover was suspended from two external crossed wires that were bent and inserted into the ground (Plate 9). This system allowed the entrance of air, water and sunlight and excluded any possible vertebrate predator of *C. zealandica*. During the experiment, all soil core units were protected from sheep cattle using wire fences (Plate 10). Five soil cores similar to those used as experimental unit were taken in an adjacent area (1 m from the experimental units) the day the experiment was set up, to evaluate background conditions. The number of *C. zealandica* larvae were recorded from these soil cores. Visual larval disease assessments and enumerations of *S. entomophila* were also made. The experiment lasted for two months in each pasture. The establishment dates were March 31th, April 7th, and April 14th and the removal dates were May 30th, June 6th and June 13th at pastures S14, A6 and A1 respectively. All experimental units were collected the same day, the five experimental units from each of the five blocks were placed together in a bucket and transported to the laboratory. These buckets were covered and stored at 5° C in darkness until an examination was made. Each one of the five blocks collected from each pasture were examined the same day. During examination, each soil core was broken down into two strata of 10 cm and passed through a set of 5.6, 4.0, 2.8 and 2.00 mm² mesh sieves. The number of living, diseased and dead larvae and the live weight of surviving larvae showing no apparent symptoms of disease were recorded. Data on other soil macro-invertebrates were also recorded from all experimental units; apart from earthworms, no other macrofaunistic group was substantially represented. No predatory carabids, staphylinid beetles or dipteran larvae were found.

3.2.4 Enumerations of *Serratia* in soil during the mesocosm experiment

Before and after the experiment, enumerations of *S. entomophila* and *S. protamaculans* populations were made using the method described by O'Callaghan and Jackson (1993). Soil samples (20 g fresh weight and <2.00 mm) from experimental units were pooled independently for each treatment and pasture to obtain a duplicated estimation of bacterial numbers for each treatment in all sites .

Plate 10

Wire fences used to protect the experimental units from sheep disturbance during the field mesocosm experiment described in Sections 3.2.3 and 5.2.2.1 and general aspect of the 4-year-old pasture (A6).



3.2.5 Soil organic matter analysis

The SOM determinations were carried out using duplicated sub-samples of pooled soil for each treatment. Total C and N determinations of different SOM fractions were conducted as described earlier (Chapter II and IV).

3.2.6 Herbage and living root DM measurements at the end of the mesocosm experiment

The dry weight (80°C 24h) of plant residues, living roots and herbage were also determined for each experimental unit at the end of the experiment. Determination of herbage DM and living root DM were made according to Nguyen and Goh (1992). Herbage and living root DM were also estimated from the extra block of soil cores taken at the beginning of the experiment. Herbage DM material collected from each experimental unit were pooled independently for treatments and sites. Herbage DM material was ground and used for measurements of total C and N from duplicated samples of each treatment and pasture by Dumas combustion (Grewal et al., 1991). Determination of total C and N in living root DM were also made following the same procedure as for herbage DM.

3.2.7 Statistical analysis

Statistical analysis were carried out following the criteria of Little and Hills (1978); Devore and Peck (1986) and D. Saville pers. comm. Statistical differences among sites and treatments were obtained by using ANOVA of raw and transformed data. A least significant differences (LSD) test was used to separate the differences between pairs of means and were calculated at a level of 95%. Arcsin transformations for percentages were performed. Square root and \log_{10} transformations were carried out for herbage and root DM yields. \log_{10} transformations were made to compare *Serratia spp* numbers. Correlations and multiple regressions among larval growth with raw and transformed data from above and below ground plant variables were carried out.

3.3.1 Mortality and amber colour of *C. zealandica* larvae at the end of the field mesocosm experiment

Results from the experimental units where larvae were excluded (treatment 0L+H₂O) showed that the background of *C. zealandica* larvae was the highest in soil from S14 (Figure 3.1 and Table 3.1). No *C. zealandica* larvae were collected at the beginning or at the end of the experiment from the soil cores that were taken at site A1. Therefore results from treatments in A1 pasture represent an ideal control group in the assessment of the effects of the presence of *C. zealandica* larvae on plant performance. Because of the background of *C. zealandica* larvae in S14 soil an average initial density of ca. 1250 larvae m⁻² was present in the experimental units at this site (Table 3.1). At least 70% (866 larvae m⁻²) of these larvae displayed no apparent symptoms of disease.

At the end of the experiment, differences in the number of healthy and amber diseased larvae were not significant among sites ($P>0.05$) (Table 3.1). However, the number of marked larvae that were not recovered plus those that were found dead or showing clear symptoms of disease were significantly higher ($P<0.05$) for S14 pasture than for A6 and A1 pastures. No significant difference ($P>0.05$) was observed between the numbers of healthy, diseased and dead larvae between the young pastures ($P>0.05$) (Table 3.1). Percentages of SOM were significantly ($P<0.05$) different for site S14 (6.8%) than for sites A1 and A6 (4.6% for both sites). This result suggests, that under field conditions, the natural regulation of *C. zealandica* larval populations by entomopathogens was greater in the soil from the oldest pasture ($P<0.05$). This statement is also supported by the observation that 75% of the initial larval population in S14 pasture was dead or diseased at the end of the experiment while only 56% and 37% were in these categories in A6 and A1 pastures respectively². Three possible explanations may account for absence of differences among pastures in levels of amber disease and larval density at the end of the experiment: (a) the longevity of amber disease infected larvae may have been lower in S14 soils than in A6 and A1 pastures; (b) the rate of decomposition of cadavers of amber disease infected larvae may have been higher in S14 than in A6 and A1 pastures and (c) microscopic assessments of symptoms of disease should give a more accurate estimation on the levels of diseases which probably were underestimated in this study. As

² However, the effect that density-dependent factors of mortality may have had in this result should also be considered (Prestidge pers. comm.).

Table 3.1

Mean values of the number of *C. zealandica* larvae present in the experimental units at the end of the mesocosm experiment at Winchmore.

Site	S14	A6	A1
	larvae m ⁻²	larvae m ⁻²	larvae m ⁻²
Healthy	312 (229)	337 (102)	402 (0)
Amber disease	172 (204)	146 (25)	102 (0)
Dead+Disease*	936 (382)	426 (50)	235 (0)
Total	1248 (611)	763 (152)	637 (0)
LSD _(0.05%)		375 (160)	

Values in parenthesis represent the larval background of *C. zealandica* before the experiment in adjacent areas.

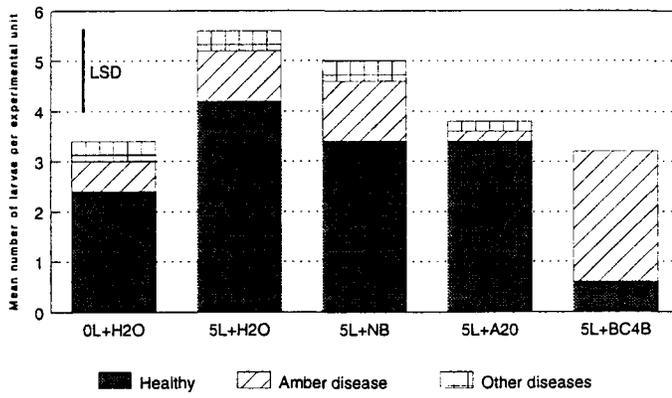
*This category includes marked larvae which were not recovered, larvae with clear symptoms of amber disease or other diseases or dead.

Figure 3.1

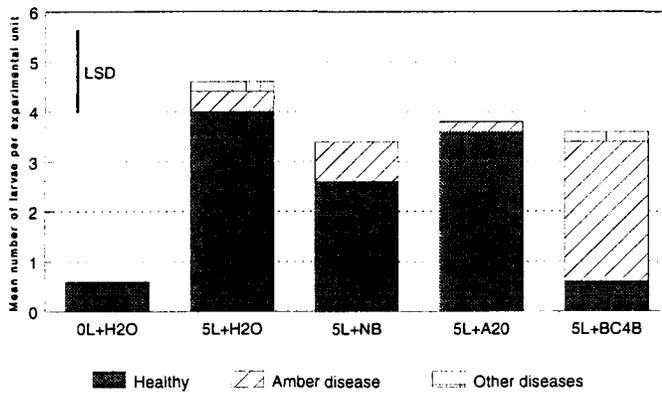
Mean number of diseased and healthy *C. zealandica* larvae recorded in each of the five experimental units at the end of the mesocosm experiment at Winchmore. See explanation of the treatments in Section 3.2.3.

LSD for amber diseased and healthy larvae ($P < 0.05$).

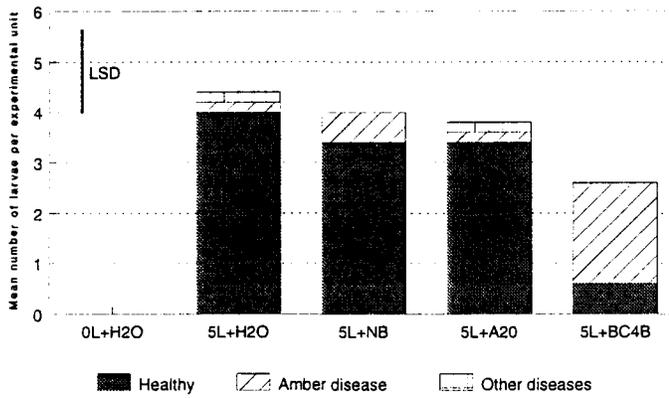
S14
(a)



A6
(b)



A1
(c)



has been pointed out by Morris (1965): "The effect of any mortality factor on population trend is influenced greatly by other factors that operate contemporaneously with the same age interval and by the extent to which interacts with the other factors". Another reason for the need to consider carefully the measurements of apparent mortality caused by amber disease is that some infected larvae may recover (T.A. Jackson pers. comm.). Nevertheless, the results from both microcosm and mesocosm experiments strongly suggest that SOM may be associated with the different rates of mortality in S14 compared with A6 and A1 pastures.

No difference was observed in the efficacy of the treatment 5L+BC4B among pastures ($P>0.05$) (Figure 3.1). In all soils, the differences between the numbers of healthy and amber disease infected larvae were significant ($P<0.05$) among treatments (Figure 3.1). As expected, soil cores in treatment 5L+BC4B showed significantly ($P<0.05$) higher levels of amber disease than the remaining treatments.

Although the differences were non-significant ($P>0.05$), more amber disease was observed in treatments 5L+NB or 5L+H₂O than in treatment 5L+A20. Evidence from the laboratory bioassays suggests that infection of amber disease is less likely to occur in larvae fed with carrot coated with non-pathogenic strains of *S. entomophila* than in larvae fed by carrot alone (Glare, 1992c). Competition within the larval gut among *Serratia* spp strains either for membrane receptors on the larval crop, for bacterial nutrients present in the lumen, or through bacterial antibiosis has been suggested (Nuñez-Valdez, 1994).

3.3.2 Enumerations of *Serratia* in soil during the mesocosm experiment

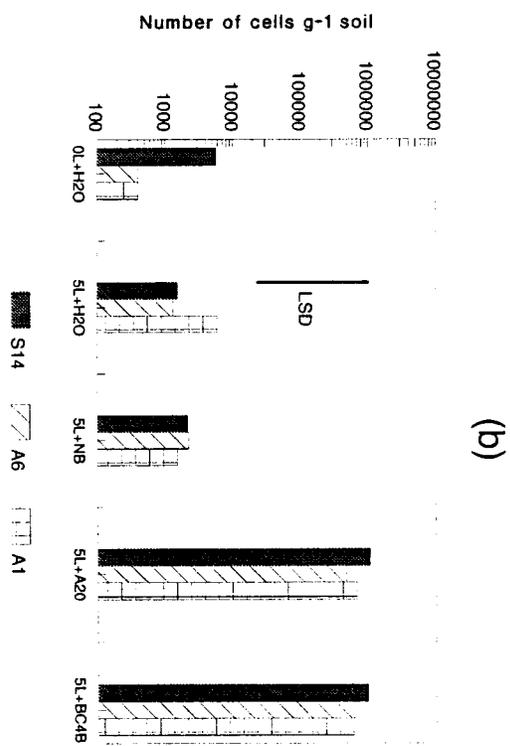
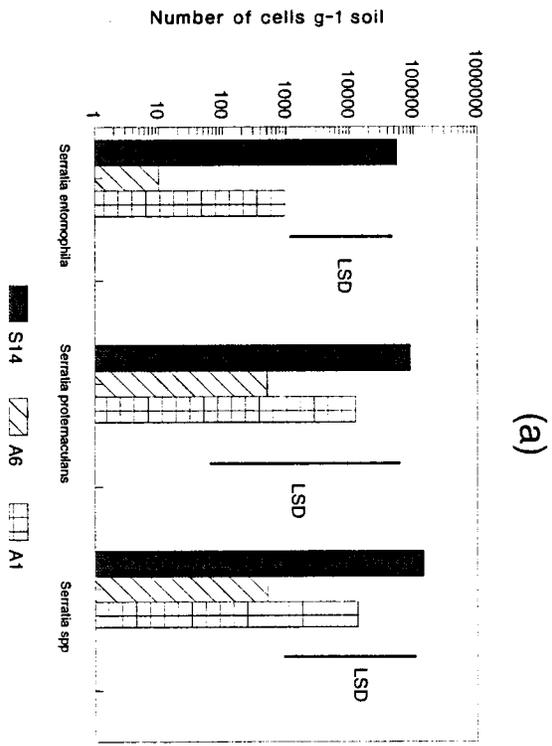
Data from Figure 3.2a shows that, at the beginning of the mesocosm experiment, the soil from S14 contained a higher background population of indigenous *S. entomophila* than soil from A6 and A1 pastures. This result confirms the pattern described in Section 2.3.2 (Chapter II) where higher *S. entomophila* numbers were found in soil from S14 than from A6 pasture. However, non-significant ($P>0.05$) differences were observed for *S. protamaculans*, another bacterial species able to produce amber disease (Grimont et al., 1988). When the densities of both bacterial species were combined, only significant ($P<0.05$) differences occurred between S14 and A6 pastures.

Figure 3.2

(a) Number of cells of *Serratia* spp present in the soil before the mesocosm experiment (April 1992).(b)

Mean of bacterial enumerations in pooled soil at the end of the mesocosm experiment (June 1992). LSD

($P < 0.05$).



Despite their agronomic history, soil from A1 presented a significantly ($P < 0.05$) higher background of *S. entomophila* than soil from A6 pasture. Nevertheless, levels of amber disease were not significantly different ($P > 0.05$) in those treatments in which only the bacterial background was present (Figure 3.1b and c). Results from the microcosm experiment suggest that the ingestion of living roots may be higher in soils with lower SOM content than in those with a high content of SOM. This preference for living roots in soils with a low content of SOM may have two possible repercussions. On the one hand, a higher ingestion of SOM by *C. zealandica* larvae may increase the probability of infection by amber disease. On the other, the ingestion of roots may have a positive effect on larval nutrition and resistance to entomopathogens. *S. entomophila* may live in the gut of healthy larvae without producing apparent symptoms of amber disease. It has been found that an average background of 8000 cells of *Serratia* spp occur in the body of healthy *C. zealandica* larvae (Jackson et al., 1993). Differences between population numbers of *S. protamaculans* or *Serratia* spp for young pastures were not significant ($P > 0.05$) either. According to Jackson et al. (1991 in Glare et al., 1993) of the *S. entomophila* isolates recovered in New Zealand soils only 50% can cause amber disease. Different degrees of pathogenicity could also explain the disparity between counts of soil-borne *S. entomophila* and the levels of amber disease observed during this experiment (Nuñez-Valdez, 1994). Furthermore, the presence of bacteriophages may inhibit the development of amber disease in New Zealand soils (Glare, 1992c; O'Callaghan and Jackson, 1993b).

In early June 1992, at the end of the mesocosm experiment, a significant ($P < 0.05$) difference was detected in the level of *S. entomophila* among treatments (Figure 3.2b). As expected, those treatments in which the pathogenic (BC4B) and non-pathogenic (A20) strains were applied contained higher numbers of *S. entomophila* than those in which no bacteria were applied. Results from the experimental units treated with *S. entomophila* showed no significant ($P > 0.05$) differences in the survival of either pathogenic or non-pathogenic strains in all soils (Figure 3.2b).

Levels of *S. entomophila* were not significantly ($P < 0.05$) different in all pastures when nutrient broth and water were applied. Results from the experimental units not treated with the bacteria showed that the larval mortality recorded during the experiment did not increase significantly ($P > 0.05$) the level

of *S. entomophila* in soil. The population of bacteria in treatment 0L+H₂O in all sites was not significantly ($P>0.05$) different than in treatment 5L+H₂O and 5L+NB. Considering that amber diseased third instar *C. zealandica* collected from the field have an average of 1.44×10^6 cells of *S. entomophila* (O'Callaghan, 1989), this result gives an indication of the extent to which pathogen recycling can be detected in soil in the short-term.

A non-significant ($P>0.05$) increase in amber disease levels was promoted by the application of nutrient broth. This effect is matched by a slight increase ($P>0.05$) in the number *C. zealandica* larvae affected by amber disease (Figures 3.1a, b and c) in all soils. The percentage of recovery of *S. entomophila* populations in soil from cores treated with both strains of the bacteria was 10% and 5% higher in soil from S14 than in soil from A6 and A1 respectively (Table 3.2). Although these differences are not significant ($P>0.05$), they may indicate a better survival of *S. entomophila* in S14 than in A6 and A1 soils. However, because of the difficulty in quantifying bacterial numbers in the soil (O'Callaghan, 1989) more replicates are needed to assess accurately bacterial survival.

3.3.3 Interactions among *C. zealandica*, *S. entomophila*, plant growth and plant DM production during the mesocosm experiment

Results of herbage and root growth were compared among treatments in Figure 3.3a and 3.3b respectively. Herbage DM production, living roots DM production as well as their content of total C and N were also compared among treatments (Table 3.3). Correlation coefficients for the growth of healthy third instar larvae of *C. zealandica* with plant variables are presented in Table 3.4 and Table 3.5. The significance of these coefficients was consistently supported by multiple regressions and correlation analyses carried out using combined or independent data from treatments 5L+NB and 5L+A20. Independent correlation analyses and multiple regressions were also performed for each site combining results from different treatments (Table 3.5). Because of the effect that the pathogenic strain had in inhibiting larval growth (Section 3.3.5), results from the treatment 5L+BC4B were excluded from these analyses. The assumption that there is a positive relationship between root growth and herbage growth was not fulfilled under the conditions of this experiment. The interactions among larval herbivory and herbage growth are

Table 3.2

Number of cells of *S. entomophila* inoculated during the mesocosm experiment; background population before the experiment (March 1992) and bacterial numbers at the end of the experiment (June 1992).

Site	Inoculum	<i>S. entomophila</i>	<i>S. entomophila</i>	Recovery
	(March 1992)*	(March 1992)	(June 1992)*	(%)**
	Cells g ⁻¹ soil	Cells g ⁻¹ soil	Cells g ⁻¹ soil	
S14	5.8x10 ⁸	5.5x10 ^{4a}	1.0x10 ^{6a}	16 ^a
A6	5.7x10 ⁸	<100 ^b	3.2x10 ^{5a}	6 ^a
A1	5.8x10 ⁸	950 ^b	6.2x10 ^{5a}	11 ^a

*Mean values from A20 and BC4B treatments

**Percentage of recovery was corrected for bacterial background. Differences among values sharing the same letter are non-significant. LSD $P < 0.05$; d.f.=3

Table 3.3

Mean value of above and below ground plant variables recorded at the end of the mesocosm experiment in all experimental units at each of the three studied sites¹

Treatments	Herbage (gm ⁻²)			Living Roots (gm ⁻²)			Amount in Living Roots (µg g ⁻¹ soil)	
	DM	%C	%N	DM	%C	%N	C	N
S14								
0L+H ₂ O	179	41.14	2.16	565	35.24	1.19	2043.5	68.78
5L+H ₂ O	178	39.97	2.16	407	39.15	1.33	1507.1	50.40
5L+NB	187	41.77	2.39	392	38.52	1.52	1186.9	46.66
5L+A20	241	41.48	2.53	689	37.39	1.09	2747.0	80.51
5L+BC4B	243	41.20	2.97	547	32.23	1.12	1830.9	64.33
A6								
0L+H ₂ O	196	43.66	3.40	382	28.04	1.42	1082.7	56.75
5L+H ₂ O	164	43.26	2.89	300	25.38	1.20	761.9	36.15
5L+NB	146	41.31	2.78	292	38.43	1.96	680.8	34.20
5L+A20	208	43.99	3.23	323	31.40	1.46	1248.0	58.39
5L+BC4B	206	43.97	3.58	321	33.06	1.73	1351.5	70.92
A1								
0L+H ₂ O	250	43.62	3.50	345	31.41	1.48	1218.3	57.33
5L+H ₂ O	291	43.29	2.81	330	35.87	1.85	633.3	32.80
5L+NB	229	41.26	2.92	348	33.49	1.35	1534.7	62.36
5L+A20	259	44.10	3.13	378	32.18	1.21	918.2	34.85
5L+BC4B	185	44.01	3.55	213	34.30	1.54	689.7	31.48
LSD _(0.05)	76.39	2.39	0.41	216	10.07	0.53	649.3	24.24

¹Refer to Sections 3.2.1. and 3.2.3 for a description of the sites and treatments respectively.

Table 3.4

Correlation coefficients (r) for the growth of healthy¹ third instars *C. zealandica* with above and below-ground plant variables at the end of the mesocosm experiment. Results from the experimental units in which larvae were incorporated and water and bacteria-free nutrient broth were applied in three pastures (n=12).

	Carbon	Nitrogen
Living roots (%)	-0.81(*)	0.02(ns)
Total amount in living roots	-0.75(*)	-0.32(ns)
Concentration in living roots g-1 soil	-0.66(ns)	-0.78(*)
Herbage (%)	0.77(*)	0.85(*)
Amount in herbage	0.09(ns)	0.29(ns)
Amount in living roots + herbage	0.04(ns)	-0.55(ns)
Living root DM production	-0.31(ns)	
Growth of living roots	0.40(ns)	
Herbage DM production	0.01(ns)	
Herbage growth	-0.16(ns)	
Living roots + herbage DM production	-0.27(ns)	

¹Combined results from healthy larvae marked and healthy larvae present in the background.

(*)= $P < 0.05$; **= $P < 0.01$ (ns)=non-significant difference.

Table 3.5

Correlation coefficient (r) for the growth of healthy¹ third instars *C. zealandica* with above and below-ground plant variables at the end of the mesocosm experiment. Main results (n=6) from the experimental units from the three pastures considered independently combining all treatments except those treated with the pathogenic bacteria (BC4B).

	S14	A6	A1
Amount of N in living roots	0.77(ns)	0.57(ns)	-0.87(*)
Amount of C in living roots	0.62(ns)	0.69(ns)	-0.91(*)
Growth of living roots	0.54(ns)	0.67(ns)	-0.83(*)
Growth of herbage	0.64(ns)	-0.24(ns)	0.24(ns)

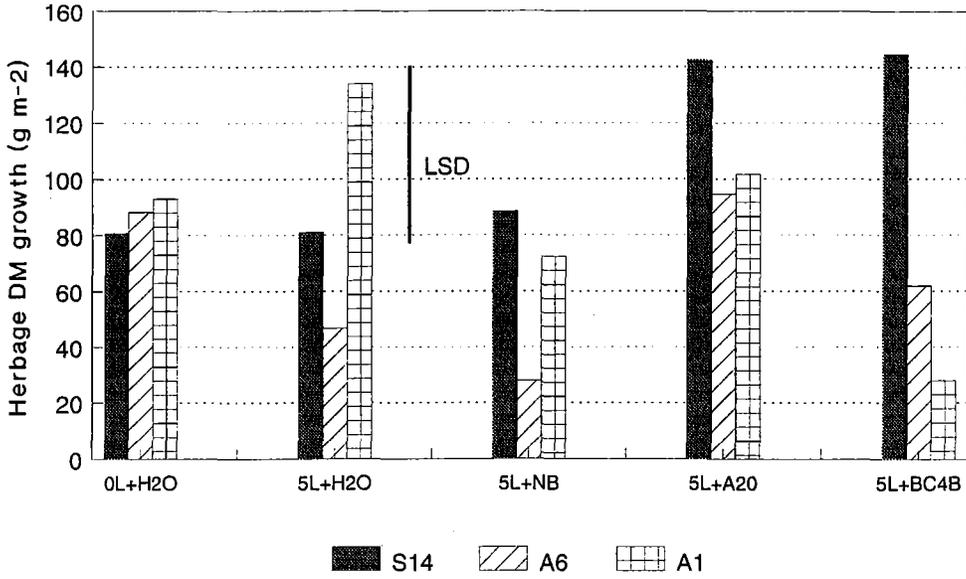
¹Combined results from healthy larvae marked and healthy larvae present in the background.

(*)= $P > 0.05$; **= $P > 0.01$ (ns)=non-significant difference.

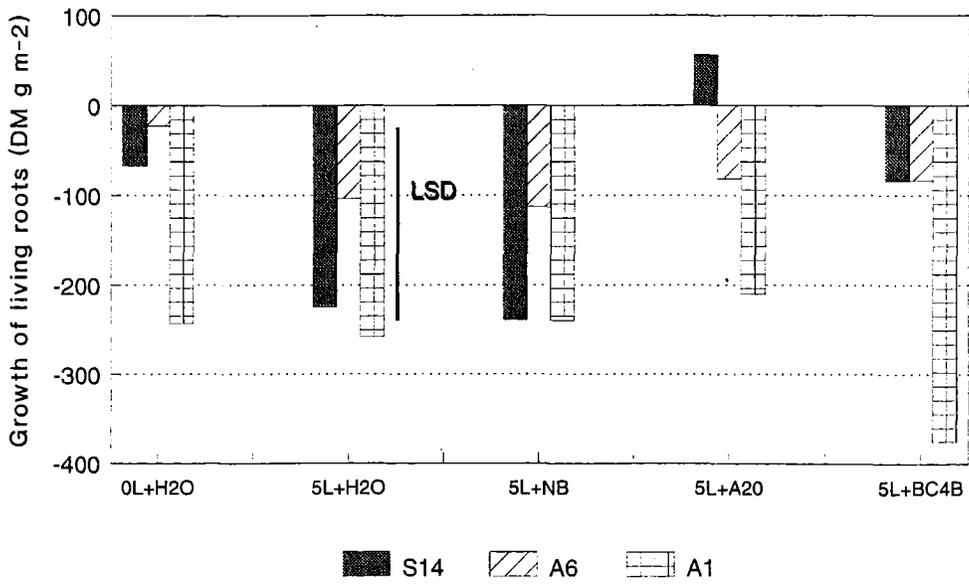
Figure 3.3

(a) Means of growth of herbage DM during the mesocosm experiment at Winchmore. (b) Means of growth of living root DM in each treatment. LSD ($P < 0.05$).

(a)



(b)



complex and a detailed description and interpretation of results follows.

3.3.3.1 Effect of *C. zealandica* on herbage DM production and growth during the mesocosm experiment

The effects of *C. zealandica* larvae on herbage and living root production among treatments are presented in Table 3.3. The effects produced by the presence or absence of *C. zealandica* larvae in the experimental units treated with water are indicative of the natural process of herbivory occurring in pastures. Surprisingly, no significant differences were observed in herbage DM production between treatments that included and excluded larvae for any of the sites of study.

Herbage DM production at the end of the mesocosm experiment was higher in most treatments in A1 pasture than in A6 and S14 pastures (Table 3.3). This result may be related to the higher content of weeds present in A1 pasture (Appendix I). Growth of dandelion may have been favoured if the preferential grazing on ryegrass and clover plants by *C. zealandica* larvae may have increased the availability of resources for this weed. Data on grass composition were only recorded at the beginning of the experiment in this study (Appendix II). Analyses of botanical composition carried out at the beginning and at the end of the mesocosm experiment would have been useful to evaluate the effect of the presence of *C. zealandica* on plant composition under field conditions.

Data on the effect of *C. zealandica* larvae on herbage DM production and growth may be safely compared in the experimental units from A1 pasture where no larval background was recorded (Table 3.3). Contrary to the common belief, herbage production was higher in some cases when the insect was included (5L+H₂O), than when it was excluded (0L+H₂O). In A1 pasture, a non-significant ($P>0.05$) but higher herbage DM production and growth occurred in treatment 5L+H₂O than in 0L+H₂O treatment. (Table 3.3 and Figure 3.3b). Moreover, and also surprising, is a significantly ($P<0.05$) higher herbage growth (80%) in treatment 5L+H₂O compared with treatment 5L+BC4B in A1. These results may, however, reflect a favourable herbage growth of weeds that possibly were not as susceptible to insect herbivory as clover and ryegrass (Appendix II).

These contradictory results may support the hypothesis that under particular conditions the insect may contribute directly or indirectly to plant growth. Brown and Gange (1990) strongly suggest that when parts of the root system are removed by soil rhizophagous insects, the replacement of plant roots is very rapid. See Section 1.2.3.6 (Chapter I) for a further discussion on this. More research is needed to reproduce the environmental conditions that cause the different patterns of damage of *C. zealandica* in Canterbury.

3.3.3.2 Effect of *C. zealandica* on the content of N and C in herbage during the mesocosm experiment.

Significantly ($P < 0.05$) lower percentages of N in herbage were observed both in A6 and A1 pasture in treatment 5L+H₂O in comparison with treatment 0L+H₂O (Table 3.3). However this result was not observed for the experimental units from S14 pasture. This result suggest that through root attack the insect may be reducing the amount of N present in herbage. This reduction was particularly evident in young pastures where clover was more abundant and SOM content was lower in comparison to that observed in the oldest pasture. In S14 pasture either the higher proportion of grass to clover or the higher SOM content may be the main factors involved in the compensation of the loss of N present in herbage produced by larval herbivory. Reductions promoted in the level of other elements in plant herbage through larval herbivory have been observed elsewhere. Leaf N, P, and K concentrations decreased linearly as the level of infestation of larvae of *Ligyris subtropicus* (Blatchley) increased in Florida sugar cane (Coale and Cherry, 1989). The role of SOM in the compensation of loss of N in herbage after larval herbivory requires further research.

Non-significant ($P > 0.05$) differences in the percentage of total C were observed for herbage between experimental units treated with water including and excluding *C. zealandica* larvae in all sites. Levels of C in herbage may be compensated regardless of insect attack by changes in the rates of photosynthesis. The levels of CO₂ in the atmosphere apparently are not a constraint for the amount of C available for biological fixation of the plants.

3.3.3.3 Effect of *S. entomophila* on herbage DM production and growth during the mesocosm experiment.

Because no treatment in which either pathogenic or nonpathogenic strains of *S. entomophila* were used alone (excluding *C. zealandica* larvae) during this experiment, little can be said about the effect of the bacteria on herbage production and growth. However, the significant ($P<0.05$) increase of 43% in DM herbage growth both in treatments 5L+A20 and 5L+BC4B suggests that bacteria alone had a positive effect on herbage production in S14 pasture (Figure 3.3b). Results from the experimental units in A6 pasture showed non-significant differences ($P>0.05$) for pasture growth. The application of the pathogenic strain of *S. entomophila* (BC4B) reduced herbage DM production in A1 pasture. Significantly lower ($P<0.05$) herbage DM values were also recorded in 5L+BC4B treatment than in 5L+H₂O treatment in A1 pasture.

Data in Figure 3.3a show the differences observed among treatments in herbage growth. At the end of the mesocosm experiment, in S14 pasture, a significant ($P<0.05$) increase (40%) in herbage growth was observed in treatments 5L+A20 and 5L+BC4B in comparison to 5L+H₂O and 0L+H₂O (Figure 3.3a). The significant ($P<0.05$) increase (70%) in herbage growth observed in 5L+A20 treatment in comparison to that observed in 5L+NB treatment suggest that *S. entomophila* produced a positive effect by itself in A6 pasture. In A6 pasture, reductions of 50-66% in herbage growth were observed in the 5L+H₂O and 5L+NB treatments respectively in comparisons with 0L+H₂O treatment. Conversely, in S14 pasture, no differences in herbage growth occurred among the same treatments (Figure 3.3a). Because of the relatively high larval background present in treatment 0L+H₂O in S14 pasture (Figure 3.1a) the comparison of the effect of larval herbivory on herbage growth between the old and the young pastures is difficult. These results, however, suggest that the effect of larval herbivory had a greater impact on herbage growth in the soil with the lowest SOM content.

3.3.3.4 Effect of *S. entomophila* on the content of N and C in herbage during the mesocosm experiment

The percentage of N in herbage was the highest in treatment 5L+BC4B in all sites (Table 3.3). Significant ($P<0.05$) differences were observed for the percentage of N in herbage between treatments 5L+BC4B and 5L+H₂O in all pastures. Overall, the percentage of N in herbage increased significantly ($P<0.05$) in herbage from treatments 5L+A20 and 5L+BC4B in comparison with the remaining treatments (Table 3.3). In treatment 5L+BC4B the level of N in herbage reached almost the same level as that

observed in treatment 0L+H₂O at sites A6 and A1 and even significantly ($P<0.05$) higher at S14 pasture. The percentage of N in herbage was on average 27%, 19% and 21% higher ($P<0.05$) in experimental units treated with BC4B than those treated with water in S14, A6 and A1 pastures respectively (Table 3.3). These differences may be explained both in terms of the inhibitory effect on larval feeding induced by the bacteria (Jackson et al., 1993) and its direct or indirect effect on root growth. Direct effects may induce root growth or SOM mineralization (Section 3.3.4 and 4.3.2 in Chapters III and IV respectively). *C. zealandica* larvae may have an effect in reducing the proportion of N in herbage as has been observed in young pastures. Results from the present study indicate that inundative applications of *S. entomophila* may have a positive effect by itself on increasing the level of N in herbage. It should be borne in mind, that this response could be confounded with that induced by the control of *C. zealandica* larvae. This statement is supported by the fact that increases in the percentage of N in herbage occurred regardless of the presence of healthy *C. zealandica* larvae in all pastures.

The application of either pathogenic or non-pathogenic strains of *S. entomophila* did not affect the percentage of C in herbage significantly ($P>0.05$). However, a significant ($P<0.05$) reduction in the proportion of C present in experimental units treated with the nutrient broth free of *S. entomophila* was observed in young pastures. The addition of low molecular weight organic compounds present in the nutrient broth which are readily mineralizable possibly had an effect on larval feeding response. Non-significant ($P<0.05$) changes in the percentage of C in herbage were observed in the soil with the highest content of SOM. A significant ($P<0.05$) reduction in the percentage of C in herbage was detected in the treatment 5L+NB at site A6 (Table 3.3.). Under laboratory conditions, amino acids, vitamins, inorganic salts and carbohydrates induced considerable feeding activity of third instar larvae of *C. zealandica* (Sutherland, 1971; 1972; Sutherland and Hillier, 1974). Therefore this reduction in the percentage of C in herbage may be associated with an increase in larval rhizophagy in treatment 5L+NB as a consequence of a high intake of C through larval herbivory.

3.3.3.5 Effect of *C. zealandica* on living root DM production and growth during the mesocosm experiment.

Results obtained at the end of the mesocosm experiment suggest that the presence of *C. zealandica*

larvae reduced slightly living root DM production and growth (Figure 3.4a and Table 3.3). Contrary to expectations, this effect was less conspicuous in A1 pasture and more pronounced in S14 pasture. Results from the microcosm experiment suggested that the root feeding activity of *C. zealandica* was higher in A6 than in S14 soil. However, the assumption that the growth of living roots is exclusively affected by larval herbivory may not be fulfilled. The effect that different degrees of larval herbivory may have in plants may be a function of the botanical composition, plant age and secondary metabolites.

Even though a higher trend for rhizophagy was expected in young pastures, additional factors that are known to reduce rhizophagy are root physical strength and the presence of compounds such as saponins that produce a feeding deterrent response in *C. zealandica* larvae (Sutherland et al., 1982; Sutherland, 1975). Detailed results on botanical composition may also provide important information about the pattern of rhizophagy followed by *C. zealandica*.

3.3.3.6 Effects of *C. zealandica* on C and N content in living roots during the mesocosm experiment.

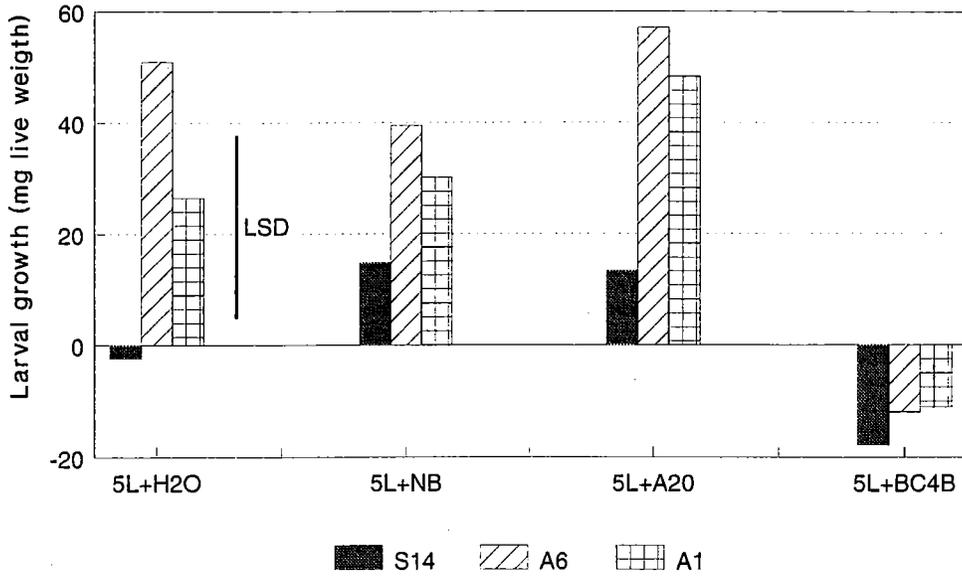
Differences between treatments 0L+H₂O and 5L+H₂O in the percentage of C and N in plant roots were non-significant ($P>0.05$) for all sites. Apparently plants can also compensate for losses in the proportion of C and N in subterranean parts caused by the rhizophagous activity of *C. zealandica*. A higher percentage of N in roots from A6 and A1 was detected than in roots from S14 ($P<0.05$). This difference was probably the result of a higher clover content in the young pastures (Appendix II). The symbiotic relationship between the N-fixing bacterium *Rhizobium* sp and clover (Rovira et al., 1990) roots may account for this difference.

A non-significant ($P>0.05$) reduction of 26%, 30% and 48% in the amount of total C contained in living plant roots in treatments 5L+H₂O when compared with treatment 0L+H₂O was observed in S14, A6 and A1 pastures respectively (Table 3.3). Only the reduction (43%) in the amount of total N in living roots between 0L+H₂O treatment in comparison with 5L+H₂O treatment in A1 pasture was significant ($P<0.05$) (Table 3.3). In A1 pasture, significant ($P<0.05$) reductions (43%) in the amount of N in living roots were recorded in 5L+H₂O treatment in comparison with 0L+H₂O treatment.

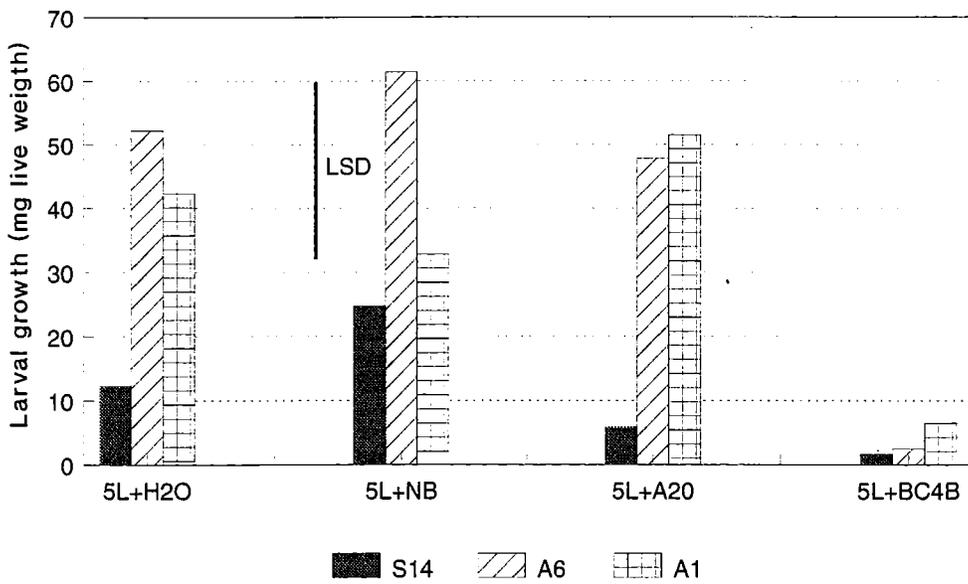
Figure 3.4

Mean larval growth (live-weight gain) of *C. zealandica* during the mesocosm experiment in different treatments and pastures. (a) growth of healthy and disease larvae considered together and (b) larval growth of marked third instars. LSD ($P < 0.05$).

(a)



(b)



3.3.3.7 Effects of *S. entomophila* on root growth during the mesocosm experiment

Several observations suggest that *S. entomophila* has a positive effect by itself in the growth of living roots: (a) In S14 pasture, the magnitude of the growth of living roots was five times higher in 5L+A20 treatment in comparison with 5L+NB or 5L+H₂O treatments (Figure 3.3b). In addition the 5L+A20 treatment promoted the highest living root growth and production in S14 pasture (Table 3.3). It should be borne in mind that, treatment 5L+A20 induced root growth regardless of the presence of healthy *C. zealandica* larvae in S14 pasture (Figure 3.1); (b) in soils from sites S14 and A6 the treatment 5L+BC4B produced a similar result in root production and root growth as the treatment 0L+H₂O; (c) in S14 pasture, a non-significant ($P>0.05$) decline of 60% in root growth occurred in treatment 5L+H₂O compared with treatment 5L+BC4B.

Three possible explanations may account for the increase in root growth attributed to the application of *S. entomophila* to the experimental units present in the soil with the highest content of SOM:

(a) production of plant hormones as cytokinins by soil microorganisms has been suggested to increase root growth (Nieto and Frankenberg, 1990). Although this effect has not been reported for bacteria of the genus *Serratia*, research is recommended to assess the significance of this process.

(b) Changes promoted by *S. entomophila* in the root feeding behaviour of *C. zealandica* may be taking place in the rhizosphere. It has been shown in previous laboratory and field experiments (Jackson et al., 1993; Jackson et al., 1986) that increases of herbage DM production are promoted by the application of pathogenic strains of *S. entomophila*. The assumption was made that these increases were due to the antifeeding effect of the bacteria on *C. zealandica* larvae. Through the addition of the non-pathogenic strain, however, the intensity and amount of nutrients taken by the insect from living roots may also be reduced. There is the possibility that the *S. entomophila* by itself may be a source of N for *C. zealandica* larvae. In fact, a rich and abundant microflora has been observed in the fermentation chamber of the gut of *C. zealandica* larvae which are thought to be a source of protein for the larvae (Bauchop and Clarke, 1975). This idea has also been suggested for other soil melolonthid larvae because of the smaller amount

of bacteria has been found in the faeces than in the gut (Davidson and Roberts, 1968a).

(c) *S. entomophila* may be an antagonist to plant rhizopathogens. In fact, *Serratia plimuthica* is a common inhabitant of the rhizosphere of various plants and has been reported as antagonist of the root fungi *Fusarium culmorum* and *Phytium* sp (Alstrom and Gerdharson, 1987 in Hornby, 1990). Moreover, competition among *S. entomophila* with other phytopathogens in the soil may reduce the likelihood of plant root diseases. Several *Serratia* isolates have proved to be antibacterial and sometimes good antifungal agents (Gerber, 1975 in Hornby, 1990).

However, the highest drop in growth of living roots that occurred during the experiment was observed in 5L+BC4B treatment at A1 pasture. The mean of the coefficient of variation of root DM for all treatments at the end of the mesocosm experiment was 47% for A1 pasture, whereas values of 27% and 39% were obtained at sites S14 and A6 respectively. The higher proportion of weeds with stronger and bigger roots is likely to be the reason for this differences. The high variability observed in root biomass in site A1 should be minimized in future experiments.

Similar results were obtained for living root DM production (Table 3.3) and growth (Figure 3.3b) between 5L+H₂O and 5L+NB treatments. This may be the net result of two opposite trends: a higher *C. zealandica* root herbivory or a higher rate of root growth that occurred after the addition of nutrients into the experimental units. Plants are dynamic systems able to respond to pressure imposed by below-ground herbivores (Brown and Gange, 1990; Trumble et al., 1993). In all sites, both living root growth and DM production, the 5L+H₂O and 5L+NB treatments were similar ($P>0.05$) to those observed in the 0L+H₂O treatment.

3.3.4 *C. zealandica* larval growth during the mesocosm experiment

Results obtained for the growth of third instar larvae of *C. zealandica* after the mesocosm experiment are presented in Figure 3.4. A slightly different picture is observed when the weight of healthy and diseased larvae were analyzed together than when they were set apart to compare the effect of the treatments and soils (Figure 3.4a and b).

When the weight of healthy and diseased third instar *C. zealandica* were considered together, the loss in live weight was significant ($P < 0.05$) in experimental units treated with the pathogenic strain in A6 and A1 but not in S14 pasture. However, when the larval growth of marked healthy larvae was set apart, lower values were observed in 5L+BC4B treatment. Larval growth was significantly ($P < 0.05$) lower for healthy larvae collected in treatment 5L+BC4B than for any other treatment in experimental units both from A6 and A1 pastures. If the assumption that larval growth is an indication of health is true, this result indicates that apparent symptoms of disease may not be a reliable estimation of the health status of *C. zealandica* larvae. Larvae showing no evident symptoms of disease in treatment 5L+BC4B possibly have reduced their rate of feeding or recovered from amber disease. As has been observed in the microcosm experiment, the growth of healthy larvae was significantly ($P < 0.05$) lower in soil from S14 than in soil from A6 pasture.

Except for the significant ($P < 0.05$) difference observed in treatment 5L+NB between young pastures, the growth of marked healthy larvae was not significant ($P > 0.05$) for any other treatment (Figure 3.4b). Experimental units treated with nutrient broth in A6 pasture offered the best conditions for larval growth. The observation that organic compounds of low molecular weight may stimulate larval feeding activity in *C. zealandica* may account for this result (Sutherland, 1971). Moreover, this result may also be linked with the richer content of C and N in living roots in A6 pasture (Table 3.3). However, it is still unclear why healthy marked larvae grew less in soil cores treated with nutrient broth from A1. According to Sutherland (1975) plant toxins such as saponins in roots of lucerne (*Medicago sativa*) and condensed tannins from root extracts have been found to be active against *C. zealandica*. These compounds in natural levels may act as growth retardants for *C. zealandica* larvae (Sutherland 1975). Therefore, the greater plant diversity observed in A1 than in A6 pasture may have imposed restrictions to larval growth of *C. zealandica*. Further research is needed to assess the importance of this and other soil factors on *C. zealandica* growth.

3.3.5 Correlations among larval growth and plant growth

Surprisingly, the growth of healthy third instar was not significantly correlated ($P > 0.05$) with herbage growth and herbage DM production at the end of the experiment (Table 3.4). Correlations and

multiple regressions of data combining the treatments 0L+H₂O from all sites suggest that, regardless of the presence of *C. zealandica* larvae, root growth and herbage growth were not significantly correlated. Neither root growth and herbage growth were significantly correlated when analysis of corrected values for insect herbivory were used. These corrected values were obtained by subtracting the result of growth of living roots and growth of herbage DM in the treatment 0L+H₂O from the value obtained in the remaining treatments. This correction is based on two assumptions: (a) the background larval densities in S14 and A6 pasture (see Figure 3.1a and 3.1b) imposed a significantly lower pressure for living roots and herbage growth than that accounted for those treatments in which larvae were included and (b) the insect was the only cause responsible for differences in plant growth. If these assumptions are real, results from Figure 3.5a and 3.5b show that the presence of the insect may have different effects on herbage production under different SOM conditions. In Figure 3.5, negative values represent the cases in which the treatment had a positive effect on living roots and on herbage growth. Damage attributable to *C. zealandica* on living roots was significantly ($P<0.05$) lower only for the experimental units treated with the non-pathogenic strain in comparison to those treatments in which no bacteria was applied (Figure 3.4a). Reductions in the growth of herbage caused by *C. zealandica* larvae were non-significant ($P>0.05$) at any of the sites studied (Figure 3.4b). Both logarithmic and square root transformations as well as raw data were used to test these correlations but none of them were found to be significant ($P>0.05$).

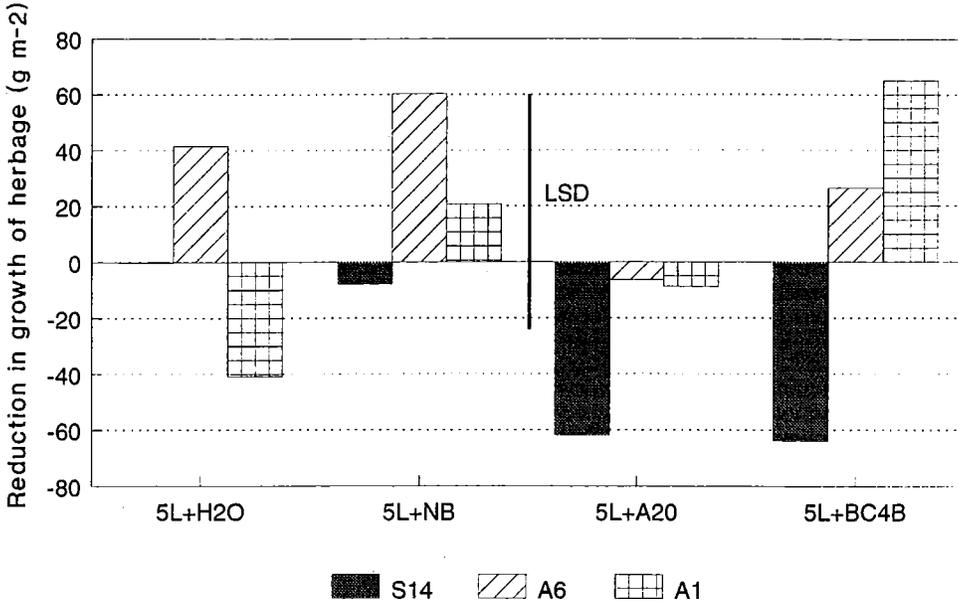
However, larval growth was negatively correlated with some plant variables and the results from Table 3.4 suggest that *C. zealandica* may be removing C and N from living roots. When the amount of N present in living roots was considered, a significant ($P<0.05$) positive correlation was observed. A significant positive correlation was also observed between larval growth with the amount of C and N present in herbage. As has been previously discussed, the higher content of clover and the lower likelihood of becoming infected by entomopathogens are favourable conditions for the growth of *C. zealandica* larvae in A1 and A6 pastures compared with S14 pasture.

Data in Table 3.5 show correlations obtained combining all treatments except 5L+BC4B. These data suggest that there is an increasing trend in the intake of C and N promoted by larval herbivory present in living roots with decreasing levels of SOM. Significant negative correlations were observed for

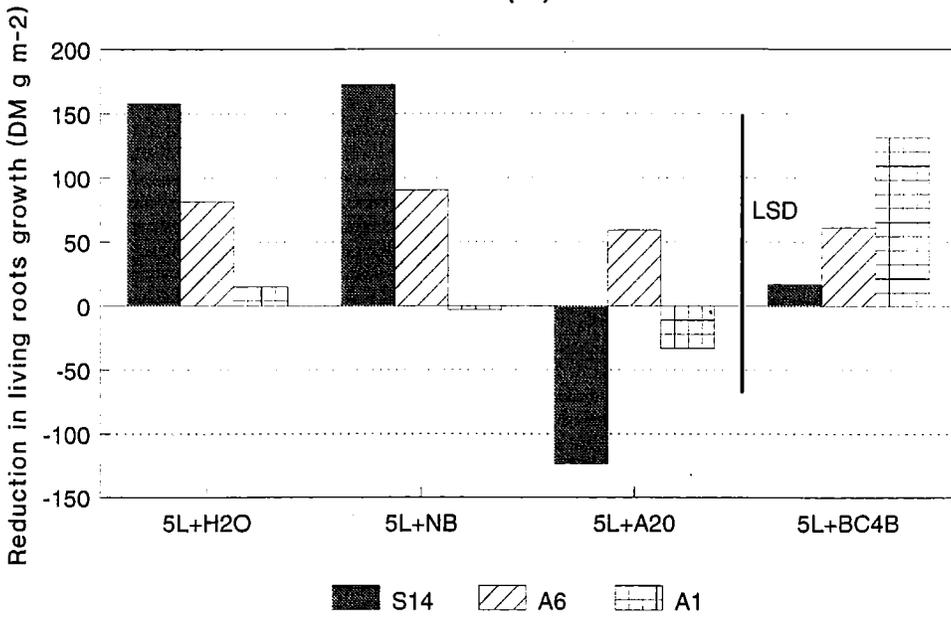
Figure 3.5

(a) Reduction in the growth of herbage caused by larval herbivory of third instars *C. zealandica* under the conditions of the mesocosm experiment. (b) Reduction observed in the growth of living roots.

(a)



(b)



the total amount of N and specially for the total amount of C present in living roots at A1 pasture. If the assumption that there is a cause-effect relationship between the reduction in levels of total C and N in living roots and larval growth is valid, these results may also suggest that the more intense the larval root consumption, the greater the larval growth (See also Section 2.3.5 in Chapter II) which may also be valid for field mesocosm conditions. When sites were considered apart, however, no significant correlations were found between larval growth and growth of herbage. None of the remaining plant variables presented in Table 3.4 was significantly correlated with larval growth for any of the pastures.

Despite the reduction of total C and N associated to larval herbivory of *C. zealandica*, the lack of correlation among growth of herbage and living roots under the experimental conditions of this study, strongly suggests that the obvious pattern of damage caused by *C. zealandica* may be mainly related to physical rather than with nutritional injury in living roots and herbage.

3.4 Conclusions

A higher natural regulation of third instar larvae was recorded in the site with the highest content of SOM. No significant differences on the visual assessments of the number of larvae showing apparent symptoms of amber disease were observed. However, mortality of *C. zealandica* larvae caused by entomopathogens was significantly higher in the soil with the highest content of SOM.

Significantly higher numbers of *Serratia* spp and *S. entomophila* were recorded in the soil with the highest content of SOM. Nevertheless, numbers of bacteria in the soil were not necessarily associated with levels of mortality or amber disease in third instar *C. zealandica*. A set of environmental conditions may, therefore, dictate if disease or death of the insect will occur.

No significant differences were observed in the efficacy of the pathogenic strain of *S. entomophila* in producing amber disease in third instars *C. zealandica* among pastures under mesocosm conditions.

The impact on growth of herbage and living roots caused by *C. zealandica* seems to be more related to the physical injury rather than with a nutritional effect. No correlation was found between

larval herbivory with the reduction of herbage and living roots growth. However, results suggest that the presence of third instar larvae of *C. zealandica* reduced total C and N from living roots of plants. Under field mesocosm conditions, removal of C and N from herbage and living roots increased as the level of SOM decreased.

Despite relatively high densities of *C. zealandica* larvae used in this experiment, no significant damage was observed on any of the pastures studied. Density was experimentally manipulated to impose a significant pressure on herbage growth, living roots growth and their content of total C and N. This density was above the action threshold level reported for *C. zealandica* larvae in New Zealand pastures. Severe plant damage caused by *C. zealandica* larvae on plants seem to be regulated by the interplay of environmental variables in the pasture ecosystem. Generalizations about pasture growth and damage caused by third instars of *C. zealandica* during late autumn and the onset of the winter need to be modified in the improvement of insect management strategies.

Under the conditions of the present mesocosm experiment, *C. zealandica* larval growth increased significantly ($P < 0.05$) in soils with low amounts of SOM where: (a) the ingestion of living roots was greater than in soil with a high content of SOM; (b) living roots were richer in N; (c) there was a lower probability of infection by amber disease. The combined effect of these factors may have repercussions for the higher susceptibility of young pastures to *C. zealandica* damage in comparisons to old pastures observed in Canterbury.

The process described in this work may partially explain the long-term reduction, below an economic threshold level, of larval density in old pastures. Results strongly suggest that SOM conditions may account for this difference.

Efforts should be made to increase the efficiency of the bacterial applications to the soil; to encourage the build up of SOM in order to reduce damage; to increase the presence of indigenous soil micro-organisms and to enhance the contribution to soil fertility that *C. zealandica* larvae may have. These actions are also recommended to prevent pasture damage. Conservation and increased soil fertility may be crucial within a sustainable pastoral agriculture.

EFFECT OF *Costelytra zealandica* (White) AND *Serratia entomophila* (Grimont *et al.*) ON SOIL ORGANIC MATTER DYNAMICS.

4.1 Introduction

Most recent research on the soil-dwelling larvae of the melolonthid *Costelytra zealandica* (White) has focused on its control (Barratt, 1990; Chapman, 1990; Jackson *et al.*, 1992) and little attention has been placed on its contribution to soil fertility (Yaacob, 1967; Pottinger, 1976). Because of the injury caused to plant roots, this insect is regarded as the main entomological problem of New Zealand pastures (Garnham and Barlow, 1993). However, research has indicated that this insect may play an active role in soil organic matter (SOM) dynamics (e.g., Yaacob, 1967; Pottinger, 1976). In addition to ingesting plant roots, *C. zealandica* larvae may also feed on SOM and complete their whole life cycle in soil devoid of living roots (D. Miller pers. comm. in Sutherland, 1971). If this SOM mineralization is significant under field conditions, *C. zealandica* larvae may play a similar role in soil fertility to that of earthworms. There is a current gap in the information gathered from field experiments on the ecological functions of economically important soil-dwelling melolonthid larvae.

Larvae of *C. zealandica* are commonly infected by the endemic bacterium *Serratia entomophila* (Grimont *et al.*). *S. entomophila* is a chronic, *C. zealandica* specific soil-borne pathogen which causes larvae to cease feeding, develop an amber coloration and eventually die (Jackson *et al.*, 1993). This pathogen has been recently commercialized as an inundative biological control agent in New Zealand (Jackson *et al.*, 1992). Apart from the information available on the soil ecology of this bacterium directly related with biological control (O'Callaghan, 1989), nothing is known about its effect on indigenous soil microbial populations, on plant roots and on the breakdown of soil organic compounds.

No previous studies have been made integrating the biology of melolonthid larvae and their entomopathogens with detailed analysis of SOM. In the present study, a fractionation scheme for SOM has

been proposed to explore the interactions among *C. zealandica* larvae, *S. entomophila* and SOM. Definition of SOM and the biochemical constitution of each SOM fraction are given in Section 1.1.3 (Chapter I).

In this chapter an attempt has been made to explore the short-term effect of *C. zealandica* and *S. entomophila* on SOM dynamics under the conditions of a mesocosm experiment conducted in pastures from Winchmore Irrigation Research Station, Canterbury, New Zealand. The main objectives of this study were to:

- (1) Determine the abundance and distribution of total C and N in SOM from pastures under similar agronomic and climatic conditions except for their content of SOM.
- (2) Evaluate the effect of *C. zealandica* early third instars on SOM dynamics.
- (3) Assess the consequences of *S. entomophila* inundative applications on SOM turnover.

4.2 Materials and methods

Experimental units from the field mesocosm experiment described in Chapter III (Section 3.2.3) were used to explore the short-term effect of *C. zealandica* larvae and *S. entomophila* applications on SOM dynamics in three pastures. These pastures were located at Winchmore and they have been earlier described (Sections 2.2.1 and 3.2.1 in Chapter II and III respectively). At the time the experiment was conducted, site S14 was a 39-year-old pasture with a dominant grass vegetation consisting mainly of perennial ryegrass (*Lolium perenne* L.). Site A6 was a 5-year-old pasture which contained mainly white clover (*Trifolium repens* L.). Site A6 received alternative cycles of 3-4 years cropping (wheat, barley, pea and oat) followed by 3-4 years of pasture. Site A1 was a 2-year-old pasture that has been subjected to annual cycles of wheat, pea, oat, grass and barley in consecutive years. White clover was the dominant plant species, however, a higher abundance of weeds such as dandelion (*Taraxacum officinale* L.) and nodding thistle (probably *Cirsium arvense*) was present. The assumption was made that the previous five years under cropping cultivation produced a significant effect on SOM depletion at site A1.

The mesocosm experiment consisted of five treatments:

(a) Five marked early third instars *C. zealandica* (L3) plus 10 ml of a pathogenic strain of *S. entomophila* (BC4B) at a concentration of 4.7×10^{10} cells ml⁻¹ resuspended in nutrient broth (5L+BC4B). The nutrient broth was a solution of raw sugar, yeast extract diet, urea, Na₂HPO₄, KCl and NH₄NO₃.

(b) As for "(a)" but a non-pathogenic strain (A20) of *S. entomophila* was used (5L+A20).

(c) As for "(a)" but 10 ml of nutrient broth free of *S. entomophila* were added (5L+NB).

(d) As for "(a)" but 10 ml of distilled water were added (5L+H₂O) and

(e) As for "(d)" but no larvae were incorporated (0L+H₂O).

Experimental conditions were as described in Chapter III (Section 3.2.3, Plates 7,8,9 and 10). At the end of the experiment, duplicated subsamples of pooled soil per treatment were used for total C and N determinations and for analysis of SOM fractions. Total C and N in soil were determined by Dumas combustion (Grewal et al., 1991). Details on the methods and techniques of SOM analysis are given in Sections 2.2.4 (Chapter II) and Appendix I. A detailed description of the methods used and biochemical compositions of each of the SOM fractions can be found in Appendix I and Section 1.1.7 (Chapter I) respectively. At the end of this mesocosm experiment plant residues dry matter (DM) were measured by the modification of the washing-flotation method described originally by Ladell (1936) for separating soil fauna. Plant material was separated from the soil by washing samples in a set of 1 mm and 650 µm sieves. Both sieves were placed on a tray containing a solution of MgSO₄ (density 1.1). Floating material was collected by using a hoop covered with a folded cotton gauze. Plant material was washed, collected, dried (80°C for 24 h) and weighed. To determine the total C and N this material was ground (<250 microns) and combusted by the Dumas method (Grewal et al., 1991) (see Appendix I for details).

4.3. Results and discussion

4.3.1 Soil organic matter fractionation

The amount and distribution of total C and N in SOM fractions from the pastures studied are presented in Table 4.1. Labile fractions are represented as protruding slices in Figure 4.1 where a graphic representation of the proportions of C and N in the studied soils is made. A significantly ($P<0.05$) higher SOM content (6.8%) was found in soils from S14 than those from A6 and A1 pastures. The same value of SOM (4.6%) was observed in both young pastures. There were also significant ($P<0.05$) differences in the amount of total N in the oldest pasture compared with the younger pastures (Table 4.1). The concentration of N in cold water extraction was significantly ($P<0.05$) higher in S14 soil than in A1 soil but not in A6 soil ($P>0.05$).

Surprisingly, the amount and distribution of C and N were very similar in both young pastures regardless of their differences in agronomic history. The effect of tillage in disturbing the natural cycle of decomposition of organic compounds in the soil is well known (Foth, 1979; Stout et al., 1981; Stevenson, 1982). According to Stevenson (1982), marked changes are brought about in the SOM content through cropping. Intensive cultivation reduces the amount of plant residues available for humus synthesis. Tillage increases aeration and accelerates microbial decomposition of the labile SOM exposed on fresh soil surfaces (Stout et al., 1981; Stevenson, 1982). Sowing of pasture in the fourth year of rotation in site A1 has probably compensated the losses of SOM from the previous years of cultivation. From 4000-5000 kg ha⁻¹ of organic matter in the top 20 cm of soil are returned to the soil by roots of grass during one year (Davies et al., 1972 in McLaren and Cameron, 1990). The annual addition of underground plant dry matter (DM) living roots, exudates and plant residues into the soil have been estimated in the range of 5500-6000 kg ha⁻¹ annually in a grazed permanent pasture (Goh and Gregg, 1980). Stevenson (1982) states that for most agricultural soils, SOM can be maintained at high levels by inclusion of a sod crop in the cropping sequence.

Table 4.1

Amount of total C and N in mg per g dry soil (mg g⁻¹ soil) in SOM fractions from three soils at Winchmore.

Site	S14		A6		A1	
Fraction	C	N	C	N	C	N
Soil microbial biomass	1.04 ^{l*}	0.15 ^{ij}	0.47 ^{KL*}	0.07 ^l	0.54 ^{k*}	0.08 ^l
Cold H ₂ O extraction	0.41 ^{KLM}	0.13 ^{jk}	0.36 ^{LM}	0.11 ^k	0.34 ^M	0.08 ^l
Hot H ₂ O extraction	3.28 ^H	0.40 ^{fg}	1.70 ^l	0.20 ⁱ	1.80 ^l	0.19 ⁱ
Humic acids	4.90 ^{FG}	0.47 ^f	3.38 ^H	0.29 ^h	3.55 ^{GH}	0.33 ^g
Fulvic acids	7.79 ^E	0.80 ^d	5.28 ^F	0.49 ^{ef}	6.28 ^{EF}	0.59 ^e
Humins	26.56 ^{BC}	2.33 ^b	17.53 ^D	1.52 ^c	16.14 ^D	1.53 ^c
Total	42.92 ^A	4.13 ^a	28.25 ^B	2.61 ^b	28.11 ^{BC}	2.72 ^b

S14=39-years-old pasture; A6=5-years-old pasture and A1=2-years-old pasture.

*Corrected from dichromat-oxidizable C values (W) to total C values (T) according to Grewal et al., (1991), where: $T=0.126+1.25W$.

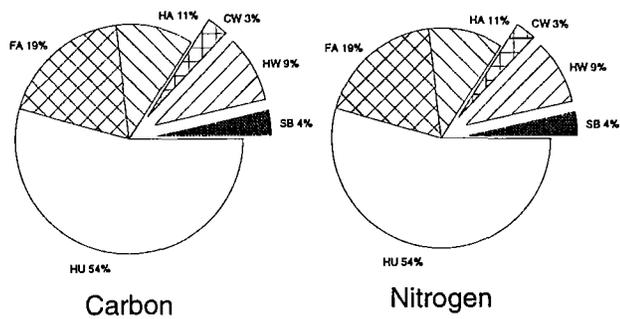
Means of 12 replicates per fraction.

Differences among values sharing the same letter are non-significant. LSD $P<0.05$ for log transformed data. Comparisons run down and across columns for the same element.

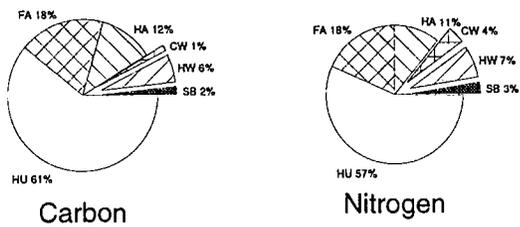
Figure 4.1

Mean (n=12 replicates) of the percentage of total C and N in different SOM fractions from the three pasture sites studied. The pie size is proportional to the value of total C and N. Nitrogen values were multiplied by a factor of 10. (a) Results from site S14 (39-year old pasture); (b) Results from site A6 (5-year-old pasture); (c) Results from site A1 (2-year old pasture). SB=soil microbial biomass, CW=cold water extraction, HW=hot water extraction, HA=humic acids, FA=fulvic acids, HU=humins,.

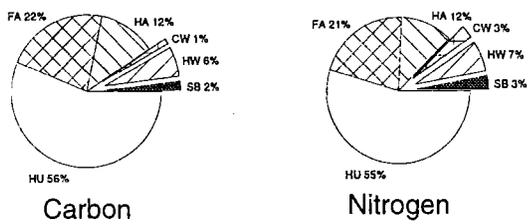
S14
(a)



A6
(b)



A1
(c)



It is possible that similar values for the SOM fractions observed in A1 and A6 pasture were a result of the long-term cropping history. Losses of SOM as resulting from cultivation are much greater during the earlier than later periods. A reduction of 25% of SOM has been observed during the first 20 years of cultivation in Missouri (Foth, 1979). In Missouri, 10% of SOM reduction occurred in the second 20 years and only about 7% in the third 20 years.

The only significant differences ($P < 0.05$) in SOM fractions between young pastures were observed in total N present in cold water extraction and humic acids (Table 4.1). The content of N in labile low molecular weight organic compounds may be the most sensitive part of SOM to cultivation under the rotation system followed at Winchmore. Because of their complexity, more research is required to explain changes in total N present in humic acids between young pastures.

In S14 soil, 88% of C and N was present in humic substances (humic acids, fulvic acids and humins), whereas 93% of C and 90% of N were allocated in humic substances in A6 and A1 soil (Figure 3.1). Stout et al., (1981) mentioned that tillage has an effect on increasing the mean age of the more stable SOM in comparison to the more labile SOM that is exposed on fresh soil surfaces.

Overall, the pattern of distribution of total C and N in SOM fractions described in Chapter II (Section 2.3.1) for soils S14 and A6 have been confirmed in this experiment in which the mean of a higher number of samples (12 replicates per fraction) were compared.

4.3.2 Contribution of *C. zealandica* and *S. entomophila* to SOM dynamics

Comparisons between treatments 0L+H₂O and 5L+H₂O were made to explore the main short-term effects that third instar *C. zealandica* had on SOM fractions. An assumption has been made that the background larval density in all soils was negligible, allowing differences to be observed when *C. zealandica*

larvae were included to the experimental units. It has also been assumed that, during the experiment, the presence of third instar *C. zealandica* had detectable direct or indirect repercussions on SOM turnover.

It should be taken into account that direct modifications of SOM by *C. zealandica* may theoretically take place through the passage of SOM into the larval gut, either by enzymes produced by the insect or its microbial associations. Indirect modifications of SOM caused by *C. zealandica* may occur either by dispersal, stimulation or grazing of microbial communities, or by comminution of complex pools of SOM which mix and expose organic materials to microbial attack.

The effect of *S. entomophila* in SOM dynamics was mainly inferred by comparing results from treatments 5L+NB and 5L+A20 in which the main difference was the presence of the bacterium. Treatment 5L+BC4B provided complementary information either to assess the contribution of *C. zealandica* and *S. entomophila* in SOM degradation that occurred during the experiment.

It should be borne in mind that during this mesocosm experiment, some assumptions have been made concerning the assessment of *C. zealandica* and *S. entomophila* as decomposers: (a) larval death has not masked significantly the transformations of SOM induced by living larvae; (b) nonpathogenic and pathogenic strains of *S. entomophila* behave in an identical way apart from their effect on the insect; (c) the addition of nutrient broth with and without bacteria do not have significantly different properties after being altered by the metabolic activity of *S. entomophila*; (d) there were not significant differences in the amount of total C and N in SOM fractions in the soil from the studied sites at the beginning of the experiment.

A detailed presentation and discussion of the results about the effects of the insect, the bacteria and their interaction on SOM dynamics is made in the next Sections.

4.3.2.1 Effect of *C. zealandica* on plant residues decomposition

Results on the amount of C and N in plant residues are presented in Figures 4.2a and b respectively. Significant reductions ($P < 0.05$) in total C and N in this SOM fraction occurred both in S14 and A6 pasture in treatment 5L+H₂O in comparison with treatment 0L+H₂O. It has been observed that

third instars of *C. zealandica* can feed on plant residues during examinations in the microcosm experiment earlier described (Section 2.2.5 in Chapter II). Larval consumption of plant residues in this experiment may therefore be inferred as reductions in their content of total C and N or in the amount of plant residues DM. Nevertheless, comparisons between 0L+H₂O and 5L+H₂O treatments in A1 pasture where no larval background was found showed that this fraction of SOM has not suffered a significant ($P>0.05$) change. The mortality of living roots in treatments 0L+H₂O and 5L+H₂O was very similar (Section 3.3.3.6 in Chapter III). As a consequence, soil in treatment 0L+H₂O from the A1 pasture may have accumulated more plant residues than soil in treatment 5L+H₂O. This accumulation may have masked the larval consumption of plant residues.

Another indication of plant residues consumption by *C. zealandica* larvae is that the amount of total C and N in this SOM fraction was significantly ($P<0.05$) higher in treatment 5L+BC4B than in the remaining treatments (Figure 4.2a). This result suggests that inhibition of larval feeding caused by amber disease was the main factor involved in the accumulation of C and N in plant residues at this site. Indeed, in S14 pasture, a higher value of total C and N ($P<0.05$) has been observed in this SOM fraction in treatment 5L+BC4B in comparison with treatment 0L+H₂O. It is possible that the presence of healthy *C. zealandica* larvae in pasture S14 (see Section 3.3.1 and Figure 3.1 in Chapter III) accounted for this difference.

A further insight about the possibility that plant residues larval consumption occurred was suggested when DM values for this SOM fraction were compared (Table 4.2). Despite the lack of significance ($P>0.05$), the presence of healthy larvae has been associated with a reduction in the amount of plant residues in most experimental units from pastures A6 and S14. Except for A1 soil, a higher value in plant residues DM was observed in treatment 5L+BC4B than in treatment 5L+H₂O ($P<0.05$).

Results of the percentage of C and N in plant residues were inconsistent among sites and experimental units in treatments 0L+H₂O and 5L+H₂O (Table 4.2). In soil from A1 pasture, significant reductions in percentage of C and N were observed in treatment 5L+H₂O compared with treatment 0L+H₂O. No significant differences were observed in pastures A6 for the same variables, whereas in S14

Table 4.2

Mean value of plant residues DM obtained at the end of the mesocosm experiment in experimental units from the sites of study.

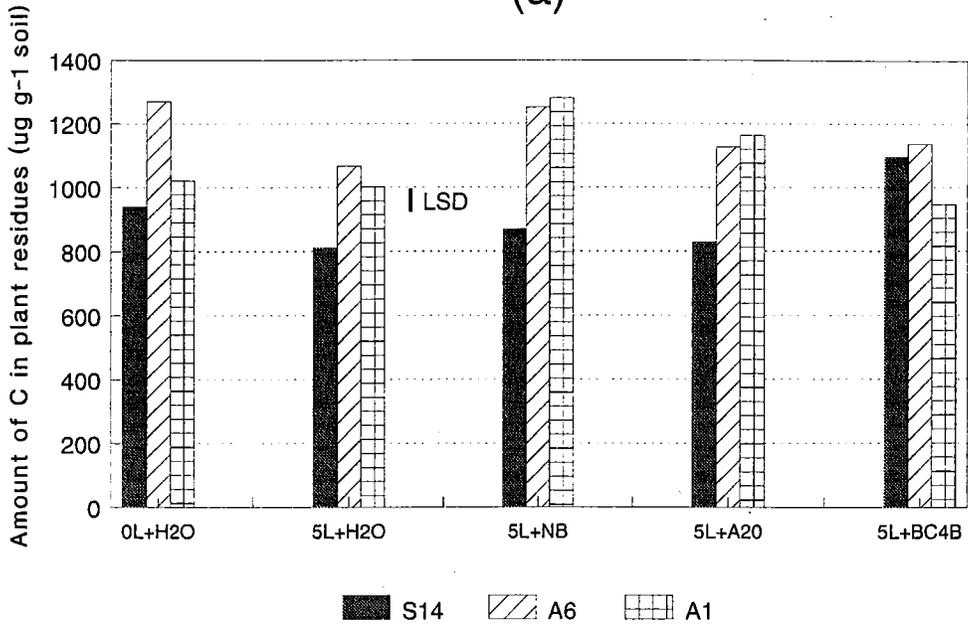
Treatments	Plant Residues (mg ¹ soil)		
	DM	%C	%N
S14			
0L+H ₂ O	2.84	33.04	1.45
5L+H ₂ O	2.15	37.71	1.49
5L+NB	2.53	34.37	1.34
5L+A20	2.24	36.90	1.53
5L+BC4B	3.31	33.10	1.46
A6			
0L+H ₂ O	4.15	30.59	1.38
5L+H ₂ O	3.47	30.73	1.34
5L+NB	3.38	37.01	1.64
5L+A20	2.30	34.18	1.57
5L+BC4B	3.02	37.63	1.76
A1			
0L+H ₂ O	3.24	31.52	1.28
5L+H ₂ O	3.98	25.16	1.02
5L+NB	3.55	36.05	1.54
5L+A20	3.31	35.15	1.46
5L+BC4B	2.67	35.47	1.44
LSD _(0.05)	1.31	1.30	0.10

S14=39-years-old pasture; A6=5-years-old pasture and A1=2-years-old pasture. For description of sites and treatments see Section 4.2.

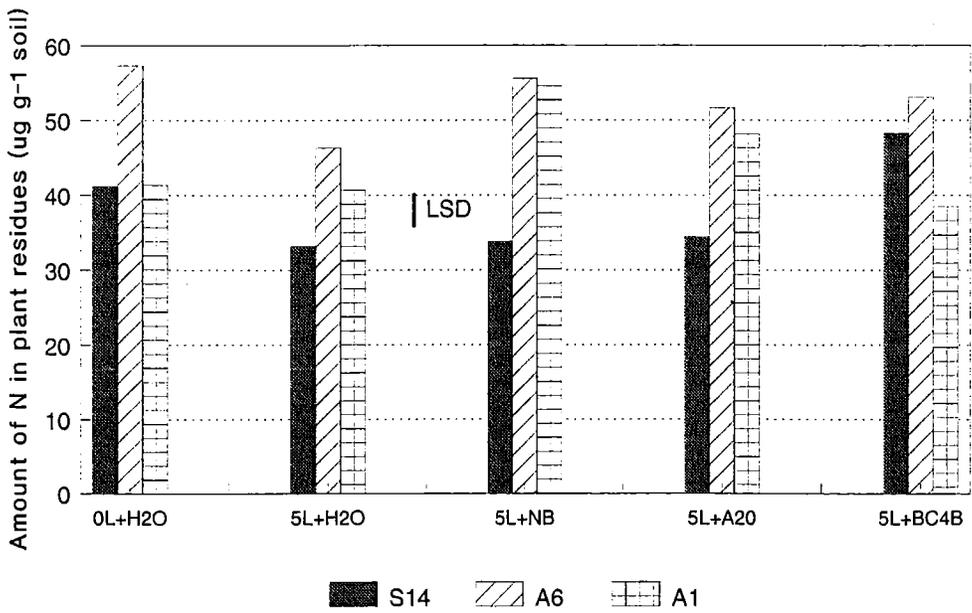
Figure 4.2

(a) Amount of total C in plant residues after the mesocosm experiment at Winchmore, (b) amount of total N in plant residues. $LSD_{(0.05)}$. For the explanation of treatments and sites see Section 4.2.

(a)



(b)



pasture a significant ($P < 0.05$) higher percentage of C was recorded in treatment 5L+H₂O than in treatment 0L+H₂O. Considering these results, *C. zealandica* larvae may be either increasing or reducing the proportion of C and N in plant residues. Increases occurred when the surrounding soil contained a high soil microbial biomass as in S14 pasture. Reductions in the percentage of C and N in plant residues were attributed to the presence of *C. zealandica* larvae in A1 pasture. Moreover, proportions of C and N in this SOM fraction may also be predetermined by the nature of the precursor living roots as in A6 pasture. Therefore, changes in percentage of C and N in plant residues seem to be mainly associated with the colonization by microbial communities. The presence of *C. zealandica* larvae may stimulate this process of colonization and decay of plant residues.

4.3.2.2 Effect of *S. entomophila* on plant residues decomposition

The available evidence suggests that, in the short-term, the degradation of plant residues caused by *S. entomophila* and other microorganisms was lower than the rate of larval consumption (Figure 4.2). In pasture S14, for example, the amount of C and N present in this SOM fraction in treatment 5L+A20 was not significantly ($P > 0.05$) different than in treatments 5L+NB and 5L+H₂O (Figure 4.2). In addition, at sites A6 and A1, the amount of C and N present in plant residues were significantly higher ($P < 0.05$) in treatment 5L+A20 than in treatment 5L+H₂O. These results suggest that the short-term direct effect of *S. entomophila* on plant residues decomposition is less important than that produced by the inclusion of the insect.

Evidence that *S. entomophila* contributed to the decomposition of plant residues in young pastures was provided by a significantly lower amount ($P < 0.05$) of C recorded in treatment 5L+A20 when compared with 5L+NB treatment. Similar results were observed for total N present in plant residues in A1 but not in A6 pasture.

Significant ($P < 0.05$) differences in the percentage of C and N in plant residues between treatment 5L+H₂O and any of those treatments in which either bacteria or nutrient broth were applied in young pastures have been observed (Table 4.2). This result may be related to the incorporation of C and N from

the nutrient broth and bacteria applied into plant residues. Either with nutrient broth or with bacterial inputs, an incorporation of C and N has been made with the application. In the case of the nutrient broth, C and N were incorporated in the form of raw sugar, yeast extract, urea, and NH_4NO_3 . In the case of the application of either pathogenic or non-pathogenic strains, C and N were incorporated through the addition of nutrient broth and bacterial cells. The two month period of the mesocosm experiment may have been too short a time to allow the incorporation of C and N applied in the inputs into biological tissues. In fact, Chanal and Warner (1965 in Stout et al., 1981) found that 25% of ^{14}C glucose was incorporated into SOM after three months and the remaining 75% was decomposed by microbial activity.

In soil from A1 there were no significant ($P>0.05$) differences in plant residues DM among treatments. If the rate of production of plant residues overcomes the rate of its consumption and decomposition, this effect may conceal differences. Results from Figure 3.5b (Section 3.3.6 in Chapter III) indicated that an important decrease in plant roots DM occurred in pasture A1. Moreover, a higher rhizophagous larval activity in soil from A1 with lower content of SOM may also promote higher root mortality and production of plant residues in comparison to those observed in S14 soil. Previous results (Sections 2.3.4 and 3.3.3.5 in Chapter II and III respectively) suggest that a low SOM content may increase significantly the intensity of larval root herbivory.

Consistently, the amount of C and N in plant residues was significantly lower ($P<0.05$) in S14 than in A1 and A6 pastures in most treatments. This effect may be mainly due to the higher clover dominance in the botanical composition of this pasture (Appendix II). However, this result suggest may also suggest that a higher rate of accumulation of C and N in plant residues occurred in the youngest pastures. Besides, a higher root mortality occurred in treatments in which larvae were included compared with that observed in treatments in which larvae were excluded (Figure 3.4b). In addition, a lower rate of decomposition was probably linked with the lower soil microbial biomass present in younger pastures than in the oldest pasture (Table 4.1). Overall, the concentration of N in plant residues from experimental units in A6 pasture was significantly ($P<0.05$) higher than that from A1 and S14 pasture.

4.3.2.3 Effect of *C. zealandica* and *S. entomophila* on total C and N in soil.

Differences in the total C and N in soil were not significant ($P>0.05$) among treatments in any pasture (Table 4.3). In all pastures, there was a significantly ($P<0.05$) higher content of C and N in stable fractions such as humins, humic and fulvic acids than in the more labile microbial biomass and water extractions (Figure 4.1). Therefore, total C and N values as an estimation of SOM content, were not suitable to detect short-term effects either of *C. zealandica* or *S. entomophila* on SOM dynamics under mesocosm conditions. As it has been discussed earlier (Section 2.3.4 in Chapter II), a more detailed fractionation scheme of SOM is required to understand better biotic interactions in soil.

4.3.2.4 Effect of *C. zealandica* in soil microbial biomass

The amount of total C present in soil microbial biomass was significantly higher ($P<0.05$) in treatment 5L+H₂O than in treatment 0L+H₂O in S14 pasture (Figure 4.3). This effect was less marked ($P>0.05$) in soils from young pastures. Under laboratory conditions, Yaacob (1967) found a stimulatory effect of *C. zealandica* larvae in soil microbial activity. Results from the present field experiment suggest that the presence of *C. zealandica* larvae may have increased soil microbial biomass and that the greater the soil microbial biomass in the soil environment, the more this effect is enhanced. The hindgut of soil melolonthid larvae harbour dense microbial populations comprising large numbers of both bacteria and protozoa (Bauchop and Clarke, 1975; Gupta and Rana, 1988). Although it has been suggested that these micro-organisms contribute significantly to larval nutrition, a number of these micro-organisms are released into the soil through faeces (Davidson and Roberts, 1968a). Soil invertebrates have been considered important as dispersal agents of microbial inoculum. In particular, the contribution of soil macroinvertebrates in the dispersal of vesicular-arbuscular mycorrhizal fungi within the soil profile has been suggested (Visser, 1985; Rabatin and Stinner, 1988). The contribution of *C. zealandica* larvae may not be very different from that attributed to earthworms in affecting the structure of soil microbial communities.

4.3.2.5 Effect of *S. entomophila* on soil microbial biomass

Contrary to expectations, soil from treatments 5L+A20 and 5L+BC4B yielded significantly lower ($P<0.05$) soil microbial biomass C and N than soil from treatment 5L+H₂O (Figure 4.3 and Table 4.3). A

Table 4.3

Amount of total C and N (mg g⁻¹ soil) present in different SOM fractions at the end of the mesocosm experiment.

Treatments	SB		CW		HW		HA		FA		HU		DC	
	C	N	C	N	C	N	C	N	C	N	C	N	C	N
S14														
0L+H ₂ O	0.76	0.11	0.47	0.13	3.23	0.41	5.02	0.47	6.70	0.70	21.40	1.96	40.84	3.93
5L+H ₂ O	1.64	0.23	0.42	0.13	3.30	0.37	4.89	0.46	7.35	0.76	24.79	2.27	39.86	3.97
5L+NB	0.76	0.11	0.38	0.11	3.50	0.44	5.26	0.51	7.91	0.79	24.21	2.29	41.35	3.98
5L+A20	0.98	0.14	0.40	0.18	3.33	0.33	4.73	0.46	7.49	0.75	23.29	2.15	39.99	3.84
5L+BC4B	1.04	0.15	0.47	0.13	3.47	0.41	4.70	0.45	8.37	0.87	23.55	2.21	40.70	3.92
A6														
0L+H ₂ O	0.43	0.06	0.38	0.12	1.64	0.18	3.58	0.31	6.01	0.55	25.63	2.25	27.97	2.51
5L+H ₂ O	0.56	0.08	0.28	0.06	1.46	0.17	3.24	0.27	5.39	0.50	24.32	2.17	27.75	2.48
5L+NB	0.65	0.09	0.35	0.08	1.67	0.22	3.34	0.30	5.57	0.51	18.17	1.65	25.27	2.41
5L+A20	0.50	0.07	0.43	0.17	1.47	0.15	3.10	0.27	4.65	0.41	19.42	1.72	26.15	2.46
5L+BC4B	0.61	0.09	0.37	0.11	2.00	0.22	3.17	0.28	5.16	0.49	25.11	2.22	26.08	2.50
A1														
0L+H ₂ O	0.48	0.07	0.28	0.08	2.08	0.21	3.65	0.34	5.02	0.47	26.72	2.31	26.41	2.54
5L+H ₂ O	0.67	0.10	0.36	0.09	1.86	0.18	3.90	0.37	6.14	0.62	18.13	1.61	27.29	2.66
5L+NB	0.51	0.07	0.25	0.06	1.62	0.19	3.73	0.35	6.47	0.63	25.52	2.17	28.21	2.75
5L+A20	0.50	0.07	0.34	0.08	1.85	0.18	3.55	0.32	6.87	0.66	18.99	1.69	27.89	2.69
5L+BC4B	0.34	0.05	0.44	0.11	1.70	0.19	3.63	0.33	5.82	0.47	21.57	1.96	26.65	2.61
LSD _(0.05)	0.36	0.05	0.05	0.01	0.22	0.04	0.06	0.01	1.01	0.09	0.80	0.07	2.41	0.22

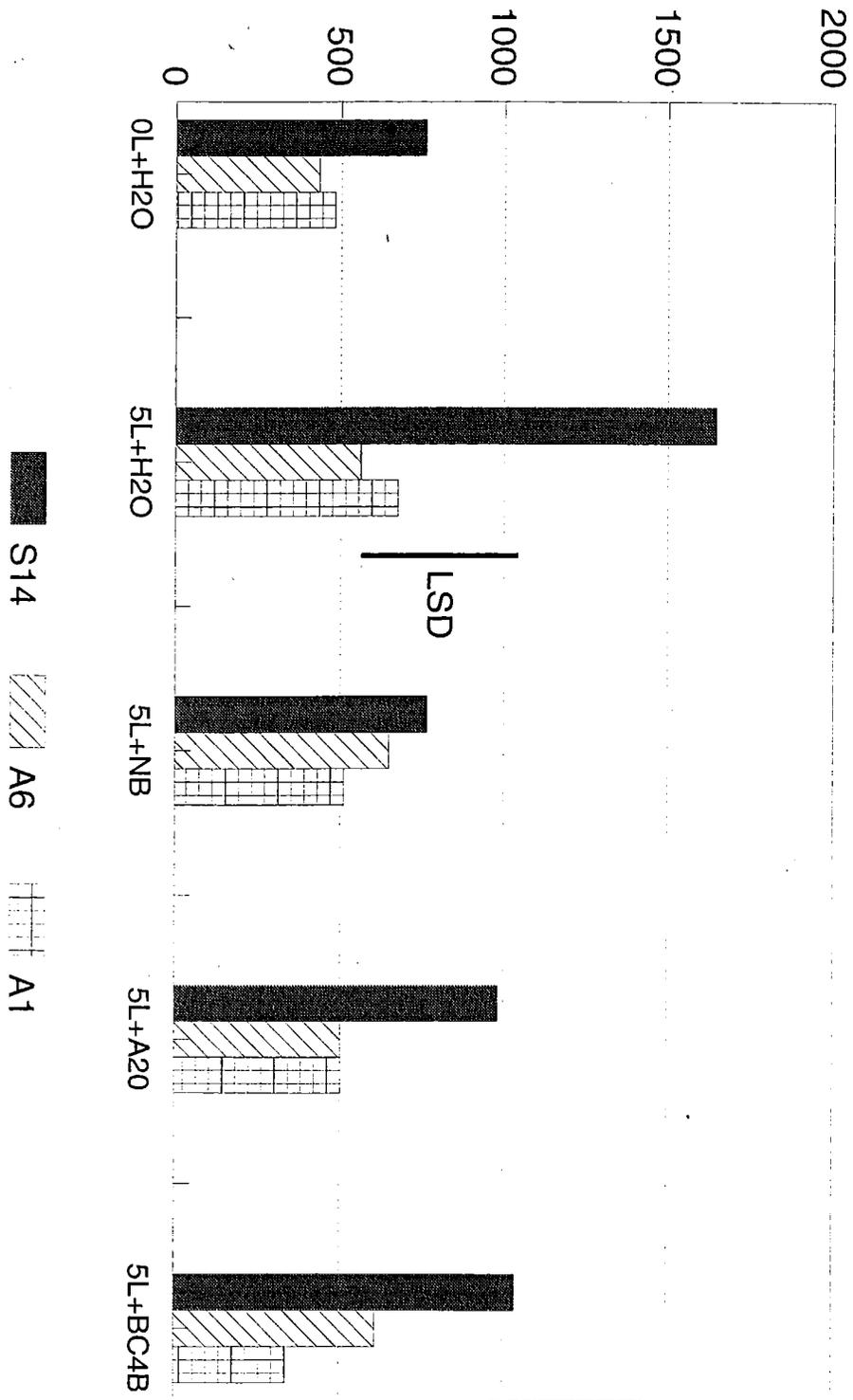
SB=soil microbial biomass, CW=cold water extraction, HW=hot water extraction, HA=humic acids, FA=fulvic acids, HM=humins,

DC=Dumas combustion. For explanation of other abbreviations see Table 4.2.

Figure 4.3

Soil microbial biomass C recorded after the mesocosm experiment at Winchmore. $LSD_{(0.05)}$. For the explanation of treatments and sites see Section 4.2.

Total soil biomass C (ug g⁻¹ soil)



possible explanation for this result is that the applications of *S. entomophila* in this experiment produced an inhibitory effect on other soil microbial populations. This statement is supported by the contention that, in pasture S14, a lower incidence of other entomopathogens was observed in larvae from treatments 5L+BC4B and 5L+A20 than in treatments 5L+NB, 5L+H₂O and OL+H₂O (Figure 3.1a). Although differences among treatments and soils were not significant the background of other diseases was higher in soil from S14 pasture where a higher build up of entomopathogens and soil microbial biomass was observed than in A1 and A6 pastures (Sections 2.3.1, 2.3.3 in Chapter II and Section 3.3.1 in Chapter III).

The possibility of antagonistic soil microbial interactions under the conditions imposed by inundative applications of *S. entomophila* must be considered. Competition and antibiosis are regarded as the most common antagonistic interactions among soil microorganisms which affect most directly the composition of a microbial community (Kas, 1966 in Vancura and Kunk, 1988). On the other hand, constraints on the growth of *S. entomophila* in soil has partially been attributed to competition with other micro-organisms (O'Callaghan, 1989). More research is required to confirm these results. This information will be useful in the management of indigenous soil biota and at the same time will help to optimise biological control measures.

4.3.2.6 Effect of *C. zealandica* in nonhumic substances

A significant ($P < 0.05$) reduction in the amount of total C in cold water extraction was observed in S14 and A6 pastures in treatment 5L+H₂O compared with treatment 0L+H₂O (Table 4.3). In A1 pasture, a significantly ($P < 0.05$) higher level of cold water extractable C was found in treatment 0L+H₂O than in treatment 5L+H₂O. No significant ($P > 0.05$) differences were observed in the amount of C present in hot water extractions between treatments 0L+H₂O and 5L+H₂O in S14 and A6 soils.

The inconsistency of these results is probably due to the interplay of several factors that are dynamically forming or degrading this fraction of the SOM. For example, immobilization by *C. zealandica* larvae of nutrients taken from this fraction of the SOM is likely to occur. Elements are immobilized in the insect body and this could be a biological mechanism which prevents leaching. Yaacob (1967) suggests that

C. zealandica larvae are selective feeders on the N rich organic matter in the soil and this selective feeding accounts for the fact that casts contain higher total and mineral N than the adjacent soil. The amount of N immobilized by *C. zealandica* larvae is about 1.2% of the live weight (Yaacob, 1967). In addition, the production and release of light molecular weight organic compounds by the insect catabolism may increase the amount of C and N in nonhumic substances. It is known that, through its catabolic activity, *C. zealandica* release N into the soil. Yaacob (1967) states that *C. zealandica* larvae influence this process in two steps: (a) by producing ammonium-nitrogen in the animal gut (ammonification) and (b) by producing nitrate-nitrogen in droppings (nitrification). Moreover, the rates of root mortality and root decomposition may have also affected to different extent amount of total C and N in water extractions in treatments 0L+H₂O and 5L+H₂O.

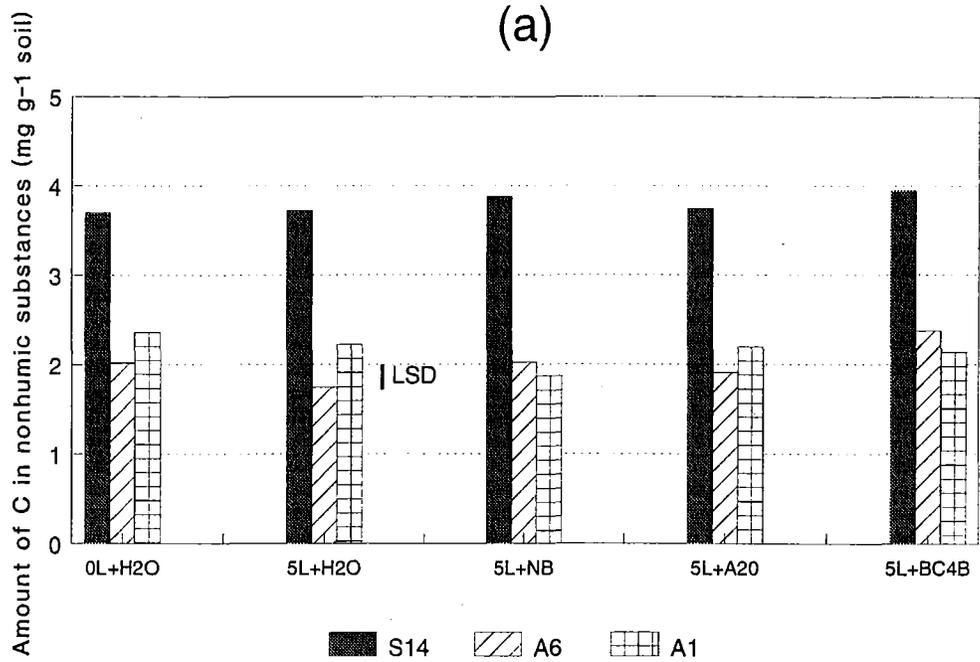
The amount of total C and N present in nonhumic substances (cold and hot water extractions combined) after the mesocosm experiment are presented in Figure 4.4. Despite the interplay of the factors described above, consistent reductions in total C and N in nonhumic substances were observed in treatments 5L+H₂O in comparison with treatment 0L+H₂O in soil from all sites (Figure 4.4). Another piece of evidence for the effect of *C. zealandica* in reducing C levels from nonhumic substances is that the amount of C in treatment 5L+BC4B was significantly higher than that of treatment 5L+A20 in A6 pasture. The reduction caused by *C. zealandica* larvae in the content of total C and N in nonhumic substances could become significant by extending the duration of future experiments.

Consistently higher values ($P < 0.05$) of C and N were recorded for nonhumic substances in soil from the experimental units at S14 in comparison with those at A6 and A1 pastures. These results suggest that the depletion of nonhumic substances in soil may increase the trend observed in *C. zealandica* larvae to attack living roots. In sites of low SOM status or where melolonthid larvae of *Rhopaea morbillosa* Blakburn and *Anoplognathus* spp feed for long periods and thus deplete soil organic matter, plant roots may be found to be a more important source of food (Davidson and Roberts, 1968a). As has been discussed earlier (Section 2.3.4 in Chapter II), depletion of nonhumic substances in the soil would partially explain the higher incidence of pasture damage caused by *C. zealandica* in young pastures (Section 2.3.5 in Chapter II). However, by promoting increases in this SOM fraction, larvae of *C.*

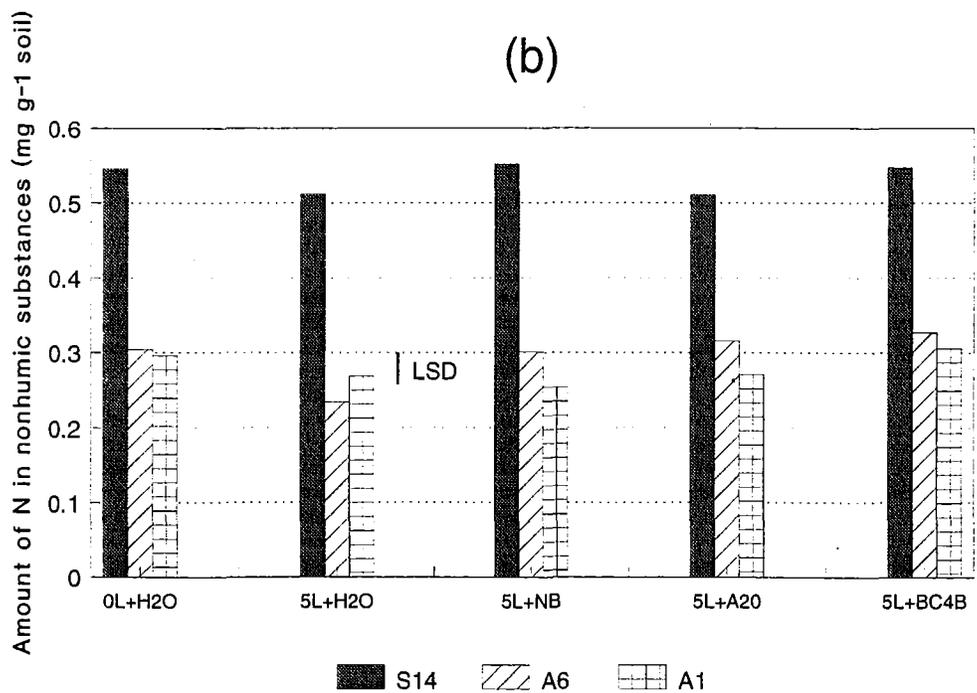
Figure 4.4

(a) Total C present in nonhumic substances after the mesocosm experiment at Winchmore. (b) Total N in nonhumic substances. $LSD_{(0,05)}$. For the explanation of treatments and sites see Section 4.2.

(a)



(b)



zealandica may contribute to its turnover and, therefore, to increase soil fertility. Therefore, the content of nonhumic substances in the soil could make the difference between the presence or absence of damage caused by *C. zealandica* larvae.

4.3.2.7 Effect of *S. entomophila* on nonhumic substances

Comparisons between treatments 5L+NB and 5L+A20 revealed that the presence of bacteria was associated with an increase ($P<0.05$) of total C and N in cold water extractions in all sites (Table 4.3). Only for total C present in this SOM fraction in soil from S14 pasture, non-significant ($P>0.05$) differences were observed. Conversely, the presence of *S. entomophila* reduced significantly ($P<0.05$) the amount of total C and N in hot water extractions (non-significant for C in A6 and S14 pasture and for total N in A1 pasture). The only exception in which an increase ($P<0.05$) in total C in hot water extraction was associated to the presence of *S. entomophila* was observed in A1 pasture.

Both cold and hot water extractions were combined in Figure 4.4b and represented as nonhumic substances. These results reveal a reduction ($P<0.05$) in total N in this SOM fraction in treatment 5L+A20 in comparison to treatment 5L+NB in site S14. However these differences were not significant in A6 and A1 pasture. This result suggests that the presence of *S. entomophila* promoted a reduction in the amount of N present in nonhumic substances only when a high SOM content was present. Decomposition of nonhumic substances by bacterial attack is more likely to occur than that of humic substances (Stevenson, 1982). Enzymatic degradation of proteins, polysaccharids and other ordered biopolymers that act as a receptive substrate is more likely than that of the disordered structure of humic substances (MacCarthy et al., 1990). The subproducts of this decomposition could then become available for plant roots or macro-invertebrates. As a consequence of bacterial activity, a depletion in C and N in nonhumic substances may be reasonably expected. A number of extracellular enzymes hydrolysing chitin, gelatin, and DNA are typical of the genus *Serratia* (Grimont et al., 1988). However, most information about the biochemical properties of this microorganism have been collected from tests made *in vitro*. *S. entomophila* has a free life stage in the soil and may use nonhumic substances as a source of C and N, contributing indirectly to their mineralization.

As discussed earlier (Section 3.3.4.7, Chapter III), a significant increase ($P < 0.05$) in the growth of living roots was associated to the decrease in total C and N from nonhumic substances in treatment 5L+A20 in S14 pasture. This result matches with the observed reduction in C and N in nonhumic substances. The fact that this result was neither observed in young pastures or in the level of total C in all sites, may be related to the higher rate of accumulation and lower rate of decomposition of nonhumic substances than in the oldest pasture.

4.3.2.8 Effect of *C. zealandica* on humic substances

The quantities of total C and N present in different fractions of humic substances (humic acids, fulvic acids and humins) are presented in Table 4.3. Comparisons of the total C and N in humic acids between treatments 0L+H₂O and 5L+H₂O in A6 and A1 pastures show that the insect have either promoted a reduction ($P < 0.05$) in pasture A6 or an increase ($P < 0.05$) in pasture A1. No significant differences ($P > 0.05$) were observed for the total C and N in humic acids between these treatments in pasture S14. Even though humic substances are considered to be stable components of SOM, evidence suggests that they also may suffer a quick SOM turnover. Marked changes in the structure of preparations of humic acids were observed after only two weeks of incubation with a bacterial culture (Gordienko and Kunk, 1984 in Tesarova, 1988). Presence of *C. zealandica* larvae may induce these changes through dispersal of microorganisms. A similar pattern to that observed for humic acids was found for total C and N in fulvic acids between the same treatments. Microbial breakdown may also release C and N from fulvic acids in the short-term. The consumption of oxygen for the oxidation of fulvic acids in a soil sample was observed immediately after their addition to the soil (Kunk et al., 1976 in Tesarova, 1988). Moreover, the presence of *C. zealandica* larvae was associated with a significant reduction ($P < 0.05$) in the content of N present in humins in A1 soil. The opposite pattern, however, was observed in S14 pasture. No significant differences were observed for N in this SOM fraction between the same treatments in A6 pasture. This release of N from humins when larvae were included in the soil from A1 pasture may have been induced by the competitive interactions of the soil biota.

Results from humic acids, fulvic acids, and humins were combined and presented in Figures 4.5a

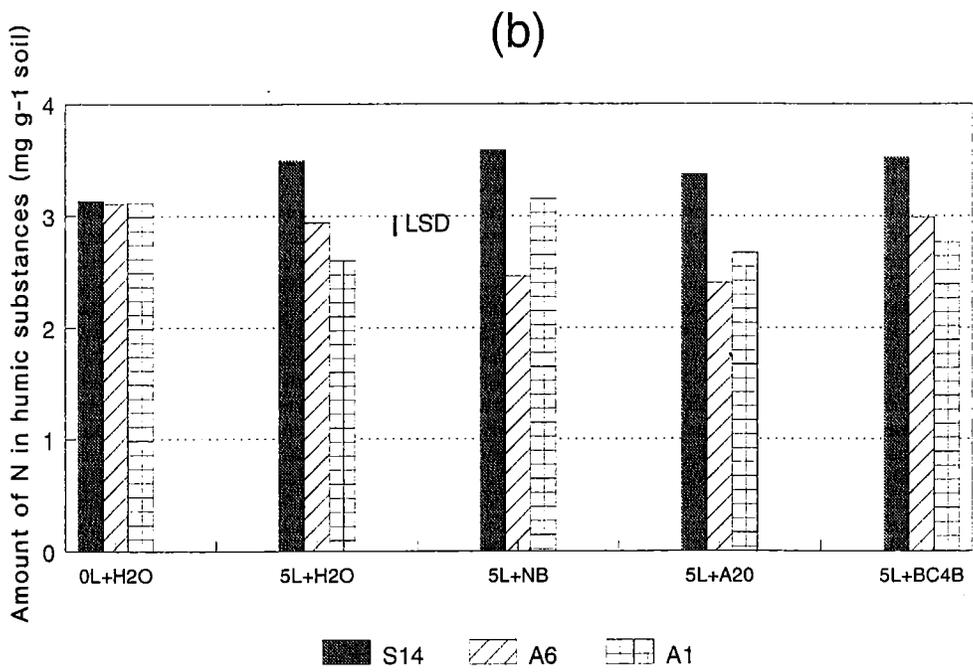
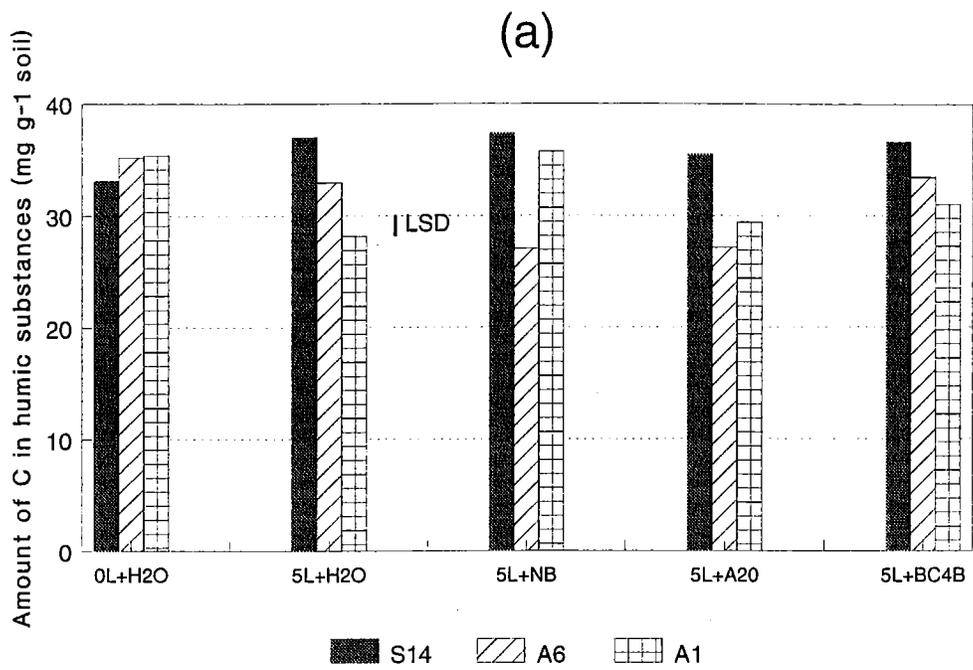
and 4.5b to observe general trends or changes in stable SOM fractions. Apparently, in experimental units from A1 and A6 the presence of the larvae induced a reduction in the amount of total C and N present in humic substances (Figure 4.5a and b). The opposite pattern, however, was observed in soil from S14. Results from the mesocosm experiment suggest that *C. zealandica* larvae are unable to make direct nutritional use of the C and N present in humic substances (Section 2.3.5 in Chapter II). This release was therefore, probably more related with the indirect effects associated with the presence of the insect described in Section 4.3.2.

On the other hand, it has been recognized that soil fauna may produce humic and fulvic acids. Through its activity, the soil biota produces organic polymers that bind the clay particles into domains and then domains into micro-aggregates (Tisdall and Oades, 1982 in Lal, 1991). Earthworms and *C. zealandica* larvae were the main macroinvertebrates present in the experimental units. No distinction between the alteration of humic substances promoted by these soil animals was made from the present experiment.

Although significant differences were observed in the total C and N in humic substances among treatments and soils, interpretation of their biological meaning is an untractable problem. Because there was no consistency between results from treatments 0L+H₂O and 5L+H₂O and among soils, a simple biological explanation cannot be provided. Moreover, many uncontrolled variables that affect the formation and degradation of these compounds were involved during this experiment. Therefore, no clear cut conclusion can be drawn about the effect of *C. zealandica* on humic substances during the mesocosm experiment. A microcosm experiment is strongly recommended to reduce environmental variability and to corroborate the validity of field results. According to MacCarthy et al., (1990), the multicomponent and random character of humic substances imposes severe restrictions on the ability to interpret experimental data measured on these materials. The opinion of these authors that an appreciation of this fact would help to minimize the frequency of over-interpretation of data has been taken into account in this study.

Figure 4.5

(a) Total C present in humic substances after the mesocosm experiment at Winchmore. (b) Total N in humic substances. $LSD_{(0.05)}$. For the explanation of treatments and sites see Section 4.2.



4.3.2.9 Effect of *S. entomophila* on humic substances

Amounts of total C and N in humic acids at the end of the mesocosm experiment in treatments 5L+NB were significantly ($P<0.05$) higher than in treatments 5L+A20 and 5L+BC4B in all soils (Table 4.3). This reduction suggests that the application of *S. entomophila* had an effect by itself in degrading this fraction of the SOM. Results obtained for total C in fulvic acids showed no significant differences ($P>0.05$) among the same treatments for all soils. However, in A1 soil where no larval background was found, a significant interaction for the level of N in fulvic acids ($P<0.05$) was observed. The highest value was observed in treatment 5L+A20 and the lowest in treatments OL+H₂O and 5L+BC4B, indicating that the interaction of larvae actively feeding and the application of *S. entomophila* promoted a greater allocation of N in this SOM fraction. Results of the amount of N present in humins in A1 soil, from treatment 5L+NB compared with the 5L+A20 and 5L+BC4B treatments show that both the bacteria and the insect may have a synergistic effect on promoting the release of N from this SOM fraction. However results from A6 pasture, where a low larval background was found, showed a significant ($P<0.05$) increase in the level of total C and N in humic substances in treatment 5L+BC4B compared with treatments 5L+A20 and 5L+NB.

Changes in total C and N present in humic substances promoted by *S. entomophila* (differences between treatments 5L+NB and treatment 5L+A20) were not consistent among soils. A significant ($P<0.05$) reduction of 4.8-10.1% in total C and of 8.6-10% of N was attributed to *S. entomophila* activity in all soils. Mostly non-significant increases or decreases in C and N present in fulvic acids were recorded. The bacteria had a effect ($P<0.05$) in degrading 3.8% of C and 6.1% of N from humins in S14 pasture whilst reductions ($P<0.05$) of 25.6% on C and 22.1% in N occurred for the same fraction in A1 pasture. Increases ($P<0.05$) of 6.4% of C and 4.1% N were observed in humins in A6 pasture. The extent to which humic substances are utilized by microorganisms is closely associated with their quality and structure. About 10-45% of fulvic acid C and 12-38% of humic acid C was utilized by soil micromycetes within two months in an experiment conducted in a soil from Europe (Dubovska and Macor in Tesarova, 1988).

Total C and N values from humic acids, fulvic acids and humins were combined in Figure 4.5b. These results also suggest that *S. entomophila* contributed to the release of C and N from humic substances. A significant decrease in the content of C and N in humic substances occurred in soil from A1 and, to a lesser extent, in treatments 5L+A20 and 5L+BC4B compared with treatment 5L+NB in S14 soil. However, in treatment 5L+BC4B, a higher ($P<0.05$) content of C and N was observed in comparison to treatments 5L+A20 and 5L+NB in A6 pasture. Another inconsistency is that in treatment 5L+H₂O in A1 soil, a lower content of C and N was observed than in treatment 5L+NB. Soil micro-organisms have a bidirectional effect on humic substances, either in their formation or in the slow release of C, N, P and S that follows their decay. Swift and Posner (1972, in Haynes, 1986) stated that during humus degradation high molecular weight units of humic acids were broken down by microbial attack and oxidation to form smaller molecules, with a preferential loss of nitrogenous material. On the other hand, the role of microbial activity in the synthesis of polyphenols, a common constituent of humic acids, was diagrammatically represented by Stout et al., (1981).

It is well known that humic substances are more resistant to microbial decomposition than nonhumic substances (MacCarthy et al., 1990). However, even though effects are low and inconsistent, net direct or indirect transformations by *S. entomophila* on either humic substances formation or degradation under the experimental conditions were significant.

4.3.3 Correlations between *C. zealandica* larval growth and SOM fractions

Correlation coefficients were calculated for the growth of healthy *C. zealandica* larvae with all SOM fractions measured after the mesocosm experiment (Table 4.4). More significant ($P<0.05$) positive correlations were observed between larval growth and the amount of total C and N present in plant residues than between larval growth and living root DM or their content of total C and N (Section 3.3.6 in Chapter III). This result is a strong indication of the important role of plant residues as a source of C and N for *C. zealandica* larval nutrition under field conditions. After dissections of the foregut of the soil melolonthid larva *Popillia japonica* (Newm.), Smith and Hadley (1926) found that the material eaten was composed of small soil particles, fresh plant tissue and small pieces of plants which were partially

Table 4.4

Correlation coefficients (r) for growth of healthy¹ third instar larvae of *C. zealandica* and SOM variables recorded after the mesocosm experiment².

	Carbon	Nitrogen
Amount in plant residues ³	0.93(**)	0.95(**)
Plant residues (%)	-0.67(ns)	-0.47(ns)
Soil microbial biomass	-0.35(ns)	-0.35(ns)
Cold water extraction	-0.96(**)	-0.93(**)
Hot water extraction	-0.93(**)	-0.88(*)
Humic acids	-0.96(**)	-0.95(**)
Fulvic acids	-0.95(**)	-0.94(**)
Humins	-0.20(ns)	-0.28(ns)
Dumas combustion	-0.88(*)	-0.94(**)

¹Combined results from healthy larvae marked and healthy larvae present in the background.

²Results from treatment 5L+H2O in the three sites of study

³Corrected for soil bulk density (n=12).

* = $P < 0.05$; ** = $P < 0.01$ (ns)=non-significant difference.

decomposed. According to these authors organic materials (mainly living and dead root particles) constitutes about 67% by weight of the total material consumed by these larvae. As is the case in *C. zealandica* (Miller pers. comm. in Sutherland, 1971) and other soil-dwelling melolonthids such as *P. japonica* (Smith and Hadley, 1926), and *Macrodactylus mexicanus* (Burm.) (Villalobos, 1992), larvae can complete their whole life cycle in soil devoid of living roots. The role that plant residues play in the nutrition of melolonthid larvae may be critical under these conditions. Bauchop and Clarke (1975) suggest that the low number of xylan- and pectin-utilizing bacteria isolated from the gut of *C. zealandica*, and the absence of demonstrable cellulose utilization indicate that structural carbohydrates are not the main source of nutrition for this insect. The nutritional value of this SOM fraction may be enriched once microbial colonization and decomposition activity take place. Feeding by melolonthid larvae on decaying parts of living roots could also induce root renewal.

Highly negative correlations were recorded between larval growth and total C and N of cold and hot water extractions, humic and fulvic acids and soil total C and N (Table 4.4). These results suggest that: (a) a trend to feed on living roots in soils with low content of SOM produced a positive effect on larval nutrition in *C. zealandica*. Smith and Hadley (1926) conclude that the presence of roots is extremely important for the development of *P. japonica* larvae and this seem to be the case also for *C. zealandica*; (b) high SOM values may increase the probability of infection by entomopathogens which may reduce larval performance. However, non-significant ($P>0.05$) negative correlations were observed between larval growth and the total amount of C and N present in soil microbial biomass and humins. Contradictory results have been found by Davidson and Roberts (1968a) in Australia. These authors found that the addition of manure to soil greatly increased live weight gains of melolonthid larvae and suggested that live weight might be related to microbial numbers in the soil. Because soil microbial communities are highly diverse and complex, more research is needed to identify micro-organisms that are critically related with larval nutrition. Moreover, differences between a natural build up of SOM and human-induced incorporation of manure into the soil have not been clearly identified and may be reason for this contradiction.

Larval mortality caused by entomopathogens was higher under the conditions of the present

experiment in soil from S14 than from A6 and A1 pasture (Table 3.1 and Section 3.3.1 in Chapter III). Therefore, measurements of soil microbial biomass by the fumigation-extraction method are not sufficiently reliable to estimate the importance of micro-organisms in larval nutrition and the load of entomopathogens of *C. zealandica* in the soil.

Although plant damage seems to be mainly related with the physical injury on sensitive parts of living roots (Section 3.3.3.5 in Chapter III), the idea that SOM depletion may increase the probability of incidence of plant damage should also be considered. When plant residues and other sources of nonhumic substances that are readily available in the soil become scarce, the trend to increase larval herbivory may also increase until the symptoms of damage become visible.

4.4 Conclusions

The pattern of SOM abundance and distribution in the sites of study during the mesocosm experiment confirm the results of the previously reported microcosm experiment (Section 2.3.1 in Chapter II). Significant differences were observed in amounts of total C and N in most SOM fractions between the old and the younger pastures. The effect of previous cropping rotations on SOM between young pastures probably promoted a significant reduction in the total N present in cold water extractions and an increase in the amount of C in humic acids.

Differences in the amount of C and N in plant residues among experimental units in the mesocosm experiment suggest that this fraction of the SOM represents an important component of the diet of *C. zealandica* third instars. Multiple correlations strongly suggest that this component of SOM is an important source of C for larval growth. Consumption of plant residues by *C. zealandica* larvae contributed to mineralization of this SOM fraction under the conditions of the mesocosm experiment.

Under mesocosm conditions, the presence of *C. zealandica* larvae increased the amount of total C and N present in soil microbial biomass. This appeared to be a synergistic effect between microbial biomass present in the soil and the ability of the insect in dispersing and stimulating soil microbial communities.

Non-significant ($P>0.05$) correlations were observed between growth of healthy third instars *C. zealandica* and soil microbial biomass. Significant negative correlations were observed between larval growth and most of the SOM fractions considered in this study. These results may be related either to a trend to feed less on living roots or a high probability to become infected by entomopathogens in soils with high natural build up of SOM.

The application of *S. entomophila* inhibited soil microbial biomass and may reduce the effect of other larval entomopathogens. Under the conditions imposed by inundative application of *S. entomophila* to the soil in the field, microbial antagonism may be enhanced and inhibitions of either natural populations of *S. entomophila* or indigenous microflora may occur. A better understanding of the microbial interactions in the soil may lead to the optimization of *S. entomophila* as a biocontrol agent.

Results from the present study suggests that intake of C and particularly of N from nonhumic substances by *C. zealandica* larvae occurred under field conditions. However, complexity in the dynamics of this SOM fraction that takes place under field conditions, precludes any conclusive statement about this phenomenon. Depletion of C and N in nonhumic substances in the soil may increase the probability of *C. zealandica* attack on living roots.

The results also suggest that *S. entomophila* inundative applications may encourage degradation of nonhumic substances. This may be one of the reasons why herbage production increases in pastures treated with this bacterium. A general trend was observed in which the presence of *S. entomophila* significantly increased total C and N from cold water extractions and decreased total C and N from hot water extractions.

In the short-term, the presence of *C. zealandica* larvae may either induce significant increases or reductions in amount of C and N allocated in soil humic substances. Significant reductions occurred in total C and N present in humins in young pastures and significant increases were observed in the old pasture. However, the complexity of these organic compounds and the multiple interactions that occurred during the mesocosm experiment impose restrictions on the interpretations derived from these results.

Overall, the application of *S. entomophila* promoted a significant release of C and a dramatic release of N in humins under low SOM conditions.

5) EFFECT OF THE APPLICATION OF COTTAGE CHEESE WHEY ON THE REDUCTION OF PLANT DAMAGE CAUSED BY *Costelytra zealandica* (White) LARVAE IN NEW ZEALAND PASTURES.

5.1 Introduction

Damage caused by *Costelytra zealandica* (White) was described in previous sections of this study (Section 3.1 and 3.3.6 in Chapter III). The beneficial role of soil organic matter (SOM) in reducing grass grub damage has also been pointed out earlier (Section 2.1 and 4.1 in Chapters II and IV). Applications of organic residues are being considered in New Zealand with the view to increasing the sustainability of the pastoral ecosystem (Blakeley, 1990).

According to Roberts et al. (1992) three factors are considered important for the successful utilization of wastes as soil amendments: (a) application rates should be balanced to the needs of the soil-plant system; (b) a regular chemical monitoring programme of the soil, plants and animals needs to be implemented, and (c) the economics of using wastes must be favourable to the agriculturalist and horticulturalist. Whey is a by-product liquid from the manufacture of cheese, casein and whey products and it seems to meet all of these requirements.

Whey and other N-containing wastes have previously been proposed as a substitute for fertilizers in pasture soils (Kell, 1992; Radford, 1992). According to Radford (1992) the major nutrients for pasture nutrition that were supplied by the recommended rate of 40,000 L lactic casein whey/ha are 56 kg N/ha (most N becomes slowly plant available from decomposing whey protein), 26 kg P/ha, 60 kg K/ha and 6 kg S/ha. Plant available P, K, and total N are more readily available from whey than from compost (Kell, 1992) and pasture yield was increased from 21-44% after the application of whey in comparison with control plots (Radford, 1992). Overall, whey has provided an excellent substitute for potassic superphosphate (Roberts et al., 1992).

Apparently, whey does not contain harmful levels of heavy metals or transmissible cattle diseases (Roberts et al., 1992). Most organic wastes produced in agriculture are characterized by being relatively bulky with a relative low nutrient content relative to conventional solid fertilizers (Roberts et al., 1992). Radford (1992) suggests that whey can be effectively stored with preservatives to prevent acidification and putrefaction and then later applied to pastures.

Radford (1992) considers that it is economically viable to apply a rate of 40,000 L ha⁻¹ year of Cheddar cheese whey to New Zealand pastures. The supply of major nutrients for pasture by this rate of application does not require additional fertilizer in dairy farms (Roberts et al., 1992). Radford (1992) states: "the New Zealand dairy industry has estimated whey volume as a fertilizer replacement was 270,437 m³ during the 1990/91 dairy season".

Additional advantages of the use of whey in pastoral agriculture are that it may be applied in combination with other farm practices such as irrigation or heavy rolling (Atkinson et al., 1992). Its use, instead of irrigation, could be a great advantage during dry periods in the Canterbury region (P. Cunningham, pers. comm.). Furthermore, no problems of soil wetness or surface soil stability are evident after whey applications (Roberts et al., 1992).

However, the possibility of underground water pollution by nitrate or the occurrence of ammonia volatilisation (Roberts et al., 1992) should be considered. Encouragement of phytopathogens in pastures is an aspect that has not been studied under New Zealand pastoral conditions. Clearly, further research on the potential harmful side effects of applying whey to pastures is necessary.

The nutrient composition of whey products varies depending on the manufacturing process generating the waste (Roberts et al., 1992). The whey used in the present study was a by-product in the Cottage cheese production.

The efficient management of *C. zealandica* larval populations and optimization of biological control measures are also important challenges for the Canterbury region. An attempt has been made to

compensate the damage caused by *C. zealandica* in New Zealand pastures using inorganic N fertilizers (Prestidge and East, 1984). Atkinson et al. (1992) have suggested that whey applications alone may reduce larval populations of *C. zealandica* by 62%, and up to 92% in combination with heavy rolling.

The idea of transforming the liability of disposal of animal wastes into an asset of increasing and promoting the conservation of soil fertility has been the driving force of the present study.

The main objectives of the study were to:

- (a) Assess the effect of whey applications on the growth and health status of *C. zealandica* larvae.
- (b) Evaluate the effect of whey on the growth and infectivity of *S. entomophila*.
- (c) Measure the effect of *C. zealandica* larval herbivory on growth and performance of the above and below-ground parts of pasture plants after whey applications were made.
- (d) Determine the effect of whey applications on the build up of labile SOM fractions (cold and hot water extractions).

5.2 Materials and methods

Two approaches were used to evaluate the effect of Cottage cheese whey on the reduction of plant damage caused by *C. zealandica* larvae. These were defined as a laboratory microcosm and field mesocosm approaches (Crossley et al., 1990).

5.2.1 Laboratory microcosm approach

This approach allowed the assessment of the effect of the whey application on the growth of *S.*

entomophila in the soil and on the reduction of plant damage caused by *C. zealandica* larvae under a controlled soil environment. The response of the variables recorded were: (a) numbers of *S. entomophila* in soil; (b) ability of *S. entomophila* to produce amber disease; (c) performance of perennial ryegrass (*Lolium perenne* L.) seedlings and; (d) build up of the total C and N in labile SOM fractions.

5.2.1.1 Description of the microcosm experiment

Soil from a 6-year-old pasture (site A6 described in Section 2.2.1 in Chapter II) was collected in early April, 1993. A total of 288 plastic sterile screw-topped pots (9 cm height x 4.5 cm diameter) containing 60 g of oven dried soil (24 h at 80°C sieved through a 2 mm mesh screen) were used as experimental units (Plate 11). One week before the experiment, ca. 65 seeds of endophyte perennial ryegrass cv. "Nui" were sown in soil contained in these pots and incubated at 20°C and 16% soil moisture (51.4% of water holding capacity). Incubation conditions were very similar to those described for the previous microcosm experiment (Section 2.2.5 in Chapter II). In late March 1993, early third instar *C. zealandica* were collected from a pasture in the Lincoln area. These larvae were assessed for their feeding activity, weighed, and introduced individually into the experimental units as described earlier (Section 2.2.5 in Chapter II). Comparisons were made in the response of variables after testing four bacterial doses, the addition of whey or distilled water and the presence or absence of *C. zealandica* larvae. The experiment was set up following a randomized block design (4x2x2 factorial with 18 blocks of 16 treatments). Eighteen replicates were used for each of the following treatments:

(1) High dose BC4B + whey + larva. The high dose consisted of two ml of nutrient broth¹ containing 9.1×10^9 cells ml⁻¹ of the pathogenic strain of *S. entomophila* (BC4B). This solution was added to a pot (experimental unit) previously treated with 7 ml of Cottage cheese in which a third instar *C. zealandica* (L) was individually placed (treatment High+Whey+L).

¹The biochemical composition of the nutrient broth has been described in Section 2.2.5 (Chapter II).

Plate 11

Experimental units used for the microcosm experiment described in Section 5.2.1.1 in Chapter V.

Differences observed in the growth of ryegrass seedling after 20 days after applications of water (left) and whey (right) were made. This experimental units excluded *C. zealandica* larvae.



(2) Medium dose BC4B + whey + larva. Similar to (1) but a solution of 9.1×10^6 cells ml^{-1} of BC4B was added (Medium+Whey+L).

(3) Low dose BC4B + whey + larva. Similar to (1) but a solution of 9.1×10^3 cells ml^{-1} of BC4B was added (Low+Whey+L).

(4) Nil dose + whey + larva. Similar to (1) but 2 ml of a nutrient broth free of *S. entomophila* was added (Nil+Whey+L).

(5) High dose BC4B + water + larva. Similar to (1) but 7 ml of distilled water were added instead of whey (High+H₂O+L).

(6) Medium dose BC4B + water + larva. Similar to (5) but a solution of 9.1×10^6 cells ml^{-1} of BC4B was added (Medium+H₂O+L).

(7) Low dose BC4B + water + larva. Similar to (5) but a solution 9.1×10^3 cells ml^{-1} of BC4B was added (Low+H₂O+L).

(8) Nil dose BC4B + whey + larva. Similar to (5) but 2 ml of nutrient broth free of *S. entomophila* were added (treatment Nil+H₂O+L).

Treatments (9) to (16) were similar to treatments (1) to (8) respectively with the exception that larvae were not added to the experimental units.

During the incubation period of this experiment and after the application of either whey or water, soil moisture reached 28% (90% of water holding capacity). The top of each pot was loosely screwed onto to minimise soil moisture but also to allow the entrance of air. Each experimental unit was inspected once; half of them were inspected after 20 days and the remaining experimental units after 30 days. During each

inspection, records of amber disease symptoms and symptoms of other entomopathogens were microscopically determined following the criteria of Jackson et al. (1993, and pers. comm.). At the end of the experiment three further feeding assessments were made to confirm the health status of larvae. For simplicity, the health status of larvae was scored into four categories as defined below:

(1) Amber disease (AD). Larvae showing amber coloration and a none-existent or deficient feeding response during the feeding assessments.

(2) Other diseases (OD). Larvae showing obvious symptoms of entomopathogens other than *S. entomophila* and a non-existent or deficient feeding response.

(3) Dead (D). Larvae that were recovered as cadavers, or which death occurred for any cause during the feeding assessments. Marked larvae that were not recovered during the mesocosm experiment described below were also included in this category.

(4) Healthy (H). Larvae that showed minimal or no symptoms of any disease and were actively feeding during the feeding assessments.

Larval live-weights were also recorded. Estimations of the dry matter (DM 24 h at 80°C) of ryegrass herbage and below-ground plant tissues were made at the end of the experiment. Living roots and plant residues dry matter (DM) were measured as a single fraction by the modification of the washing-flotation method earlier described (Section 4.2 in Chapter IV). Total C and N in living roots-plant residues and herbage DM herbage were determined by Dumas combustion (Grewal et al., 1991). At the end of the experiment, cold and hot water SOM extractions were made as described in Section 2.2.4 (Chapter II) and Appendix I from pooled soil belonging to each treatment. Independent determinations were made from duplicates of bulked soil belonging to two groups of four experimental units examined after 20 days. Determinations of the total C and N from SOM extractions and living roots-plant residues were made from these duplicates following the techniques earlier described (Appendix I). The quantity of whey applied to the experimental units was approximately equivalent to the rate recommended by Radford (1992) for New

Zealand pastures. Fresh whey was collected from South Island Dairy Farmers (Christchurch) and stored at 4°C until use.

5.2.2 The field mesocosm approach

This approach allowed an evaluation of plant growth after the application of different treatments under field conditions. The effects of each treatment on the status of *C. zealandica* larvae and on the build up of labile SOM fractions were also evaluated through this approach.

5.2.2.1 Description of the field mesocosm experiment

Pastures S14 and A6 previously described (Section 2.2.1 in Chapter II) were selected for this experiment. Five replicates of experimental units similar to those described for the previous mesocosm experiment (Section 3.2.3 in Chapter III) were placed in the field. A randomized block design (5 blocks x 4 treatments x 2 pasture soils) was used to test the following treatments:

(1) Application of 35 ml of whey (a rate equivalent to that recommended by Radford, 1992) plus five larvae (WHEY+5L).

(2) Application of 35 ml whey without larvae (WHEY).

(3) Application of 35 ml of distilled water plus five larvae (H₂O+5L).

(4) Application of 35 ml of distilled water with no larvae (H₂O).

Experimental conditions, collection of experimental units at the end of the experiment and recording of data were similar to those earlier described (Section 3.2.3 in Chapter III). Feeding assessments were made to evaluate the larval health status using the methods described for the microcosm experiment (Section 5.2.1.1.). At the beginning of the experiment, five soil cores were taken to assess the botanical

composition of the pasture and comparisons were carried out with the composition observed at the end of the experiment. At the beginning and at the end of the experiment, measurements of total C and N were made for grass herbage DM and clover herbage DM by Dumas combustion (see Appendix I for details). At the end of the experiment SOM analysis were made to determine the effect of the treatment on the content of total C and N in cold and hot water extractions. Data were statistically analyzed as earlier described (Section 3.2.7 in Chapter III).

5.3 Results and discussions

5.3.1 Effect of whey on *C. zealandica* larval health status

Results from the microcosm experiment suggest that whey may have a significant ($P<0.05$) effect on increasing the level of amber disease produced by inundative bacterial applications. This effect was particularly significant ($P<0.05$) when comparisons between treatments Medium+Whey+L and Medium+H₂O+L were made (Figure 5.1). No larvae showed amber disease symptoms in the experimental units from Nil+Whey+L and Nil+H₂O+L treatments either at 20 or at 30 days. Similar percentages of healthy larvae were recorded in these two treatments. In treatment High+Whey+L evidence of other disease was not found. The Medium+Whey+L treatment reduced significantly ($P<0.05$) the number of healthy larvae and increased significantly ($P<0.05$) the number of larvae affected by amber disease (Figure 5.1).

These results suggest that amber disease may be enhanced if a moderate dose of the bacterium is combined with a pre-applied whey incorporation to the soil. As will be discussed further in section 5.3.2, this effect may be linked with the ability of BC4B to grow and survive better with the application of whey. This increase in bacterial numbers may also be inducing a higher probability of larval infection through the ingestion of a higher volume of soil by the larvae. Whether incorporation of whey has stimulated the larvae to ingest more soil or the bacterial growth has increased the probability of increasing amber disease remain to be explored.

At the end of the mesocosm experiment, when only marked early third instars were considered, it was observed a significant ($P<0.05$) interaction between whey and the level of SOM on reducing the number of healthy larvae (Figure 5.2). A significant difference ($P<0.05$) in the proportion of amber disease infected

Figure 5.1

Health status of *C. zealandica* early third instars recorded after the microcosm laboratory experiment. Data from inspections at 20 and 30 days were used as replicates. WH=whey+high dose; WM=whey+medium dose; WL=whey+low dose; WN=whey+nil dose; HH=H₂O+high dose; HM=H₂O+medium dose; HL=H₂O+low dose; HN=H₂O+nil dose. %AD=percentage of amber disease infected larvae; %OD=percentage of larvae affected by other diseases; %D=percentage of dead larvae; %H=percentage of healthy larva. LSD=least significant differences at 95%.

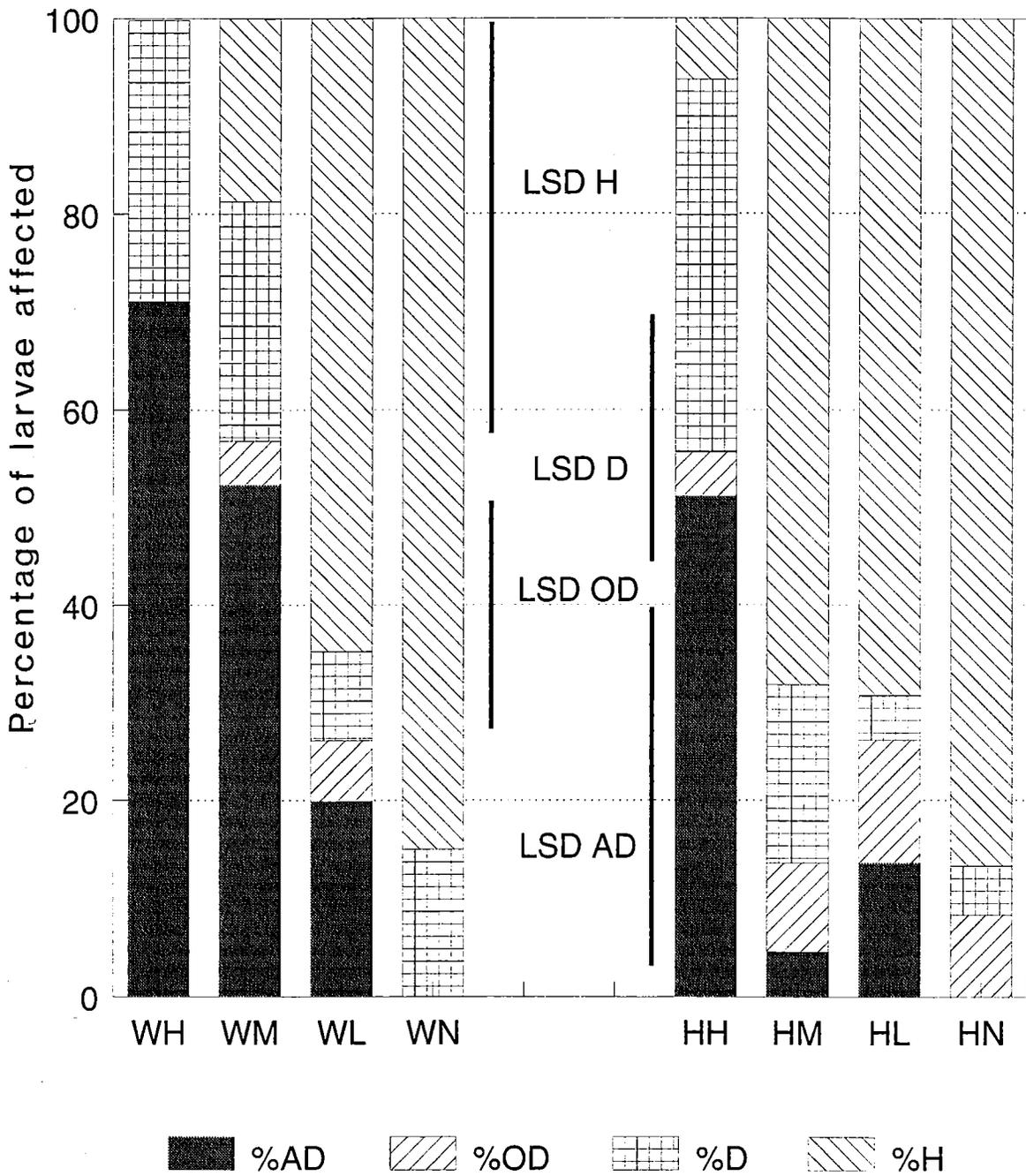
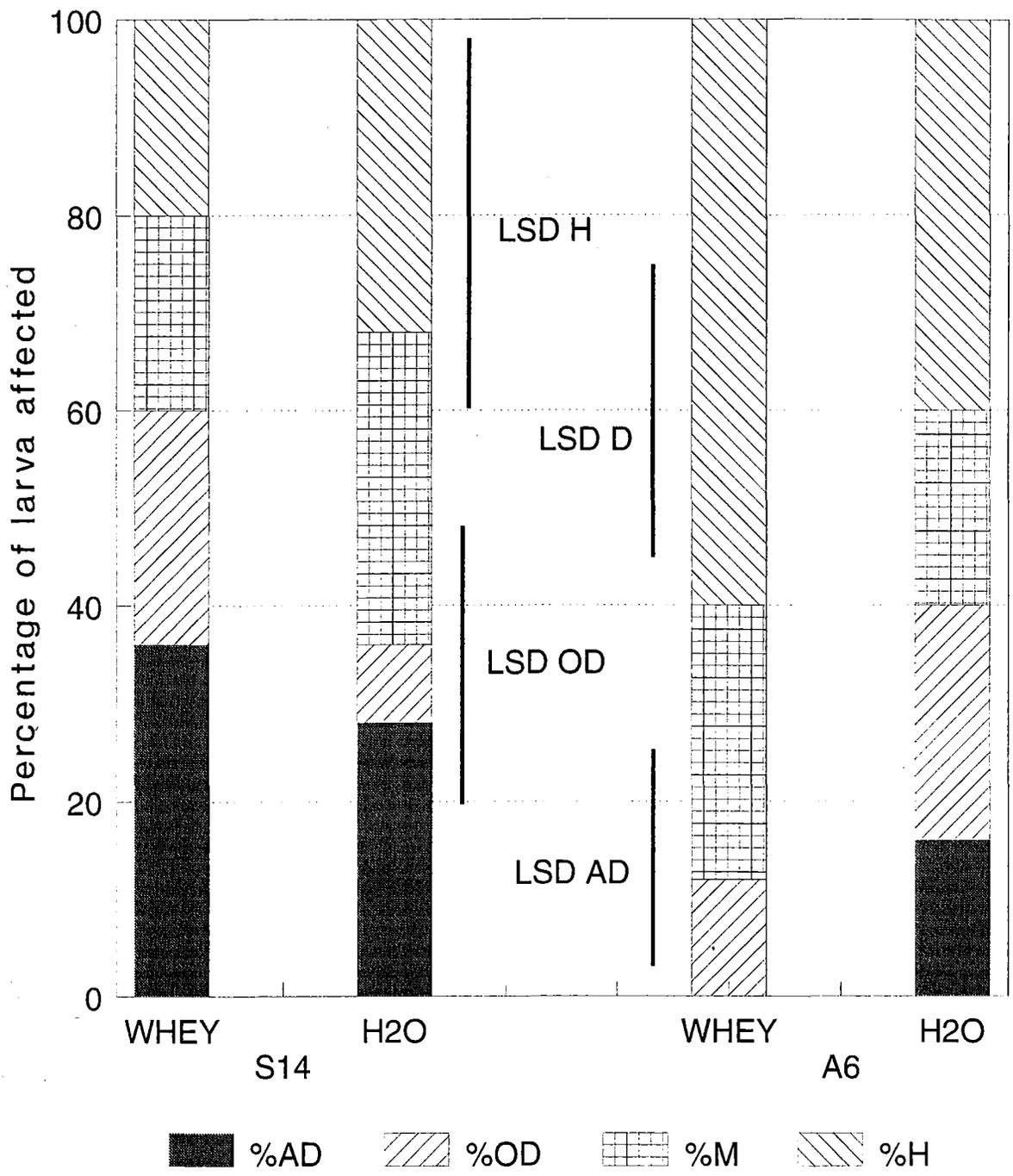


Figure 5.2

Health status of marked early third instar of *C. zealandica* recorded after the mesocosm field experiment.

WHEY=application of whey; H₂O=application of distilled water. S14=40-year-old pasture; A6=6-year-old-pasture. LSD=least significant differences at 95%.



larvae and healthy larvae occurred between soils from S14 and A6 pastures when whey was applied. As it has previously been shown (Sections 2.3.1 and 4.3.1 in Chapters II and IV respectively) soil from S14 pasture contained a significantly ($P<0.05$) higher content of SOM and a significantly ($P<0.05$) higher level of *S. entomophila* than soil from A6 pasture (Sections 2.3.2 and 3.3.2 in Chapters II and III respectively). These differences may account for the interaction observed.

An assumption was made that, at the beginning of the mesocosm experiment, larval density was not significantly ($P<0.05$) different for any health status category in the background soil among experimental units. As suggested by the data in Table 5.1, this assumption was fulfilled. When the total number of larvae (marked and not marked) was considered (Table 5.1), a significantly ($P<0.05$) higher number of larvae showed amber disease symptoms in the WHEY+5L treatment in comparison to the H₂O+5L treatment in S14 pasture. A significantly ($P<0.05$) higher number of healthy larvae was recorded in the experimental units receiving the WHEY+5L treatment in comparison to those from H₂O+5L treatment. However, in A6 soil, a significantly ($P<0.05$) higher density was recorded for dead larvae and for total larval density in experimental units treated with water in comparison to those treated with whey in S14 pasture but not in A6 pasture.

These results suggest that whey application can enhance the infectivity of soil-borne larval entomopathogens of *C. zealandica* during the early third instar. Third instar larvae are considered the main stage responsible for pasture damage during the autumn-winter period (Barratt et al., 1990). Provided the soil has a high background level of entomopathogens, the positive effect of whey in reducing *C. zealandica* numbers by itself may be beneficial. Even when a higher number of healthy larvae was found after the application of whey in the soil from A6 pasture, there were positive effects of whey on plant growth that may compensate larval herbivory. Prestidge and East (1984) found that applications of urea fertilizer to pasture soils compensated for *C. zealandica* damage, were more cost-effective than the use of insecticide and were unlikely to have detrimental effects on the natural regulation of larval populations. Atkinson et al., (1992) have suggested that the application of casein whey reduced *C. zealandica* numbers by lowering soil oxygen levels. However, these authors did not mention the effect that N inputs to the soil might have on the entomopathogenic activity against *C. zealandica*. According to these workers, a

Table 5.1

Mean densities (larvae m⁻²) of the total number of third instars of *C. zealandica* within the experimental units obtained with different treatments and in the background soil before and after the field mesocosm experiment.

Soil	Treatment	Amber disease	Other diseases	Dead	Healthy	Total
S14	WHEY+5L	331	127	153	153	764
	H ₂ O+5L	178	229	306	255	968
	WHEY	25	25	0	25	76
	H ₂ O	0	76	0	76	153
	Initial	51	178	0	76	306
	Final	51	153	25	76	306
A6	WHEY+5L	51	102	178	382	713
	H ₂ O+5L	102	204	127	255	687
	WHEY	25	25	0	25	76
	H ₂ O	0	51	0	0	51
	Initial	0	153	0	0	153
	Final	102	76	0	0	178
LSD _(0.05)		125	168	148	127	188

S14=40-year-old pasture; A6=6-year-old-pasture.

WHEY+5L= application of whey + 5 third instars *C. zealandica*;

H₂O+5L= application of distilled water + 5 third instars *C. zealandica*;

WHEY= application of whey only; H₂O=application of distilled water only.

LSD_(0.05)=least significant differences at 95%

Initial=larval background in late April 1993; Final=larval background in early June 1993

Means of 5 experimental units per treatment.

single application of whey to the prepupal stage reduced soil oxygen levels up to 4% and, as a consequence, the number of *C. zealandica* adults in the next mating season was reduced by 40% and by 64% when whey applications were combined with heavy rolling.

The results of this study suggest that the higher mortality enhanced by the application of whey was also associated with a higher rate of decomposition of larval dead bodies. As has been presented earlier (Sections 2.3.1 and 4.3.1 in Chapter II and IV respectively) significant ($P < 0.05$) differences were observed in soil microbial biomass between both pastures.

5.3.1.1 Effect of whey on larval growth²

Results from the microcosm experiment showed that there were significant ($P < 0.05$) differences in larval growth with the dose of *S. entomophila* applied (Figure 5.3a and b). After 30 days larvae from the experimental units treated with the high dose of *S. entomophila* suffered significant ($P < 0.05$) reductions in larval growth in comparison to those treated with bacteria-free nutrient broth (Figure 5.3b). The observation that *C. zealandica* larvae infected by amber disease lost weight has previously been made by Trought et al. (1981). This observation has also been confirmed in previous experiments (Sections 2.3.5 and 3.3.5 in Chapters II and III respectively).

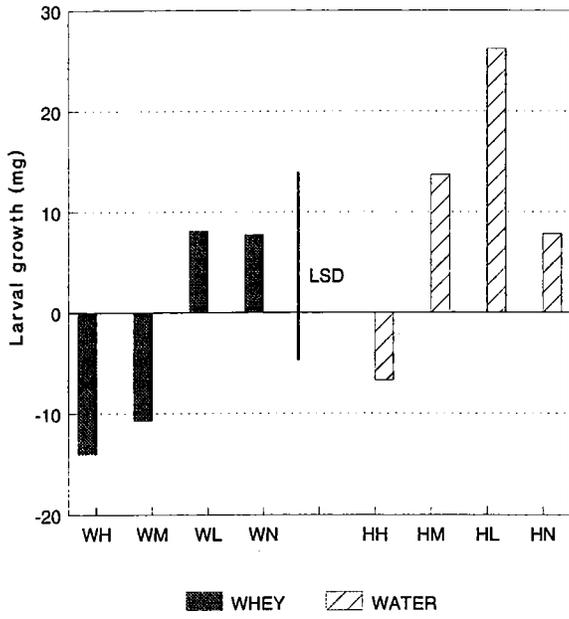
At the end of the mesocosm experiment, significant ($P < 0.05$) higher larval growth (34%) was recorded in marked larvae from A6 pasture (62 mg) in comparison to that observed at S14 pasture (41 mg). This result confirms previous observations (Sections 2.3.5 and 3.3.5 in Chapter II and III respectively) which suggest that larval growth is higher in the soil from A6 than from S14 pasture. According to Prestige and East (1984) there is no evidence that *C. zealandica* larvae are limited by the levels of N in the roots of pasture plants. These authors suggest that the lack of long-term effects of N fertilizers applications on *C. zealandica* larval populations reflects the ability of the insect to compensate for poor quality in the diet by increasing consumption rate. In a laboratory experiment carried out by these workers, similar

²Larval growth is defined here as the live weight gain of early third instar *C. zealandica* from the beginning to the end of an experiment.

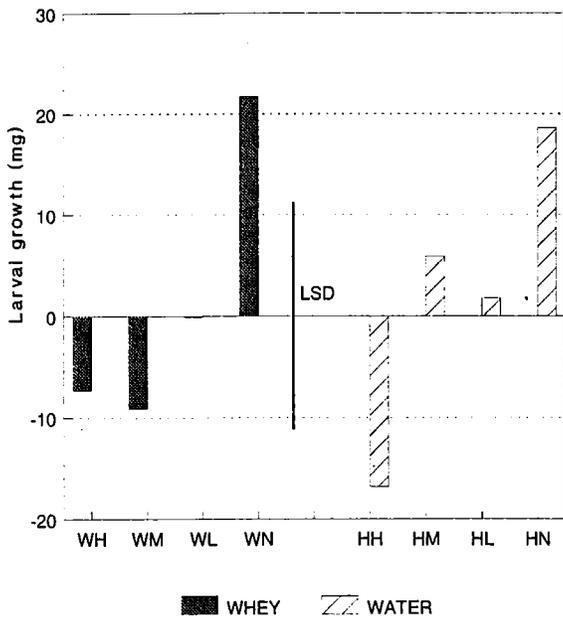
Figure 5.3

(a) Larval growth of early third instar *C. zealandica* after 20 days in the microcosm experiment in which different doses of *S. entomophila* were applied. (b) Results after 30 days (for a description of treatments see captions in Figure 5.1).

(a)



(b)



larval growth rates were recorded regardless of dietary quality. These workers have also observed that larvae ceased feeding and moved down the soil profile in readiness for pupation at different times during mid to late winter and early spring. With this behaviour larvae may reach minimum prepupal body weight (Kain and Atkinson, 1977) even when growth rates are low. Results of larval growth from the present study suggest that *C. zealandica* larva did not gain any short-term advantage from the N or other major nutrients incorporated into the soil through whey inputs either.

5.3.2 Effect of whey on soil *S. entomophila* populations

Under microcosm conditions, populations of *S. entomophila* enumerated after the experiment were significantly ($P<0.05$) higher in experimental units treated with whey than those treated with water (Table 5.2). These differences occurred regardless of the presence of *C. zealandica* larvae in the experimental units. A striking observation is that when the net change in the numbers of *S. entomophila* in the soil was considered, significant bacterial growth was detected both in the nil and low doses (Table 5.2). The initial population in the soil treated with the bacteria-free nutrient broth corresponded to the background population of *S. entomophila* after being oven dried for 24 h at 80°C. This value was 5% of the population estimated in the fresh soil. Drying and rewetting cycles are known to release organic nutrients into the soil matrix (Griffin and Birch, 1961). O'Callaghan (1989) has observed that sterilization of soil permitted rapid growth of *S. entomophila* and this growth has been attributed to an increased nutrient supply, following the sterilization of soil.

Net changes in the number of *S. entomophila* in the soil were not necessarily related to the percentage of larvae affected by amber disease. Even when a significantly ($P<0.05$) higher *S. entomophila* growth occurred in soil from Whey+Nil+L compared with the H₂O+Nil+L treatment, this effect was not associated with a higher percentage of amber disease infection (Figure 5.1). However, the higher survival of *S. entomophila* observed in Medium+Whey+L treatment compared with the Medium+H₂O+L treatment was matched with the significant ($P<0.05$) increase in the percentage of larvae affected by amber disease. These results suggest that the application of the bacterial strain BC4B was more critical in causing amber disease than the total (soil-borne + applied) number of *S. entomophila* recorded. The lack of

Table 5.2

Mean number of *S. entomophila* bacteria (cells g⁻¹ soil) in the experimental units at the beginning (initial population) and at the end (population recovered) of the microcosm experiment.

Treatment	Initial population ¹	Population recovered	Net change
Nil+Whey+L	3.98x10 ²	5.72x10 ⁵	5.72x10 ⁵
Nil+H ₂ O+L	3.98x10 ²	2.90x10 ⁴	2.86x10 ⁴
Nil+Whey	3.98x10 ²	4.36x10 ⁵	4.36x10 ⁵
Nil+H ₂ O	3.98x10 ²	1.28x10 ⁴	1.24x10 ⁴
Low+Whey+L	7.04x10 ²	5.13x10 ⁵	5.13x10 ⁵
Low+H ₂ O+L	7.04x10 ²	1.42x10 ⁵	1.42x10 ⁵
Low+Whey	7.04x10 ²	4.40x10 ⁵	4.40x10 ⁵
Low+H ₂ O	7.04x10 ²	2.32x10 ⁴	2.24x10 ⁴
Medium+Whey+L	3.06x10 ⁵	2.20x10 ⁵	-8.60x10 ⁴
Medium+H ₂ O+L	3.06x10 ⁵	1.54x10 ⁴	-2.90x10 ⁵
Medium+Whey	3.06x10 ⁵	2.08x10 ⁵	-9.80x10 ⁴
Medium+H ₂ O	3.06x10 ⁵	1.79x10 ⁴	-2.88x10 ⁵
High+Whey+L	3.06x10 ⁸	7.88x10 ⁵	-3.05x10 ⁸
High+H ₂ O+L	3.06x10 ⁸	3.74x10 ⁵	-3.06x10 ⁸
High+Whey	3.06x10 ⁸	7.39x10 ⁵	-3.05x10 ⁸
High+H ₂ O	3.06x10 ⁸	3.75x10 ⁵	-3.06x10 ⁸
LSD _(0.05)		1.89x10 ⁵	

¹Values obtained adding the quantities of bacteria applied in the dose and the bacterial background.

Means of 2 replicates per treatment

Nil=2 ml bacteria-free nutrient broth; Low=2 ml 9.1x10³ cells of *S. entomophila*; Medium=2 ml 9.1x10⁶ cells of *S. entomophila*; High=2 ml 9.1x10⁹ cells of *S. entomophila*;

Whey=7ml of Cottage cheese whey; H₂O=7 ml of distilled water. L=one third instar *C. zealandica*.

correspondence between bacterial numbers in the soil and levels of amber disease was earlier discussed (Section 3.3.2 in Chapter III).

At the end of the experiment, a significant ($P<0.05$) decline in the initial population of *S. entomophila* was observed in the soil from all experimental units that received the medium dose. This decrease was even greater in soil from experimental units treated with the high dose. O'Callaghan (1989) has suggested that density dependent bacterial intraspecific competition may explain declining patterns of populations of *S. entomophila* in the soil.

The numbers of bacteria and net changes from the field mesocosm experiment are presented in Table 5.3. Data from this experiment confirmed the earlier results (Sections 2.3.2 and 3.3.2 in Chapters II and III respectively) that significantly ($P<0.05$) lower numbers of *S. entomophila* occurred in soil from A6 than in soil from the S14 pasture. Besides, when numbers of *S. entomophila* and *S. protamaculans* were combined, significantly ($P<0.05$) higher bacterial survival occurred in soil from WHEY+5L treatment in S14 pasture than in any other treatment. According to O'Callaghan (1989) and Jackson et al. (1993) *S. entomophila* is also present in apparently healthy third instars *C. zealandica*. Furthermore, these authors suggest that the insect is important not only as a means by which bacteria can be returned to the soil inoculum pool, but also as a mechanism for survival. A significant ($P<0.05$) decrease occurred in the number of both bacterial species from the beginning (late April) to the end (early June) of the experiment (Table 5.3). This change was probably associated with the low temperature that prevailed during this time of the year. According to Milner et al. (1980), temperature is a critical factor in the infection and spread of entomopathogens such as *Bacillus popilliae* Dutky in the soil-dwelling Scarab *Popillia japonica* Newman. Survival of the bacterial species that produce amber disease may, however, be enhanced by the application of whey in a soil containing high SOM level and high number of *C. zealandica* larvae.

5.3.3 Effect of whey on herbage DM production and growth

The ryegrass herbage DM production resulting from the different treatments under microcosm conditions is represented in Figure 5.4. These results indicated that the presence of the larvae did not

Table 5.3

Mean numbers of *S. entomophila* and *S. protamaculans* (cells g⁻¹ soil) at the beginning (initial population) and at the end (final population) of the mesocosm field experiment.

Soil	Treatment	Initial Population ¹	Final Population ²	Net Change
S14	WHEY+5L	2.75x10 ⁴	1.84x10 ⁴	-9.05x10 ³
	WHEY	2.75x10 ⁴	3.57x10 ²	-2.71x10 ⁴
	H ₂ O+5L	2.75x10 ⁴	7.72x10 ³	-1.97x10 ⁴
	H ₂ O	2.75x10 ⁴	1.16x10 ²	-2.74x10 ⁴
A6	WHEY+5L	2.41x10 ⁴	6.11x10 ³	-1.80x10 ⁴
	WHEY	2.41x10 ⁴	3.16x10 ²	-2.38x10 ⁴
	H ₂ O+5L	2.41x10 ⁴	1.74x10 ³	-2.23x10 ⁴
	H ₂ O	2.41x10 ⁴	2.20x10 ²	-2.39x10 ⁴
LSD _(0.05)			1.78x10 ⁴	

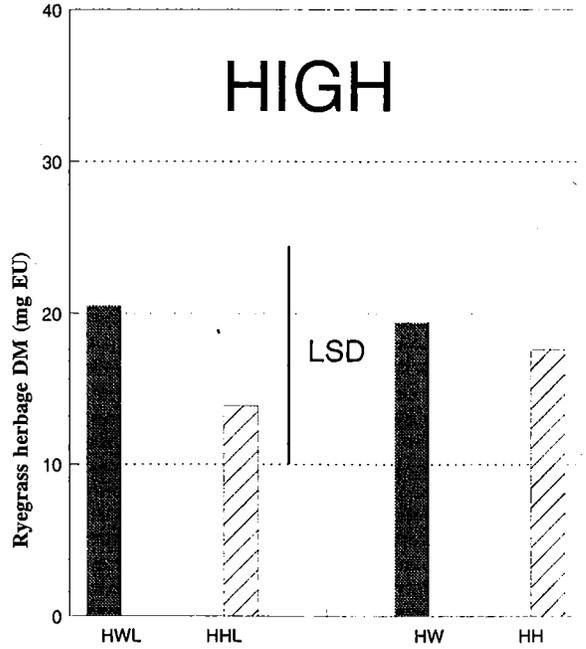
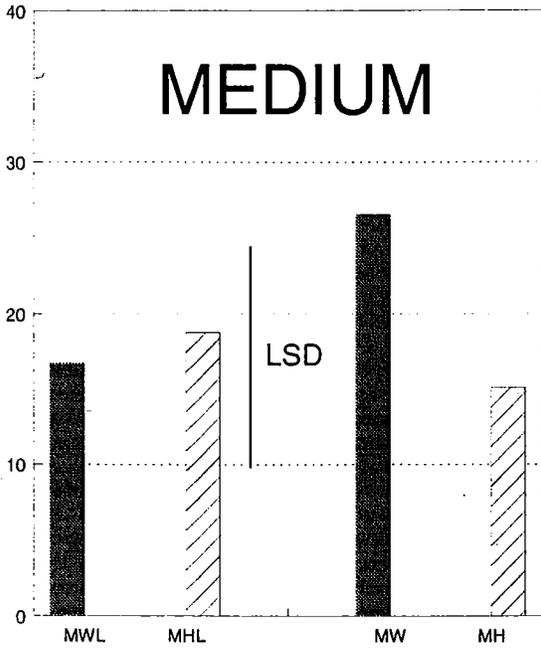
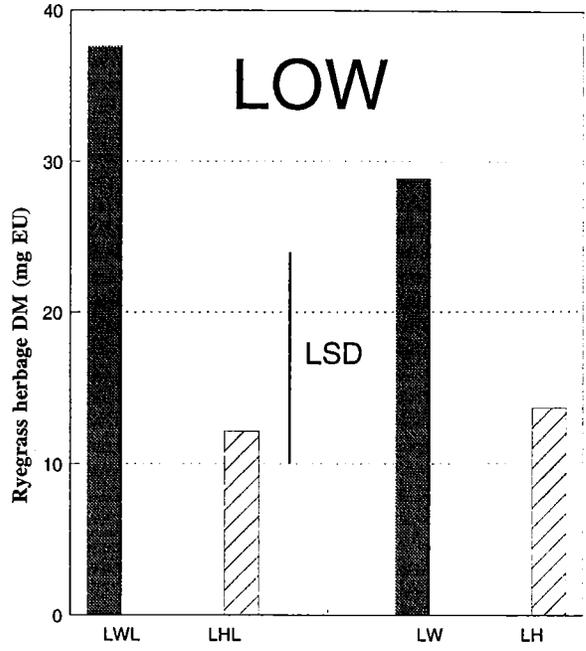
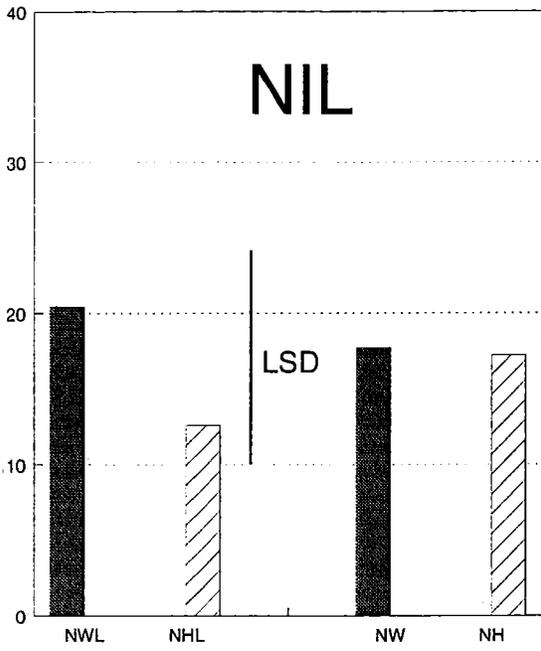
For a description of treatments and sites see captions in Table 5.1

¹Data from late April 1993; ²Data from early June 1993

Means of 2 replicates per treatment

Figure 5.4

Mean DM production of ryegrass herbage obtained per experimental unit using four doses of the bacterium *S. entomophila*, combined with the application of whey or water and in the presence or absence of *C. zealandica* third instars after 20 days under microcosm conditions.



significantly ($P < 0.05$) affect herbage production with any of the doses tested. This result confirms previous observations about the lack of correlation between the underground herbivory by *C. zealandica* larvae and herbage production (Section 3.3.6 in Chapter III). Overall, the application of whey increased significantly ($P < 0.05$) herbage DM production by 40%. Data from Figure 5.4 showed that those experimental units treated with the low dose of *S. entomophila* and whey produced the highest herbage DM production, regardless of the presence of the larva.

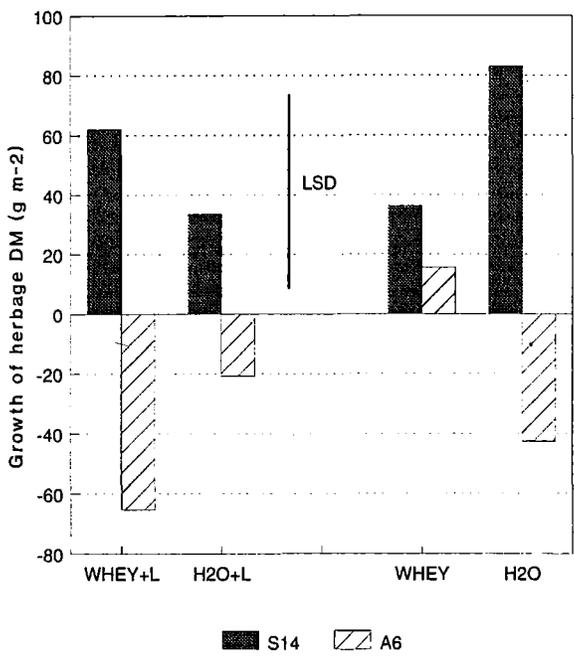
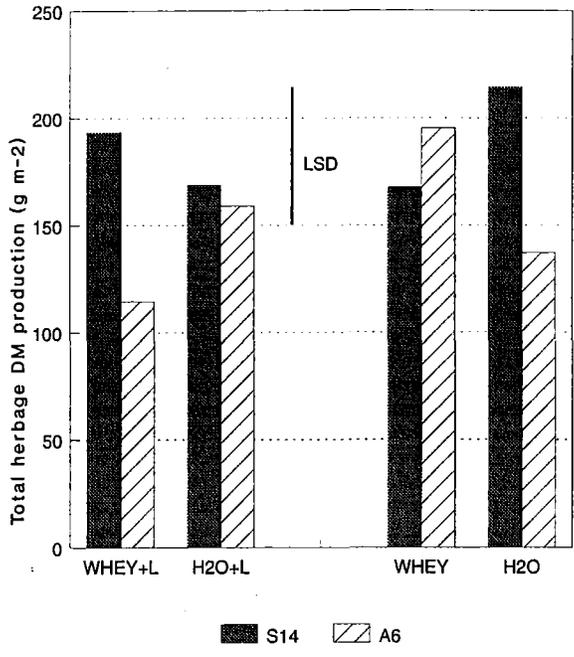
Results obtained under field conditions suggest that different responses in grass production and growth may be observed in old and young pastures. A significant ($P < 0.05$) reduction in herbage production (Figure 5.5a) and growth (Figure 5.5b) was observed in the WHEY+5L treatment in A6 pasture. The H₂O+5L treatment reduced significantly ($P < 0.05$) the herbage DM of clover (7-28% in S14 and A6 pastures respectively) in comparison with the H₂O treatment. However, the WHEY+5L treatment promoted an even higher significant ($P < 0.001$) reduction in clover herbage DM (72-60% in S14 and A6 pasture respectively) in comparison with the WHEY treatment. These results suggest that larval herbivory of *C. zealandica* was enhanced through the application of whey in A6 pasture. Radford (1992) suggests that pasture plots treated with whey tended to have a higher proportion of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) than untreated plots¹.

In both pastures, despite the significantly ($P < 0.05$) different larval density (Table 5.1), non-significant ($P > 0.05$) differences in herbage growth were recorded between H₂O+5L treatment in comparison with H₂O treatment at the end of the mesocosm experiment (Figure 5.5a). Differences were non-significant ($P > 0.05$) either in the growth of herbage among the same treatments for a particular pasture site (Figure 5.5b). These results confirm previous observations (Section 3.3.3.1 in Chapter III) that soil conditions may determine whether plant damage is produced or not by *C. zealandica* larvae. Therefore, larval density may not be as critical in explaining the pattern of plant damage caused by this insect. Indeed, Thomson and Roberts (1981) reported that autumn applications of N in the Taranaki region gave a much higher percentage increase in pasture production with a density of 120 *C. zealandica* larvae m⁻² than in pastures with 13 larvae m⁻².

¹The direct effect that N inputs as a result of the whey application has had on the growth of clover and/or its symbionts should be further explored.

Figure 5.5

(a) Mean total herbage DM production obtained by the different treatments after the mesocosm experiment. (b) Mean values of the growth in total herbage DM obtained after these treatments. LSD ($P < 0.05$).



Both in microcosm and mesocosm experiments, no visually evident symptoms of *C. zealandica* plant damage were observed for any treatment and pasture. These results and those obtained at the end of the previous mesocosm experiment (Section 3.3.3.1 in Chapter III) suggest that the causes of severe plant damage symptoms (Kain, 1975) require further investigation and interpretation.

5.3.3.1 Effect of whey on total N and total C in herbage

At the end of the microcosm experiment, the presence of the insect and the interaction whey and *C. zealandica* larvae promoted a significant ($P < 0.05$) increase in the percentage of N (Table 5.4). Percentages of N in ryegrass herbage were significantly ($P < 0.05$) higher when whey was added than when water applied (Table 5.4). In the Nil+Whey+L treatment an average of 40% increase in ryegrass herbage DM occurred in comparison to those receiving treatments Nil+H₂O+L or Nil+H₂O (Figure 5.4). Moreover, the total amount of N and C in herbage increased significantly ($P < 0.05$) in Nil+Whey+L treatment, in comparison to all remaining treatments in which the nil dose was used (Table 5.4). Two possible explanations can be suggested for these results: (a) larvae may promote directly or indirectly a significant mineralization of the N incorporated in whey which may then become available in the short-term to plants and; (b) given the incorporation of the major nutrients in the soil, ryegrass seedlings are able to compensate larval root herbivory. Results from the previous mesocosm experiment (Section 3.3.3.1 in Chapter III) support the idea that *C. zealandica* larvae may have a short-term effect in plant growth. Brown and Gange (1990) suggest that underground herbivory may produce positive effects on plant growth (Section 1.2.3.6 in Chapter I). Result from this microcosm experiment may also help to explain the pattern observed by Thomson and Roberts (1981) who found that at a high rate of N (75 Kg/ha) pasture growth increased by 100% on a high grass grub area but only by 42% on a low grass grub area.

Another striking result is that the highest percentage of herbage N (4.96%) was observed in the Low+Whey+L treatment ($P < 0.001$) (Table 5.4). This treatment produced the highest values in the amount of total N (1.86 mgN per experimental unit) and C (14.56 mgC per experimental unit) in ryegrass herbage under microcosm conditions (Table 5.4). It is worth noticing that, at the end of microcosm experiment, more than 60% of the larvae remained healthy with the application of the low dose of *S. entomophila*

Table 5.4

Mean values of percentage of N, percentage of C, total amount of N and total amount of C in ryegrass herbage DM after the microcosm experiment.

Treatment	%N ¹	%C ¹	mgN per EU ²	mgC per EU ²
Nil+Whey+L	4.68	39.74	0.96	8.12
Nil+H ₂ O+L	3.73	39.28	0.47	4.94
Nil+Whey	3.67	39.05	0.65	6.95
Nil+H ₂ O	3.42	39.13	0.43	4.98
Low+Whey+L	4.96	38.70	1.86	14.56
Low+H ₂ O+L	4.66	37.53	0.56	4.55
Low+Whey	4.62	39.37	1.33	11.37
Low+H ₂ O	3.27	40.36	0.44	5.50
Medium+Whey+L	4.69	35.25	0.78	5.90
Medium+H ₂ O+L	4.57	38.54	0.86	7.23
Medium+Whey	4.25	39.83	1.13	10.55
Medium+H ₂ O	4.25	40.89	0.64	6.18
High+Whey+L	4.19	37.57	0.86	7.70
High+H ₂ O+L	3.80	39.81	0.53	5.52
High+Whey	4.10	39.27	0.80	7.62
High+H ₂ O	4.09	37.51	0.72	6.61
LSD _(0.05)	0.42	3.36	0.07	0.54

For a description of treatments see captions in Table 5.2

¹Duplicated estimations per treatment

²Mean of 8 experimental units (EU)

(Figure 5.1). Moreover, in the soil from Low+Whey+L treatment the second highest *S. entomophila* population growth was observed (Table 5.2). It is possible that the microbial activity has been enhanced, either by the presence of the insect (Section 4.3.2.4 in Chapter IV), or by the growth of *S. entomophila* which has used the whey as a substrate. In this respect, Griffiths and Birch (1961) state: "it is well established that maximum physiological activity occurs during the lag and early low phase in the growth of a bacterial culture and it is clear that in the present instance maximum carbon dioxide production coincides with this part of the growth curve of rods rather with point of maximum number". To confirm this hypothesis it may be recommended that soil microbial biomass analyses be performed at several periods of time during a similar microcosm experiment.

In the field, the WHEY+5L treatment, resulted in a significant ($P<0.05$) increase (43-45%) in the net change of the amount of total N and C (36-38%) in grass herbage in comparison to the H₂O+5L treatment in A6 and S14 pastures respectively (Table 5.5). This observation suggests that larvae have contributed to the mineralization of whey. Evidence that *C. zealandica* larvae promote SOM mineralization has been previously found by Yaacob (1967) and discussed in Section 1.2.2.4 (Chapter I).

At least for the effect on grasses, whey may compensate for *C. zealandica* damage. However, the results obtained for clover herbage, show that the whey-insect interaction had a significant ($P<0.05$) detrimental effect on the net change of N and C content (Table 5.5). The net reduction observed both in C and N in clover herbage was significantly higher ($P<0.05$) in the A6 than in the S14 pasture. It is likely that this difference is the result of the combined effect of four factors: (a) the lower SOM content in A6 than in S14 pasture which may have increased the probability of larval herbivory on clover (Section 3.3.3.5 in Chapter III); (b) the higher proportion of clover to grass plants in A6 than in S14 pasture may have increased the probability of root larval grazing on clover. At the beginning of the experiment the proportion grass DM to clover DM was 88%:12% in S14 and 32%:68% in A6 pasture; (c) roots of white clover may be selectively grazed by *C. zealandica* larvae. White clover has been reported as a preferred host of third instar *C. zealandica* in comparison to many grass species (Dymock et al., 1989) and; (d) whey supplied available N and this may have decreased biological N fixation by clover plants. Cookson et al., (1989) reported that during a field experiment, N derived from the atmosphere significantly decreased

Table 5.5

Mean values of percentage C and N and the net change in the total amount of C and N in grass and clover herbage DM obtained with different treatments after the mesocosm experiment

Soil	Treatment	Grass				Clover			
		%N	%C	Net change (gN m ⁻²)	Net change (gC m ⁻²)	%N	%C	Net change (gN m ⁻²)	Net change (gC m ⁻²)
S14	WHEY+5L	3.29	46.04	2.68	37.56	3.02	44.16	-0.16	-2.36
	H ₂ O+5L	3.25	43.86	1.47	19.79	3.46	42.85	-0.09	-1.11
	WHEY	3.73	45.89	1.89	23.30	3.80	42.39	0.17	1.90
	H ₂ O	3.47	44.68	2.42	31.19	3.35	42.41	-0.07	-0.88
A6	WHEY+5L	3.28	43.08	2.68	31.13	3.84	41.10	-0.21	-2.20
	H ₂ O+5L	3.40	44.39	1.53	20.03	4.15	44.85	-0.11	-1.16
	WHEY	3.54	42.93	1.80	21.80	4.02	43.53	0.18	1.95
	H ₂ O	3.45	42.33	2.41	29.54	4.30	44.25	-0.09	-0.92
	LSD _(0.05)	0.82	5.08	0.48	2.89	1.47	3.24	0.05	0.12

For a description of treatments and sites see captions in Table 5.1

Means of 5 experimental units.

Net change=difference in the amount of total N or total C at the beginning and at the end of the mesocosm experiment.

when ^{15}N tracer was applied daily (87.2%) or at a 30-day interval (76.3%) after the first 30 days of regrowth.

On the contrary, in experimental units with background densities of *C. zealandica* larvae (Table 5.1), whey applications have increased significantly ($P<0.05$) the gain of total N and C in clover herbage and reduced significantly ($P<0.05$) the incorporation of N and C in grass herbage (Table 5.5). This effect may be another indication of the higher susceptibility of clover plants to *C. zealandica* root herbivory.

Results from the mesocosm experiment suggest that the application of whey may have bidirectional consequences on grass composition. It seems that ryegrass will be favoured in pastures with a high population of *C. zealandica* larvae. On the other hand, in areas with a low *C. zealandica* population, whey applications may increase the nutritive value of clover and reduce levels of C and N in grasses. Thus in applying whey to pasture soils, consideration may be required of the effect that this input could have on grass composition in the short and long-terms and the associated advantages and disadvantages.

5.3.4 Effect of whey on the production of living roots-plant residues DM

Results from the microcosm experiment (Table 5.6) show that after 20 days the presence of *C. zealandica* larvae promoted a significant ($P<0.05$) reduction (16%) on the combined values of living roots-plant residues DM production. However, larval herbivory was not the only factor involved in ryegrass herbage production. Considering only the treatments within the same *S. entomophila* dose, in those experimental units in which the insect was excluded, herbage DM did not increase significantly ($P>0.05$) in comparison to those that included the insect, either for whey or water inputs.(Figure 5.4).

In Nil+Whey+L treatment a significantly ($P<0.05$) higher reduction in living roots-plant residues DM was observed in comparison to that observed in Nil+Whey treatment (Table 5.6). Such disappearance may, therefore, be mainly attributed to larval herbivory and/or larval consumption of plant residues. The indirect larval effect through the stimulation of microbial activity on plant residues decomposition may have also accounted for this difference (Section 4.3.2.1 in Chapter IV). The whey-insect interaction reduced

Table 5.6

Mean values of ryegrass living roots-plant residues DM (mgEU), percentage of N and C, and total amount of N and C in this fraction after the microcosm experiment.

Treatment	DM (mgEU)	%N ¹	%C ¹	mgN EU ²	mgC EU ²
Nil+Whey+L	134	1.84	42.21	2.48	56.78
Nil+H ₂ O+L	168	1.78	40.59	2.99	68.26
Nil+Whey	223	1.83	39.06	4.09	87.17
Nil+H ₂ O	215	1.73	37.92	3.73	81.73
Low+Whey+L	174	1.70	37.84	2.96	65.70
Low+H ₂ O+L	191	1.72	39.95	3.28	76.16
Low+Whey	253	1.73	37.55	4.38	95.61
Low+H ₂ O	222	1.84	39.53	4.10	88.06
Medium+Whey+L	205	1.74	38.30	3.56	78.57
Medium+H ₂ O+L	222	1.63	37.87	3.62	83.93
Medium+Whey	233	1.72	37.46	4.16	87.19
Medium+H ₂ O	240	1.78	39.44	4.14	94.70
High+Whey+L	200	1.74	37.48	3.48	75.10
High+H ₂ O+L	229	1.76	39.83	4.03	91.27
High+Whey	215	1.67	41.01	3.60	78.67
High+H ₂ O	211	1.82	36.52	3.84	86.38
LSD _(0.05)	36.50	0.21	4.27	0.44	9.14

For a description of treatments see captions in Table 5.2

¹Duplicated estimations per treatment

²Mean of 8 experimental units (EU)

significantly ($P < 0.05$) the amount of living roots-plant residues DM. Overall, in soil from Nil+Whey+L treatment, 23% lower content of living roots-plant residues DM was observed compared with the soil from the Nil+H₂O+L treatment (Table 5.6). This result may be due to an increase in larval feeding through the addition of whey. It is possible that the low molecular weight organic compounds present in the whey have stimulated the feeding response of larvae. Under laboratory conditions, light carbohydrates, amino-acids, ascorbic acid and inorganic salts can stimulate the feeding response of *C. zealandica* and *Heteronychus arator* Fab. larvae (Sutherland, 1971; Sutherland and Hillier, 1974; Sutherland, 1983). This result also supports the previous observation on the stimulatory effect that the nutrient broth had on larval herbivory in the field (Section 3.3.3.5 in Chapter III).

Another significant ($P < 0.05$) interaction was found between the dose of *S. entomophila* applied and the presence of *C. zealandica* larvae. Low and medium doses produced the highest living roots-plant residues DM values in the absence of the insect (Table 5.6). However, in the soil from the Medium+Whey+L and Medium+H₂O+L treatments, significantly ($P < 0.05$) higher plant living roots-plant residues DM were recorded compared with those observed in Nil+Whey+L and Nil+H₂O+L treatments. This result may also indicate the *S. entomophila* inhibits soil microbial decomposition as mentioned in Section 2.3.6 (Chapter II).

As observed in the previous mesocosm experiment, data from the present microcosm experiment showed that the application of *S. entomophila* (BC4B) increased living root production by itself (Section 3.3.3.7 in Chapter III). Soil from the Medium+H₂O treatment contained a higher living roots-plant residues DM than those from Nil+H₂O treatment. Particularly in treatments which included larvae, these differences were significant ($P < 0.05$) (Table 5.6). It is worth remembering that similar percentages of healthy larvae were observed at the end of the experiment in the Medium+H₂O+L (70%) and Nil+H₂O+L (85%) treatments (Figure 5.1). Therefore, it is assumed that differences in the magnitude of larval herbivory between these treatments were minimal. This suggests that the interaction between healthy *C. zealandica* larvae and the bacterium, under microcosm conditions, produced a positive effect on the growth of plant roots. There seems to be an optimum dose (under microcosm conditions a concentration of about 1.82×10^4 cells ml⁻¹ of BC4B gave the best results) at which the bacterium can produce this positive effect. Assuming a uniform bacterial distribution, this dose corresponded to a initial population of 7.04×10^2 cells g⁻¹ soil.

Whether this phenomenon is associated with a particular strain or is widespread in the species is unknown. The observation that the application of bacterial strain BC4B by itself may have a positive effect on plant production should be explored further.

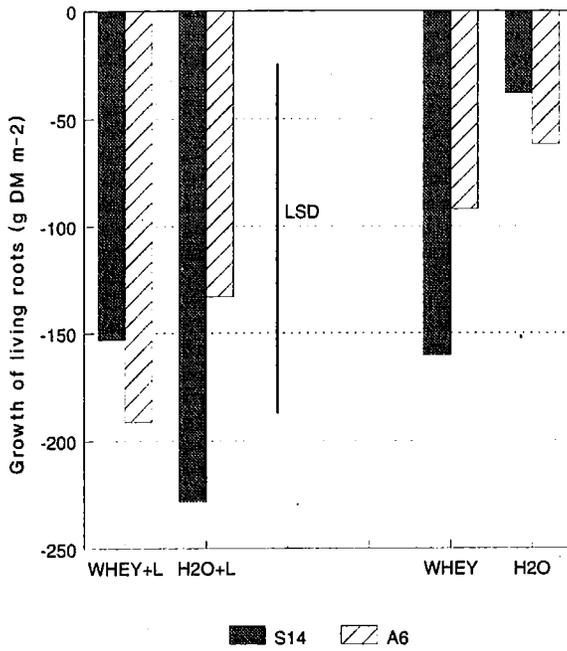
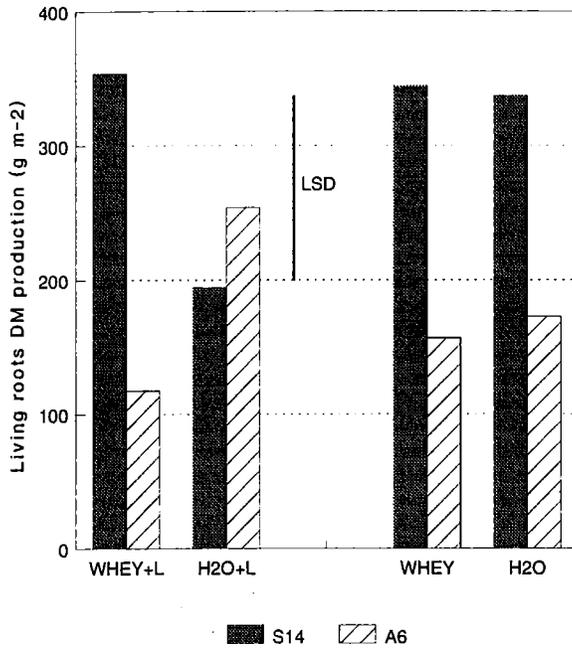
Results of the DM production of living roots after the mesocosm experiment (Figure 5.6) showed a significant ($P<0.05$) difference between the WHEY+5L and H₂O+5L treatments. In S14 pasture, about 45% more living root DM resulted from WHEY+5L treatment compared with H₂O+5L treatment. Apparently, in experimental units from S14 pasture, the whey application has compensated for *C. zealandica* larval herbivory (Figure 5.6a). The opposite pattern was observed in A6 pasture in which almost 50% more living root DM was recorded in H₂O+5L than in WHEY+5L treatment. The only significant ($P<0.05$) difference for the growth of living roots was observed between the H₂O+5L and H₂O treatments in S14 pasture. The impact of larval herbivory was enhanced by the application of whey in A6 pasture. Results from the microcosm experiment support this contention which also occurred for ryegrass living roots-plant residues DM. However, in the microcosm experiment, the enhancement of microbial decomposition by the whey application cannot be dissociated from the stimulation of larval feeding activity. The interaction of a higher susceptibility of clover roots and a more intense larval herbivory under low SOM conditions as previously suggested (Section 2.3.4 in Chapter II) may have accounted for this output. However, the effect that whey may have on clover root pathogens should also be considered and evaluated in future experiments. Rovira et al. (1990) states: "Fertilizers applied for crop production might also serve as a source of mineral nutrition for root pathogens during their saprophytic existence in soil and during penetration growth *en route* to infection..."

5.3.4.1. Effect of whey on the content of total C and N in living roots-plant residues and living root DM

A significantly ($P<0.05$) higher (15%) total amount of C in living roots-plant residues was recorded on average in soil from experimental units treated with whey in comparison to those treated with water (Table 5.6). Overall, the presence of larvae promoted a significant ($P<0.05$) decline of N (17%) and C (15%) in living roots-plant residues. Significantly ($P<0.05$) higher content of N and C (14%-15% respectively) occurred in living roots-plant residues from experimental units treated with the medium dose

Figure 5.6

(a) Living root DM production recorded after the mesocosm experiment. (b) Growth of living roots. For an explanation of the meaning of the labels see caption in Figure 5.2.



than those not treated with bacteria (Table 5.6). This result is another indication that the medium dose produced the best effect and of the need to optimize bacterial application rates. During inundative bacterial applications in the field, it is recommended to reduce the current commercial rate. According to Jackson et al., (1992), the commercial rate of *S. entomophila* applications is about 4×10^{10} bacteria ml^{-1} leading to a product volume of 1 lt ha^{-1} . Previous results (Section 4.3.2.5 in Chapter IV) suggest that high doses may have deleterious effects on the indigenous soil microbiota and may delay microbial decomposition (Section 2.3.6 in Chapter II). Several *Serratia* isolates have proved to be antibacterial and sometimes good antifungal agents (Hedges and Messens, 1990). Under conditions of high interspecific competition in the soil *S. entomophila* may also produce antagonistic compounds against other soil micro-organisms.

At the end of the mesocosm experiment significant ($P < 0.05$) differences were observed in the percentage of N in living roots between pastures (Table 5.7). Pasture A6 presented a higher percentage of N as a result of its higher proportion of clover plants. Differences for the percentage of C were only significant ($P < 0.05$) for the interaction between whey and SOM content factors. A trend was detected in which living roots from S14 soil tended to reduce their percentage of C as a consequence of the whey application. On the contrary, living roots in soil cores from A6 pasture, tended to increase their proportion of C with the application of whey.

At S14 pasture, the $\text{H}_2\text{O}+5\text{L}$ treatment produced the highest drop in the levels of total N and C present in living roots (Table 5.7). This drop was less marked in those experimental units from the WHEY+5L treatment than in those from the $\text{H}_2\text{O}+5\text{L}$ treatment, a result that supports the idea that this input had a compensatory effect for larval herbivory. However, in A6 pasture, the highest negative change in the levels of total N and C present in living roots was observed in experimental units treated with whey. The possible explanation for this result has been previously discussed (Section 5.3.4 in this Chapter).

5.3.5 Effect of whey on the build up of labile SOM fractions

Results from SOM determinations of the total N and C present in cold and hot water extractions of SOM at the end of the microcosm experiment are presented in Figure 5.7. Cold water extractions

Table 5.7

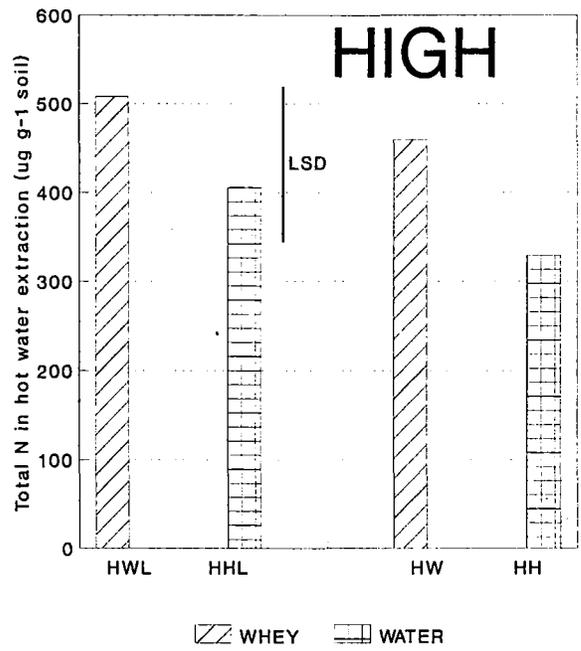
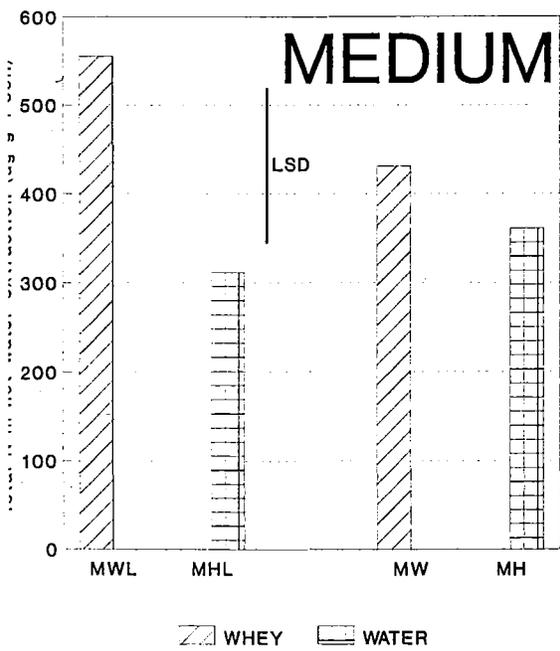
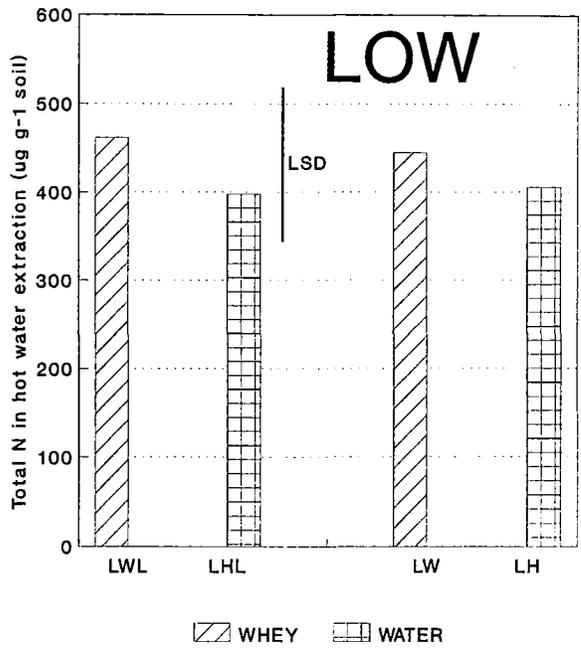
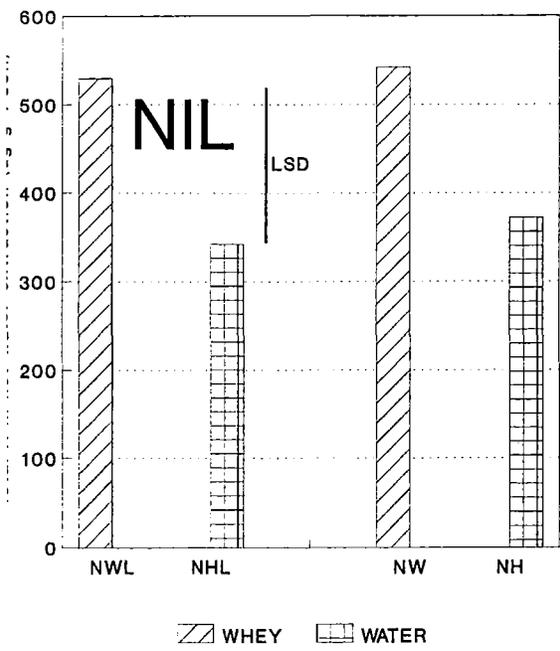
Percentage of N and C and net change in the total amount of N and C present in living roots obtained after the mesocosm field experiment.

Soil	Treatment	%N	%C	Net change (gN m ⁻²)	Net change (gC m ⁻²)
S14	WHEY+5L	1.29	43.34	-1.05	-35.53
	WHEY	1.65	43.08	-1.50	-39.11
	H ₂ O+5L	1.26	42.28	-3.04	-102.40
	H ₂ O	1.43	42.17	-1.41	-41.42
A6	WHEY+5L	2.19	41.44	-4.56	-86.19
	WHEY	1.86	41.19	-3.15	-70.01
	H ₂ O+5L	2.08	42.59	-1.49	-30.50
	H ₂ O	2.49	43.66	-3.81	-66.76
LSD _(0.05)		0.54	1.55	2.35	60.86

For a description of treatments and sites see captions in Table 5.1
Means of 2 replicates per treatment.

Figure 5.7

Amount of total N present in hot water extraction of SOM from the different treatments after 20 days in the microcosm experiment.



contain free biochemical groups as amino-acids, sugars or other monomeric labile substances which are readily mineralizable. Hot water extractions contain simple organic compounds, slightly decomposable carbohydrates and the compounds released after the death of soil micro-organisms. Compounds present in both extractions may be quickly metabolized by microorganisms, soil fauna and plants and, therefore, are considered as the labile fraction of the SOM. Overall, the application of whey has significantly ($P<0.05$) increased the level of N present in hot water extractions. A 25% increase was observed in the amount of total N in soil from experimental units treated with whey in comparison to the level recorded in soil treated with water.

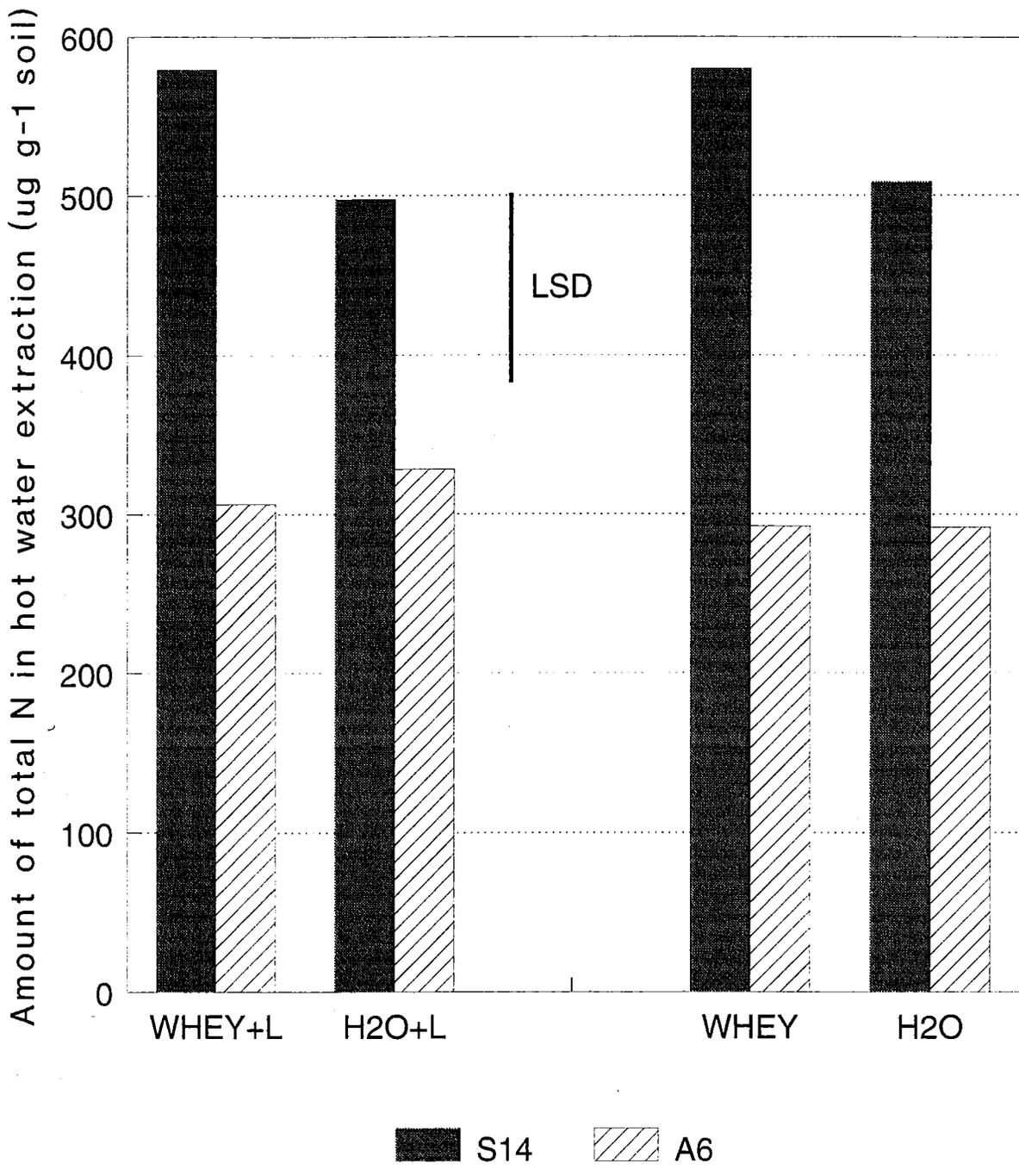
Reductions of 16%-20% were observed in the total N present in hot water extractions under microcosm conditions in soil exposed to the Medium+Whey and High+Whey treatments respectively in comparison to those from treatment Nil+Whey (Figure 5.7). According to O'Callaghan (1989), at high densities, populations of *S. entomophila* may deplete soil nutrients more rapidly than smaller populations. Under microcosm conditions, with medium and high doses of *S. entomophila*, a fifth of the amount of whey incorporated is being mineralized by the bacteria.

The highest level of N in hot water extractions was also observed in Medium+Whey+L treatment. Therefore, field bacterial applications should be complemented with the addition of whey to manage efficiently *C. zealandica* populations in young pastures, as well as promoting pasture sustainability. Radford (1990) has found that whey applications to pastures also promote substantial increases in the concentration of P and K in soil.

Under field conditions, whey applications also caused a significant ($P<0.05$) increase (15%) in the total amount of N in hot water extractions (Figure 5.8a) in soil from the S14 pasture; however, the same effect was less conspicuous in soil from the A6 pasture (7%). A significant ($P<0.05$) interaction occurred between factors whey and SOM content. These results suggest that in soils with a low content of SOM, the application of whey may have a slower impact on increasing levels of labile SOM. A previous determination of the SOM content in a pasture will help to optimize whey applications in pastures. The significant ($P<0.05$) differences in the amount of total N present in hot water extractions between pasture

Figure 5.8

Amount of total N present in hot water extraction of SOM from the different treatments after the mesocosm experiment. For an explanation of the meaning of the labels see caption in Figure 5.2



soils obtained in previous experiments (Sections 2.3.1 and 4.3.1 in Chapters II and IV respectively) were confirmed in this study.

In the present study, the application of whey induced a sudden increase in the total N present in hot water extractions in pasture soils. This sudden input of nutrients may, however, increase the probability of rhizopathogenic infections (Rovira et al., 1990), particularly in micro-environments with low diversity. The intervention of *C. zealandica* larvae on regulating directly or indirectly the composition of microbial communities (Section 1.2.2.4 in Chapter I) may contribute to reduce the probability of infection by root pathogens. Research on the appropriate application method, rate and careful selection of the pasture sites to be treated should also be encouraged.

5.4 Conclusions

Although the application of whey alone showed no significant short-term effect on the health status of *C. zealandica* larvae under microcosm and mesocosm conditions, this input interacted positively with its entomopathogens. Levels of amber disease could be increased significantly either by combining the whey application with a moderate dose of *S. entomophila*, or by enhancing the indigenous background of entomopathogens present in soils with high SOM content. Growth of soil-borne populations or artificially applied low doses of *S. entomophila* may be encouraged by whey inputs and this should be considered when attempting to optimize biological control measures.

Larval growth was not significantly affected by the whey application, suggesting that *C. zealandica* either do not take short-term advantage of this input or compensate for low quality food by modifying its nutrient consumption rates.

Findings from the laboratory microcosm and field mesocosm experiments supported results of the previous mesocosm experiment that there is no direct linear correlation between plant aerial production and *C. zealandica* larval herbivory (Section 3.3.6 in Chapter III). On the contrary, at least under the soil microcosm and mesocosm conditions, the plant-insect interaction may have a positive effect on plant

production. If moderate doses of bacteria are combined with whey inputs, the positive interaction between the insect and the plant may even enhance the diversity and productivity of the soil system. The whey-insect interaction under low SOM conditions had a significant effect on reducing the proportion of clover.

Symptoms of severe plant damage caused by *C. zealandica* larvae were not reproduced either under mesocosm or microcosm conditions. This result supports the idea that soil conditions rather than larval densities are the main cause of severe plant damage.

Whey applications produced a positive short-term effect in the build up of the total C and N in labile fraction of SOM extracted by hot water under field conditions. The addition of N and other nutrients in a short-term available form, seems to be crucial in the dynamics of the microcosm studied.

As inferred from results of the microcosm and mesocosm experiments, to increase the production and quality of ryegrass herbage and living roots and to encourage diversity, an optimum bacterial dose is likely to be midway between the low and medium doses utilized. If a suitable pre-application of whey is made, growth of indigenous *S. entomophila* could be expected. Moreover, the enrichment and accumulation of SOM will provide productive and sustainable soil conditions.

Survival of the soil-borne bacterial species that produce amber disease in *C. zealandica* may be enhanced by whey applications in soils with high larval populations and high SOM content.

The combination of animal or plant residues containing non-toxic compounds, with a rich and diverse amount of organic compounds is strongly recommended for New Zealand pastoral agriculture. This practice may contribute to multiple beneficial effects including the encouragement of natural enemies, or optimization of *S. entomophila* applications against *C. zealandica* under field conditions.

GENERAL DISCUSSION

6) SUSTAINABLE MANAGEMENT OF *Costelytra zealandica* (White) LARVAE IN NEW ZEALAND PASTORAL AGRICULTURE

6.1 Definition of sustainable management of *C. zealandica* larvae in New Zealand pastoral agriculture

Results from the present study suggest that soil organic matter (SOM), apart from having multiple beneficial effects on soil fertility (Allison, 1973; Stevenson, 1982) plays a key role in the sustainable management of larvae of *Costelytra zealandica* (White) in New Zealand pastures. These results have led to the design of a strategy of insect management to fit within the paradigm of sustainable agriculture.

In the present study, sustainable management of *C. zealandica* larvae in New Zealand pastures is defined as the strategy including the principles outlined below:

(a) Modification of economic or action threshold levels that take into account SOM content and quality, moisture, temperature and texture in pasture soils.

(b) Evaluation of the relationship between plant traits (e.g., size and biomass of living roots, content of C and N in living roots and plant diversity) and status of larval population (e.g., larval spatial distribution, larval density, larval development, larval health status).

(c) Search for available soil organic matter inputs to be used in a regular and rational basis as amendments of soil fertility.

(d) Encouragement of the diversity of soil macro-invertebrates and soil microbiota including indigenous entomopathogens.

(e) Prevention of insect outbreaks by increasing the probability of occurrence of larval diseases;

(f) Profitable management of *C. zealandica* larvae through the encouragement of their beneficial activities as stimulation of soil microbial activity, SOM mineralization and root-pruning;

(g) Long-term selection of evolutionary traits in larvae of *C. zealandica* determining saprophytic activities rather than encouraging rhizophagous feeding habits;

Although, the search for short-term solutions is important, emphasis should be placed in long-term economically and ecologically viable preventative measures through the build up of SOM in pastures.

6.2 Interactions among SOM, amber disease and mortality of *C. zealandica* larvae

Pastures with a high SOM content, or soils that have been submitted to a minimum tillage regime, may harbour a higher background of active *C. zealandica* entomopathogens and, therefore, may increase the likelihood of epizootics. In the present study, under both microcosm (Section 2.3.2 in Chapter II) and mesocosm conditions (Section 3.3.2 Chapter III), a significantly ($P < 0.05$) higher population of *S. entomophila* was present in fresh soil from the pasture with the highest SOM content. The role that SOM may play in the formation of entomopathogen reservoirs and the particular importance that these reservoirs may have in the survival of non-spore forming bacteria such as *S. entomophila* should be taken into account (Section 2.3.3 in Chapter II).

Although bacterial numbers were not always directly correlated with levels of amber disease under field conditions (Section 3.3.1 and 3.3.2 in Chapter III), under microcosm conditions a significantly ($P < 0.05$) higher level of amber disease occurred in the soil with higher SOM content than in the soil with lower SOM content (Section 2.3.3 in Chapter II). After 30 days of incubation, larval mortality was closely associated with amber disease and was 50% higher in the older than in the younger pasture.

While there was no significant ($P > 0.05$) difference in the visual assessments of the levels of amber

disease observed among pastures with different SOM content in the field, total mortality of *C. zealandica* larvae caused by entomopathogens was significantly ($P < 0.05$) higher in the soil with the highest content of SOM. Therefore, both microcosm and mesocosm approaches have indicated that SOM is an important factor associated with the natural regulation of *C. zealandica* populations.

Survival of *C. zealandica* from egg to adult has been estimated as 17% under field conditions (Plunket and Kain, 1979). In a tropical grassland, the survival from egg to adult under field conditions was calculated to be between 5-8.5% for five main species (Villalobos, 1991). Predation, parasitism and starvation under average field conditions appeared to be less important than entomopathogenic diseases in the regulation of populations of soil scarab larvae. By contrast, *C. zealandica* diseases have been postulated as the main biotic factor in explaining larval mortality in several places throughout New Zealand (Cameron and Wigley, 1989).

Under glasshouse conditions, addition of manure to soil increased the survival of two soil scarab species from Australian pastures (Davidson and Roberts, 1968). This result indicates the importance of adding or maintaining inocula of soil scarab larval entomopathogens. Different types of SOM inputs may have different effects on larval population dynamics when compared with SOM materials accumulated in the field through normal agronomic practices in pastures. When comparing larval survival among different treatments or instars, distinctions should be made between the nature and quality of different SOM sources. It is also recommended that a variety of research approaches will be needed to arrive at more valid conclusions (Section 1.4 in Chapter I).

The application of whey by itself showed no significant short-term effect on the health status of *C. zealandica* larvae, under microcosm and mesocosm conditions (Section 5.3.1 in Chapter V). However, whey interacted positively with the entomopathogenic activity of soil-dwelling natural enemies of *C. zealandica* larvae. The need to preserve soil-borne entomopathogens of *C. zealandica* larvae in the soil is highlighted by the fact that under field mesocosm (Section 5.3.1 in Chapter V) a higher number of healthy larvae were recorded in soil cores treated with whey in the soil with the lowest SOM content. Levels of amber disease increased significantly either by combining a whey application with a moderate dose of *S.*

entomophila, or by enhancing the indigenous background of soil entomopathogens under high SOM conditions. Growth of soil-borne or artificially applied low doses of *S. entomophila* populations should be encouraged through whey inputs and to optimize microbial biological control agents.

6.3 Interactions between SOM and plant damage caused by *C. zealandica* larvae

Soils with a high SOM content may reduce the intensity of *C. zealandica* larval herbivory and may delay larval growth. Under microcosm conditions, the different SOM contents between the old and the young pasture (Section 2.3.4 in Chapter II) accounted for a 40% reduction in the frequency and/or intensity of insect larval carrot consumption. However, results from the mesocosm experiment described in Chapter III suggest that the impact of larvae on the growth of herbage and living roots may be more importantly related with the severing of roots within the top 5 cm rather than to larval removal of C and N in living roots in deeper parts of the plant (Section 3.3.3).

Although high densities of *C. zealandica* larvae were incorporated into the experimental units under mesocosm conditions, no apparent symptoms of plant damage were observed for any of the studied pastures soils (Section 3.3.3 in Chapter III). Results from a field mesocosm experiment (Sections 3.3.3.1; 3.3.3.5; 3.3.6 in Chapter III), have shown no clear relationships between the amount of living roots removed by *C. zealandica* larvae and pasture herbage production. This observation confirms previous results obtained by Ridsdill-Smith (1977) who found that during a glasshouse experiment the reduction in root yield was greater than the estimated larval consumption of *Sericesthis nigrolineata* (Boisd.) at several insect densities and was proportional to the control yield. According to this author, the greatest reduction in root yield of ryegrass turf occurred between the plants with no larvae and those with the lowest insect density. Higher insect densities had comparatively less influence on plant yield. Davidson (1969) suggested that, under glasshouse conditions, 50% of the roots of pasture plants could be lost due to feeding by scarabaeid larvae before there was a significant reduction in foliage yield.

Results obtained in the present study suggests that, under field conditions, the presence of *C. zealandica* larvae reduced total C and N from living roots of plants and this removal increases as the level

of SOM decreases (Section 3.3.3.5 in Chapter III). According to Brown and Gange (1990), laboratory trials have indicated that root-feeding can seriously reduce the vegetative growth of plants often in the region of a 70% loss in biomass. Such trials can overestimate plant damage, but frequently may be translated to field situations where 50% loss in crop yield may occur (Brown and Gange, 1990). Larval feeding of *S. nigrolineata* caused water stress in the foliage, as measured by a reduction in leaf relative water content, which probably reduced the growth of plants (Ridsdill-Smith, 1977). However, there are a number of ways in which plants may compensate for the loss of parts of a root system due to insect feeding. Having access to an increased water or mineral supply, for example, can mitigate the effects of the attack. The transport of the photosynthate from the foliar parts to the roots may occur and this may aid in the proliferation of lateral roots. Indeed, at modest levels of insect attack, proliferation may overcompensate with the result that plant stability, or water or mineral acquisition are enhanced. In this way, root-feeding insects at certain densities may even benefit the plant (Brown and Gange 1990). According to Jackson (1992) relatively high number of soils scarabs can be tolerated in pasture before damage is evident. Dense aggregations of soil scarab larvae are not always associated with visible damage to pastures as reported at Armidale, Australia by Davidson (1969).

According to Kain (1975), plant damage caused by *C. zealandica* larvae can arise in distinct but interrelated ways. The most obvious type of plant damage is where plant cover is partially or completely destroyed leaving bare ground into which weed species establish. A less obvious type of damage occurs where pasture production is reduced without an associated change in the botanical composition of the pasture. The third and least spectacular type of damage is an insidious change in botanical composition. Changes in botanical composition occur though the invasion of undesirable plant species into bare ground induced by insect damage and in response to preferential feeding by the insect or differential recovery rates of damaged plant species (Kain 1975).

A plausible explanation for the pattern of severe damage reported by East (1972) and Kain (1975) is that larval aggregations occur both in a vertical and horizontal plane having an effect on the sensitive parts of the root system. These aggregations are determined by environmental changes in the soil. Movement of *C. zealandica* larvae seems to be primarily a response to soil moisture gradients (Kelsey,

1970). Indeed, soil moisture has been considered as the most important ecological factor that determines larval spatial distribution (Dumbleton, 1942). Either by increasing the probability of egg-laying and larval hatch or by eventual larval aggregations during the third instar a gradient of soil moisture may be a critical factor in explaining *C. zealandica* soil distribution patterns. According to Dumbleton (1942), in areas of dry soil such as Central Otago, the larvae are usually found in the moister localities. Jackson and Townsend (1991) found that in mid-Canterbury, the total area damaged by *C. zealandica* larvae in dryland pastures was approximately 20% of the surface area compared to 5% in irrigated pastures present in the same area. Therefore, the fact that larval aggregations occur, as a response to soil moisture gradients, in the top soil would partially explain why through irrigation a more scattered distribution may be induced and the likelihood of pasture damage may be reduced.

In cases of severe *C. zealandica* pasture damage sections of the grass can be easily rolled back after the roots have been severed by intensive root pruning (East 1972). In these cases, high densities of active larvae evidently appear in the top soil. East (1972) reported that second and third instar larvae are commonly found in the top 5 cm of soil during autumn and winter (March-August) at Winchmore, Canterbury. A similar pattern of plant damage has been reported in Australian pastures for other soil scarab species (Davidson and Roberts, 1969). In this case, the recognition of the depth of feeding was seen as a critical factor in producing this effect. Davidson and Roberts (1969) suggested that depth of feeding had a highly significant effect on the relative root damage and foliage yield and significantly greater root damage occurred under conditions more favourable for pasture growth. Their experiment was carried out by confining larvae (at a density equivalent to 430 larvae m⁻²) artificially between two wire-mesh screens 5 cm apart in a pot filled with soil under glasshouse conditions. Despite these manipulations, results from the experiment of Davidson and Roberts (1969) strongly suggested that larval aggregations in the top 5 cm of soil may be more destructive than larval feeding at greater depths even when fertilised plants and optimum soil conditions are present. Therefore, the physical effect of larval feeding activity may be the main cause of plant injury in the obvious pattern of plant damage caused by *C. zealandica* larvae.

An additional explanation for the lower susceptibility of *C. zealandica* attack in old pastures is the massive growth of living roots. According to Davidson and Roberts (1969) the generalization that the

greater the concentration of roots near the soil surface, the less the damage caused by root-feeding scarabs apply widely. Efforts should be made to encourage a more scattered distribution of *C. zealandica* larvae either by irrigation or by increasing the water holding capacity of the soil in areas prone to the attack of the insect.

Depletion of organic compounds present in the cold and hot water extractions of the SOM may increase the likelihood of larval herbivory and to induce plant damage (Section 4.3.2.6 in Chapter IV). Results from the present study suggest that the amount of organic compounds present in the labile fractions of SOM (cold and hot water extractions) plays an important role in determining the obvious pattern of pasture damage caused by *C. zealandica* larvae. Amounts of total C and total N present in cold and hot water extractions combined, showed that in young pastures there was 45% less C and 37% less N than the oldest pasture. The same proportion (about 10%) of the total C and N was present in the labile SOM fraction of both young and old pastures. The labile fractions of SOM have a quicker incorporation into the biological soil systems than that of humic substances (Stevenson 1982). Stable SOM fractions (humic acids, fulvic acids and humins) therefore, are likely to have less influence on the feeding behaviour of *C. zealandica* larvae and the process of amber disease infection. However, the relationships between labile and stable SOM fractions needs to be explored further. Microcosm experiments, may be of great value in understanding the complex relationships observed between humic acids, fulvic acids and humins with the soil biota.

An important factor that has been excluded during mesocosm experiments in the present study was the stock grazing pressure on pasture plants. Wightman (1979b) simulated this factor in a mathematical model and suggested that its interaction with the plant damage caused by *C. zealandica* larvae may lead to a complete depletion of above ground primary production. Ridsdill-Smith (1977) has found that under glasshouse conditions, in defoliation treatments, larvae of *S. nigrolineata* reduced green foliage yield by 15-40% with 3-12 larvae per pot respectively. This author reported on similar cases in which the root-pruning by scarabaeid larvae reduced foliage production in proportion to the frequency of defoliations. Frequent defoliation and other consequences of grazing, such as stratification of soil nutrients, could influence root patterns sufficiently to affect their susceptibility to scarab feeding (Davidson

Although sheep grazing and an average background density of 230 healthy larvae m⁻² occurred simultaneously in the pasture with the highest content of SOM at Winchmore, no obvious symptoms of plant damage were observed in the present study. Severe plant damage was not observed either during or after the mesocosm experiments in all studied field sites in two years of the present study (i.e. 1992 and 1993). (P. Cunningham, comm. pers.). According to Jackson et al., (1989), a viable option to minimise *C. zealandica* larval damage is to adopt an "old pasture" permanent pasture system. This strategy has been successful on the low input farmlet (S-Block) at Winchmore (Moss, 1987). According to Jackson et al. (1989), pastures in this farmlet, are now more than 30-years old and have shown high production levels and little *C. zealandica* damage despite the fact that high densities that have been recorded.

The effect of the manipulations during the mesocosm experiments was probably more detrimental than beneficial on plant growth and vigour. This effect may account for the contention that even during unfavourable conditions, plants were able to stand the high pressure imposed by larval herbivory of *C. zealandica* larvae. Growth of living roots, growth of herbage and the content of C and N in aerial and below-ground plant parts should have been determined also from soils in the adjacent area of the mesocosm experiment. Changes in this variables would give an indication of the effect that the manipulations had on plant growth. Comparisons between defoliated and non-defoliated treatments under different *C. zealandica* larval densities may contribute to simulate the effect of different stocking rates. It should be borne in mind, however, that experimentally induced defoliation may not be a faithful simulator of animal grazing.

The average pasture in Canterbury is usually cultivated after 8 years and during this time maximum plant losses due to larvae of *C. zealandica* have accumulated (Jackson et al., 1989). By incorporating soil amendments, at appropriate rates, in young pastures or by including pastures in the middle of cropping rotations, at least for one year, the levels of SOM may be maintained or increased.

Applications of whey to the soil, as a source of organic compounds may, by itself, increase pasture

production (Section 5.3.3 in Chapter V). At least under soil microcosm and mesocosm conditions, a positive interaction between herbage production and quality with the presence of *C. zealandica* larvae occurred after whey applications were made. If moderate-dose bacterial applications are combined with whey inputs, the positive interaction established between the insect and the plant may even enhance the productivity of the soil system.

Davidson and Roberts (1968a) suggested that any increased vigour through fertilization of ryegrass and clover did not enable these plants to outgrow soil scarab root feeding or to increase herbage production. These authors mentioned that the effect of additional nutrients and moisture tended to create deeper roots, and by so doing the damage at any fixed depth was greater in the vigorous than in the less vigorous plants. Up to 80% damage to roots was observed when *R. morbillosa* larvae were feeding at the top 2.5 cm regardless of the fact that a fertiliser input has been applied (Davidson and Roberts, 1968a). These authors acknowledge: "This apparently anomalous result may apply only where larvae eat all available roots at a certain depth". Under field conditions, the assumption that larvae eat all available roots at a certain depth may only be fulfilled for particular patches of pasture where high larval aggregations occur in the top five cm of soil. This effect, therefore, may be the result of either low larval dispersion during the early third instars or soil moisture gradients in the case of *C. zealandica* larvae. Even though old pastures have a lower probability of displaying *C. zealandica* larval damage, larval aggregations in the top 5 cm of soil may take place regardless of the SOM status and produce pasture damage (East and Kain 1982).

Plant damage caused by *C. zealandica* larvae seems to be dependent on the interactions of several environmental factors that occur simultaneously in the pasture ecosystem. The mechanism of damage caused by this insect should be better understood to improve forecasts of possible outbreaks.

6.4 Interactions between SOM and *C. zealandica* larval growth and survival

Larvae of *C. zealandica* adapt their feeding habits and rate of consumption to the surrounding soil but they seem to obtain a benefit from rhizophagy. Growth during the third instar was 23% higher in the

soil with the lowest SOM content under microcosm conditions (Section 2.3.5 in Chapter II). This result suggests that larval carrot consumption contributed to larval nutrition. This was particularly evident in the healthy larvae from the control group in the soil with the lowest content of SOM. According to Wightman (1972b), examination of mid-gut size and contents in *C. zealandica* larvae suggest that they feed primarily on living plant material and ingest little soil. However, Wightman (1972a) recognized that the addition of SOM to rearing media has beneficial effects on larval survival. Addition of sieved horticultural peat was found to stimulate feeding and increase weight gain of larvae of *Heteronychus arator* (Fab.) and in this study organic matter was considered to be a phagostimulant (King 1977).

A positive relationship between body weight in *C. zealandica* larvae, pupae and adults, with fecundity and development has been suggested by Farrell (1972 and 1973). Therefore it can be suggested, that a higher root herbivory in *C. zealandica* larvae may also be associated with an increase in survival, fecundity and development rate. Larvae of *S. nigrolineata* selectively ingested SOM from soil with no plants but the presence for living roots produced increases in the amount of organic matter in the gut of larvae, the amount of SOM removed from the food in the gut, the rate of ingestion and the rate of larval growth. It was suggested that larvae of *S. nigrolineata* preferentially select and feed on living roots (Ridsdill-Smith, 1975).

Under field mesocosm, *C. zealandica* larval growth increased significantly in soils with low amounts of SOM where the ingestion of living roots was greater and a lower level of disease has occurred (Section 3.3.5 in Chapter III). Significant negative correlations were observed between larval growth and most of the SOM fractions studied. These patterns may be due either to a reduced ingestion of living roots or to an increase in the probability of infection by entomopathogens in soils with high SOM content (Section 3.3.5 in Chapter III). In the laboratory, Davidson and Roberts (1968a) observed that scarab larvae of *Rhopaea morbillosa* Blackb. and *Anoplognathus* spp. gained live weight where 4% of soil was replaced with manure and the reduction of live weight gain in the absence of plants was not significant.

During the present study, although the incorporation of major nutrients was made to the soil through the addition of whey, larval growth was not significantly affected (Section 5.3.1.1 in Chapter V).

This result suggests that *C. zealandica* larvae either do not take short-term advantage of this input or compensate for the low nutritional quality in the food by modifying the rate of consumption. Third instar larvae of *S. nigrolineata* are able to reduce their feeding rate in dry soil when roots were not available. These larvae gained weight in the presence of plants and maintained their initial weight in wet soil and lost weight in dry soil in the absence of plants (Risdsill-Smith and Porter, 1980). Soil melolonthid larvae may have developed the ability to compensate their nutritional status by adjusting larval development in seasonal patterns. Kelsey (1970) states: "When third instars of *C. zealandica* collected in the field from sites in the North and South Islands of New Zealand and reared in an artificial soil in a Nelson laboratory, there was a surprisingly uniform emergence period for adults from the five Districts' material". The synchronization of the life cycle in *C. zealandica* populations in particular localities according to the seasonality may be a selective trait for a reproductive success during the mating period.

Although non-significant correlations were obtained between the growth of healthy *C. zealandica* larvae and soil microbial biomass under field mesocosm conditions (Section 4.3.2.4 in Chapter IV), soil micro-organisms are expected to be an essential component for the biology of this insect. Sterilized soil produce deleterious effects on growth and survival of *C. zealandica* larvae in the laboratory (Wightman 1972a). However, soil melolonthid larvae such as *C. zealandica*, (Miller, in Sutherland, 1972) *Anomala orientalis* Waterh. (Bianchi, 1935), *Popillia japonica* Newman (Ludwig and Fox, 1935), *Othonnius batesi* Olliff (Davidson and Roberts 1968b) and *Macroductylus mexicanus* (Burm.) (Villalobos, 1992) are able to complete their whole life cycle by living on the organic matter in soil in the absence of living roots under laboratory and glasshouse conditions. It seems that other soil scarab larvae such as *Heteronychus arator* (Fab.) (King 1977), *R. morbillosa*, and *Anoplognathus* spp. also have this ability. Soil-ingesting melolonthid larvae would undoubtedly digest soil microbial matter, whether or not there is a specific microflora in the fermentation chamber (Davidson and Roberts, 1968a). However, results from the present study failed to find any correlations between larval growth and soil microbial biomass (Section 4.3.2.10 in Chapter IV). The fumigation-extraction method used in the present study may only give a rough estimation of the total microbial activity in the soil. Future research should address specific components of the soil microbial community, particularly in the rhizosphere.

In Chapter I (Section 1.2.3), a review of the spectrum of alternative sources of food that *C. zealandica* larvae may use in the soil under field conditions has been made. Richter (1958) and Morón (1983) have recognized differential feeding preferences on living roots or organic matter among subfamilies of Scarabaeoidea. Halffter and Edmonds (1982) and Cambefort (1991) suggest that in Scarabaeinae, the saprophagous habits are a primitive character of the family. This evolutionary trait may also be shared by their Melolonthidae relatives in which a trend to increase the degree of rhizophagy would be the modern character (Crowson, 1981; M.A. Morón pers. com.). Through agronomic practices, the environment of *C. zealandica* larval populations may be manipulated to encourage the build up of SOM and the diversification of their feeding habits.

The higher susceptibility of young pastures to *C. zealandica* larval herbivory in comparisons to old pastures may be partially due to their SOM content. During the mesocosm experiment described in Chapter III, higher values of C and N were recorded for nonhumic substances in soil from the experimental units in the oldest pasture in comparison with the younger pastures (Section 4.3.1). These results showed that the depletion of nonhumic substances in soil was associated with an increase in the larval herbivory of *C. zealandica*. In sites of low SOM status or where melolonthid larvae of *R. morbillosa* and *Anoplognathus* spp feed for long periods and thus deplete SOM, plant roots may be found to be a more important source of food (Davidson and Roberts, 1968a). Ridsdill-Smith and Roberts (1976) have observed a decrease in the percentage of SOM in the foregut and in the assimilation of SOM from guts with increasing density in the melolonthid larvae of *S. nigrolineata*. These results suggest that the depletion of the labile fractions of SOM may partially induce larval aggregations in *C. zealandica* and therefore severe pasture damage. However, through artificial application of SOM, increases in this labile fraction may be obtained and by promoting its turnover, *C. zealandica* may contribute to soil fertility. Therefore, the content of nonhumic substances in the soil may be critical in determining the appearance of plant damage caused by *C. zealandica* larvae.

6.5 The contribution of *C. zealandica* larvae to soil fertility

Larvae of *C. zealandica* play an important role in SOM mineralization in New Zealand pastures.

Plant residues (and the micro-organisms associated with their decomposition), as a fraction of the SOM, represent an important component of the diet of *C. zealandica* larvae. Consequently, the insect contributes to their mineralization. Kain (1975) recognized the contribution of *C. zealandica* larva in the mineralization process. Davidson and Roberts (1968b) considered that the primary source of SOM in Australian native pastures used by soil scarab larvae were the roots of grasses, fallen leaves and animal excreta. Evidence from mesocosm experiments in this study suggest that plant residues are an important source of C for larval growth (Section 4.3.2.1 in Chapter IV). Moreover, the presence of *C. zealandica* stimulated the soil microbial biomass under field mesocosm conditions (Section 4.3.2.4. in Chapter IV). This result confirms previous observations made by Yaacob (1967) that *C. zealandica* larvae may stimulate soil microbial activity in a similar manner to earthworms. At the end of the mesocosm experiment, in this study, a synergistic effect was observed between the microbial biomass present in the surrounding soil and the stimulatory effect of *C. zealandica* on microbial communities (Section 4.3.2.4 in Chapter IV).

The assimilation of C and N present in nonhumic substances by *C. zealandica* larvae was observed under field mesocosm conditions (Section 4.3.2.6 in Chapter IV). Wightman and Whitford (1979a) suggested that soil particles contribute about 45% of the dry weight of the gut contents and estimate that 2.5% of the total organic intake is SOM. Because of this estimation, Wightman (1979b) regarded SOM as an environmental factor of minor influence in the biology of *C. zealandica* larvae. However, the fact that this insect remains active in the soil for at least nine months should be taken into account in the estimation of the actual amount of SOM consumed.

In the short-term, the presence of *C. zealandica* larvae in the soil may either induce significant synthesis or catalysis of humic substances. Significant decreases occurred in total C and total N present in humins in young pastures, while significant increases were observed in the old pasture. These opposite effects may be an indirect contribution of larvae through the stimulation of the soil microbial communities. Microcosm experiments may also be useful for clarifying these intricate relationships.

6.6 The effect of whey applications to the soil in pasture sustainability

Whey applications produced a positive effect on the build up of the total C and N present in hot water extractions of SOM under field conditions (Section 5.3.1 in Chapter V). The addition of N and other major nutrients in a short term available form, is likely to be important in affecting the dynamic relationships established among soil, plant, micro-organisms and insects. However, whey applications may impose dramatic changes on the soil dynamics of the pastoral agroecosystem. Significant increases in total C and N present in hot water extractions of SOM were observed under mesocosm conditions (Section 5.3.1 in Chapter V). Accumulation of the labile fraction of SOM in pasture soils should be encouraged and a better knowledge of its dynamics and relationships with more stable SOM fractions is required. Input of major nutrients may increase the probability of occurrence of plant root diseases (Rovira et al., 1990). Plants have particular soil nutrient status. To optimize application rates in strategies including or excluding *S. entomophila* is highly recommended. Investigations are needed on appropriate application rates and methods in relation to the particular conditions of pastures. Decisions about which pasture to be treated should consider its SOM status and priority must be given to soils which are intensively cultivated. Undesirable environmental consequences have occurred through abrupt manipulations of the agroecosystems including biological control (Pimentel et al., 1984).

6.7 Contributions to the optimization of the biological control tactics against *C. zealandica* larvae

An integrated decision-making process will help to optimize the biological control of *C. zealandica* in pastures. Larval densities should not be considered as the exclusive criterion to decide whether control is required or not. Results from the microcosm experiment suggested that a low dose of *S. entomophila* produced an effect similar to that of the high dose. Therefore, research conducted to find an optimum dose for specific soil conditions may be valuable. A higher diversity of indigenous soil entomopathogens should be encouraged. Biocontrol tactics should take into account the possibility of evolution of resistance in *C. zealandica* larvae to *S. entomophila*.

Insect resistance either to chemical or biological insecticides may develop in the short-term. *C. zealandica* larvae have developed resistance to DDT (Hoy 1965), while Colorado Potato Beetle *Leptinotarsa decemlineata* (Say) has developed resistance to the CRYIIIA coleopteran specific delta

endotoxin of *Bacillus thuringiensis* Berl. (M.E. Whalon et al., pers. comm.). Insect susceptibility is a valuable resource that has been squandered and it is becoming increasingly expensive to develop new insecticides (Mallet, 1989).

Inundative applications of entomopathogens may have detrimental effects on the environment (Pimentel et al., 1984). Under mesocosm conditions, inundative applications of *S. entomophila* inhibited the soil microbial biomass and may reduce the effect of other larval entomopathogens. A better understanding of the microbial interactions in the soil may lead to optimize the use of *S. entomophila* as a biocontrol agent.

Beneficial aspects related to *S. entomophila* applications not previously considered, were found in the present study (Section 3.3.3 and 3.3.7 in Chapter III). Herbage and living root production may increase in pastures treated with *S. entomophila* regardless of its pathogenic effect on *C. zealandica* larval populations. *S. entomophila* may encourage the short-term degradation of nonhumic and humic substances, releasing N for plants and soil biota. This effect was particularly evident in soil with a high content of SOM. Synergistic interactions of soil entomopathogens should be better understood and encouraged. Other factors such as secretions of plant hormones by microorganisms may also be involved in the positive effects observed in plant production after *S. entomophila* applications (see Sections 3.3.3.3 and 3.3.3.7 in Chapter III).

The combination of low inputs of animal or plant residues with applications of moderate doses of *S. entomophila* is recommended. Organic inputs containing clean, rich and diverse amounts of organic compounds may be of great benefit for New Zealand pastoral agriculture and the environment. This practice may contribute to plant nutrition, diversification of the soil biota, including *C. zealandica* natural enemies or optimizing *S. entomophila* applications under field conditions (Section 5.2.3 in Chapter V). Survival of the soil-borne *S. entomophila* and *Serratia protamaculans* may be enhanced by applying whey to the soil in pastures containing high larval densities and a high SOM status. The search for an optimum application rate of whey to pasture soils should be conducted under particular situations.

On the basis of the results from the present study, a *S. entomophila* dose of 1.5×10^5 cells g^{-1} soil (between the Low and Medium doses from the microcosm experiment described in Sections 5.3.1, 5.3.2 and

5.3.3 in Chapter V) is recommended in combination with a rate of 40,000 L ha⁻¹ of whey. These inputs might result in a moderate reduction (40-80%) of *C. zealandica* herbivory and the encouragement of the saprophytic and entomopathogenic activity of the indigenous soil biota. All these effects may actually produce an increase in plant production and may not be detrimental for the natural regulation of the insect. These actions may also increase the build up of the quantity and quality of SOM and may result in an increase in pasture sustainability. The use of whey as a cheap substrate in fermentations of *S. entomophila* should be investigated and this may reduce costs in the preparation of the commercial product.

6.8 The problem of the economic or action threshold levels

An essential step in any rational insect pest management strategy is the evaluation of an economic or action threshold levels. According to Kain and Atkinson (1975), the major problem associated with detailed studies on insect pest assessments studies is the translation of losses in pasture quality and quantity into animal production. This situation has also been recognized for Australian pastures where few studies provide density thresholds as a criterion to determine the economic need to control insects, or to extrapolate the pasture losses into estimates of the total losses (Allen 1987). The problem of establishing accurate economic threshold levels for grassland insects is aggravated by the low value of the pasture crop and the relatively high cost of insecticidal control (Kain and Atkinson 1975). This together with the unpredictable nature of agricultural prices makes the establishment of accurate economic threshold levels extremely difficult. Chapman (1990) considers that rather than using an economic threshold level an action threshold level may be used as a guide to indicate when controls should be applied, but not whether the controls would improve economic return. Action threshold levels are derived from the relationship between pest density and losses of pasture quality and yield, and usually involve no analysis of the value of pasture or animal production losses or cost of control methods (Chapman, 1990). Garnham and Barlow (1993) consider that for individual farms with average densities of *C. zealandica* greater than 150 larvae m⁻² significant losses may occur. Within the framework of the sustainable management of *C. zealandica* proposed in this study, a reliable indicator of insect damage would have to take into account soil environmental conditions and should be based more on experimental work combining different approaches

under laboratory and field situations than on theoretical estimations. This combination of experimental approaches, during the present study, helped enormously to correct the interpretation of isolated facts. Apart from the cost of insecticides, an economic value should also be calculated for the cost of repairing negative ecological consequences that the control practices for this insect may have. The positive contributions on soil fertility that the insect may produce and the cost of disposal of organic wastes may also have to be expressed in economic terms. By gradually increasing levels of SOM through appropriate pasture-cropping rotations or SOM inputs, the ability of the plants to withstand higher densities of larvae before evident symptoms of plant damage become apparent would be expected to increase.

6.9 Conclusions

Results from the present study showed that possible ecological processes involved in the reduction of plant damage caused by *C. zealandica* observed under high SOM conditions in pastures are: (a) SOM is an alternative direct or indirect source of food for the larvae; (b) SOM encourages the presence of *C. zealandica* natural enemies; (c) SOM offers better nutritional conditions for plants, contributing to compensate larval herbivory; (d) the spatial distribution of *C. zealandica* larvae is less likely to produce plant damage in high SOM conditions. However, considering the results of the present study, an additional factor must also be considered: (e) the larval herbivory and other below-ground activities of *C. zealandica* may have a positive direct or indirect overall effect on plant herbage production and quality, particularly in the presence of a rich SOM environment. In the present study, an attempt was made to isolate the independent effect of each one of these factors. It was found that while these factors acted independently, interactions among them were complex and dynamic. The SOM effects occur in a multifactorial way in nature and their overall effects are greater than those due to the addition of the individual components. The combination of different research approaches is essential to understand better these relationships.

To enhance the biological diversity in the soil, a moderate dose of *S. entomophila* would be recommended in biological control inundative applications. If a complimentary application of whey is made, the growth of indigenous *S. entomophila* could be expected. Moreover, in young pastures, the enrichment and accumulation of SOM will provide productive and sustainable soil conditions.

Replications of sites in future experiments would be recommended to achieve a more general conclusion about the effect of pasture age on *C. zealandica* damage. Because of the limitations of achieving reliable replication in pastures with unique agronomic histories these experiments may also give unexpected outputs. The effect that inorganic compounds such as micronutrients, and other soil physio-chemical properties not associated to the SOM on these dynamic interactions remain to be explored (Villani com. pers.) These complex interactions are, therefore, a challenge for experimental researchers.

An increased diversity in our perceptions of nature may help to promote diversity in the soil, and by increasing soil diversity through human activity, the diversity in the cultural environment of New Zealand may also be enhanced. The wide-spread use of panaceas should be avoided as negative consequences in the environment are often produced by this approach. To think globally and to act locally should also make sense in the management of *C. zealandica*. The complementarity of experimental approaches adopted during the present study, confers the strength to the statement that soil organic matter does matter in the sustainable management of grass grubs in New Zealand pastoral agriculture.

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METHODS OF ANALYSIS AND QUANTIFICATION OF SOIL ORGANIC MATTER**Sampling of Soil Organic Matter**

Soil from selected pastures was collected to determine total C and total N in different soil organic matter (SOM) fractions. In the laboratory, fresh soil was kept at 5°C before soil microbial biomass and soil moisture were measured. A subsample of this soil was air-dried, or dried at 80°C for 24 h, and was used to determine total C and N in SOM fractions and plant residues. This subsample was rolled and subsequently passed through 2 mm followed by a 1 mm mesh sieves. Soil from the experimental units was pooled and a duplicated determination of total C and N was taken for each SOM fraction.

Soil moisture

Soil moisture estimations are important to refer the results of the different components of the SOM analysis on an oven-dried basis. This determination is based on the method of difference in weight between wet and oven-dry soil (105°C, 24 h) using the formula:

$$\% \text{ Soil moisture} = \frac{W_1 - W_2}{W_2} \times 100$$

Where:

W_1 = fresh weight

W_2 = oven-dry weight

Total C and N in soil

The method of Dumas for combustion of dry soil was used to determine total C and N in soil (Grewal et al., 1991). This method gives an actual measurement of total C and N using a continuous flow C-N analyzer, connected to an isotope ratio mass spectrometer (ANCA-MS, Roboprep, Europa, United Kingdom (Grewal et al., 1991; K.M. Goh pers. comm.). This procedure involved the following steps:

- 1) Take a sample of 10 g fresh soil.
- 2) Oven-dry overnight at 80°C.
- 3) Pass through 2 mm followed by 1 mm mesh sieve.
- 4) Grind the soil to pass through a 250 microns sieve.
- 5) Take a small sample, weigh and determine total C and total N in the mass spectrometer.

Soil Field Capacity

Soil field capacity was determined by using the Haines' apparatus. Suction was applied to the soil water in a hanging water column to determine the relationship between the soil water content and the soil water potential from saturation down to about -100 cm of the column. This relationship allowed the calculation of field capacity, which was estimated for a field soil as the water content in a soil sample (sieved in a 2 mm screen) at -0.1 bar=-100 cm (K. Cameron pers. comm.). This determination was used in all soil microbial biomass estimations and involved the following steps:

- 1) Take a 50 g fresh soil sample.
- 2) Fill the tube and the funnel of the Haines' apparatus with water and calibrate the burette to 0 and the water level a few mm below the beaker's filter.
- 3) Pack the soil in the beaker with a rubber bung.
- 4) Move up gradually the burette and wait until the water level is just below the soil surface.
- 5) Wait for 15 minutes until the soil is saturated with water.
- 6) Move down the burette and place it at 50 cm (measure with a ruler) from the 0 mark.

- 7) Wait for 30 minutes and record the water level in the burette. This measure will be equivalent to the porosity of the soil sample.
- 8) Move down the burette and place it at 100 cm from the 0 mark.
- 9) Wait for 1 h to allow the soil moisture and water level to equilibrate and record the water level.
- 10) Take a sample of this soil and make a moisture determination (24 h at 105°C). This value represents the 100% of the water holding capacity (field capacity).

Soil microbial biomass

The fumigation-extraction method proposed by Jenkinson and Powlson (1976) modified by Vance et al. (1987); Ocio et al. (1991) and L. Nguyen (pers comm.) was used. Before soil microbial biomass determinations were made, soil collected from the field was analyzed either immediately, stored overnight, or stored until use at 5°C. The following steps were carried out:

- 1) Use fresh or stored samples (overnight at 5°C).
- 2) Sieve through 2 mm mesh screen.
- 3) Take a measure of moisture determination on a soil sample as described above.
- 4) Determine Water Holding Capacity (W.H.C.).
- 5) Incubate at 25°C for 10 days at a W.H.C. of about 40%.

Non-fumigated soil

- 6) Take duplicated 5 g samples of soil after incubation.
- 7) Take a soil moisture determination.
- 8) Extract with 20 ml 0.5M K_2SO_4 (1:4 soil:extractant ratio) by shaking for 1 hour.
- 9) Filter through Whatman No. 1 filter paper.
- 10) Recover filtrate.
- 11) Take an aliquot of 1 ml and place in a capsule tin in MacPherson's Nitrogen Inert Atmosphere Evaporator (Plate 4) until total dryness is reached.
- 12) Roll up the sample into the shape of a round ball to analyze in the mass spectrometer for total C and

N.

13) If a large number of samples is to be analyzed, take a known volume of the filtrate (20 ml) and follow the method of Walkey and Black (1934) for a measure of oxidizable C.

Fumigated soil

14) Simultaneously follow steps 1 to 7, take a duplicated subsample of 250 g moist soil after incubation,

15) Introduce each sample into a 400 ml glass beaker.

16) Introduce both samples in a large desiccator (30.5 cm i.d.) lined with moist filter paper.

17) Place a beaker containing 50 ml alcohol free chloroform and few anti-bumping granules in the centre of the desiccator.

18) Connect the desiccator to a water pump and evacuate.

19) Close the tap after the CH_3Cl boils vigorously for 2 min.

20) Store in the dark at 25°C for 18-24 h.

21) Remove the beaker containing CH_3Cl .

22) Make 6 evacuations of 10 min each (or more if the remaining CH_3Cl has not yet been removed), with the water pump.

23) Follow steps 8 to 12.

Determination of total oxidizable organic C

Soil microbial biomass extracts were analyzed using the tritiation method of Walkey and Black (1934). This method determines the amount of oxidizable C which is only a part of the total organic C. Grewal et al. (1991) suggested an empirical factor to convert oxidizable C to total organic C. According to these authors, the relationship between total organic C (T) from the Dumas combustion method and oxidizable C obtained by Walkey and Black (1934) (W) is described by a linear equation:

$$T=0.126+1.25W$$

Determination of total N

Aliquots of 1 ml of the extracts were evaporated an inert N gas atmosphere using the MacPherson's apparatus. Total N in soil microbial biomass extracts was measured by the Dumas combustion method.

Soil microbial biomass C and N calculations:

$$\text{Biomass C} = 2.64 E_C$$

where: E_C = difference between C extracted from fumigated and non-fumigated soil expressed as $\mu\text{gC/g}$ of oven-dried soil (Vance et al., 1987).

$$\text{Biomass N} = 2.22 E_N \text{ (Ocio et al., 1991)}$$

where E_N = difference between total N extracted by 0.5 M K_2SO_4 from soil fumigated and total N extracted from non-fumigated soil for 24 h (Vance et al., 1987).

Note: Extracts should be preferably analyzed immediately after extraction although they can be stored at 1-2°C for 1 or 2 weeks if necessary. A white precipitate (presumably hydrated CaSO_4) forms on storage from some soils. A few drops of a solution of 50 ppm of HgCl_2 helped to prevent anaerobic growth of micro-organisms. Bremner and Douglas (1971) found that between 40-50% of urease activity was inhibited by an equivalent concentration of HgCl_2 in soil.

Soil organic matter fractionation

According to Stevenson (1965), the methods used to fractionate SOM can be placed into two categories: (1) those which used to fractionate and estimate compounds characteristic of plant tissues, and (2) those based on the division of the humus into subclasses possessing similar solubility characteristics. The great difficulty in all fractionation procedures is that the methods employed either separate out products which are not definite chemical entities, or they form artifacts which do not have the properties of the original material. Complete extraction of all the SOM components by classical reagents appears to be impossible and each reagent may alter differently the physicochemical properties of some of these

components (Mortensen 1965 in Stevenson, 1965). Nevertheless, the various fractionation procedures have proved useful for studying SOM (Stevenson, 1965). In the present study, a gross chemical fractionation of SOM was employed using the following procedures (K.M. Goh, pers. comm.):

Cold and Hot water-soluble C and N

"Free" biochemicals such as amino acids and sugars (Stevenson 1982) and other monomeric labile compounds which are readily mineralizable (Goh pers. com.) are usually grossly extracted by a "cold-water" (20°C) extraction. The hot water extraction of the SOM contains simple organic compounds and slightly decomposable carbohydrates including microbial biomass. It reflects the level of organic matter supply to the soil and by this the ability of soils for releasing available N. Water-soluble total C and N were determined by modifying the procedure described by Haynes et al. (1991) for the cold water extraction and the method for the hot water extractable C followed by Korschens et al. (1990) (K.M. Goh pers. comm.).

Cold water extraction

To determine the amount of total C and total N present in the cold water extraction of SOM, the following steps were carried out:

- 1) Determine soil moisture on a subsample of soil.
- 2) Collect 10 g air-dried soil (correct for oven-dry weight).
- 3) Add 200 ml cold water (20°C).
- 4) Shake for 16 h.
- 5) Centrifuge at 2000 rpm for 20 min.
- 6) Filter 5 ml on a Whatman No. 42 filter paper.
- 7) Set aside the solid fraction.
- 8) Collect the filtrate.
- 9) Add one or two drops of H₂SO₄ and check for red colour with a litmus pH paper.
- 10) Take 40 ml of the filtrate and place it in a boiling bath for slow evaporation.
- 11) Transfer filtrate (adjust to 2 ml in a volumetric flask).

- 12) Add one drop of a 50 ppm solution of HgCl_2 and store in the refrigerator until determination of total C and total N are made.
- 13) Before mass spectrometer determinations, add small particles of a chopped pellet of NaOH to the extracts until the pH reaches 5-6.
- 14) Take an aliquot of 120 μl and place it in a previously weighted capsule tin containing 6 pieces of absorbent paper.
- 15) Place the capsule tins in an evaporator for 24-48 hours until total dryness is reached. Evaporation could be accelerated by placing a few grams of phosphorous pentoxide in a Petri dish in the bottom of the evaporator.
- 16) Roll up the capsule tins carefully into the shape of a round ball.
- 17) Measure total C and total N in the mass spectrometer.

Hot water extraction

- 1) Take the solid fraction remaining after filtration in step 5 of cold water extraction
- 2) Prepare a proportion of extraction by using 1:5 soil-water ratio.
- 3) Boil the soil under reflux for 1 h at 95°C.
- 4) Centrifuge at 2000 rpm for 20 min.
- 5) Filter 5 ml on a Wathman No. 42 filter paper.
- 5) Set aside the solid fraction for humic substance determination.
- 6) Take 20 ml of the filtrate, add one or two drops of H_2SO_4 concentrated and proceed as for the cold water extraction.
- 7) Follow the steps 11 to 17 of the cold water extraction procedure.

Humic substances

Humic acids

Stevenson (1982) define humic substances as a series of relatively high molecular weight, brown to black coloured substances formed by secondary synthesis reactions. These materials are dissimilar to

the biopolymers of micro-organisms and higher plants including lignin). The method proposed by Goh and Molloy (1978) was followed to separate these compounds. The extraction of humic substances involved the following steps:

- 1) Take the solids from step 5 of the hot water extraction.
- 2) Add 400 ml 0.1M $\text{Na}_4\text{P}_2\text{O}_7$:0.1M NaOH.
- 3) Shake the suspension for 5-10 min by hand.
- 4) Allow the suspension to settle overnight in an atmosphere of N_2 by passing N_2 gas into the bottle to displace air.
- 5) The next day centrifuge the suspension at 10,000 rpm/10 min (or 5,000 rpm/20 min).
- 6) Recover the supernatant and take the solid fraction to determine humins.
- 7) Add 6M HCl gradually to bring the pH to 1.0 and record the total volume incorporated (use 20 ml for Winchmore samples).
- 8) Allow the precipitate (humic acid) to settle overnight.
- 9) Centrifuge (10 min at 5,000 rpm) the suspension and recover the humic acid.
- 10) Wash the humic acid with 40 ml 3M HCl to recover the soil.
- 11) Shake by hand for 2 min.
- 12) Centrifuge for 10 min at 5,000 rpm.
- 13) Repeat twice the steps 10, 11 and 12.
- 14) Wash once with 40 ml distilled water.
- 15) Centrifuge and add 20 ml of distilled water to collect the precipitate.
- 16) Weigh an empty 50 ml glass beaker.
- 17) Dry overnight in an oven at 70°C.
- 18) Weight after drying.
- 19) Collect the precipitate and grind in a mortar.
- 20) Measure total C and total N in the mass spectrometer.

Fulvic acids

- 21) Take the filtrate from step 9.

- 22) Filter if there are solid particles remaining.
- 23) Take a sample of 20 ml.
- 24) Place the samples in a boiling bath for slow evaporation.
- 25) Follow steps 11 to 17 of cold water extraction.

Humins

- 26) Take the solid fraction from the step 6 of humic acid extraction.
- 27) Dry this fraction at 105°C for 24 h.
- 28) Grind a sample of this fraction in a mortar.
- 29) Measure total C and total N in the mass spectrometer.

Plant residues

Dry matter of plant residues was separated after washing the soil in a set of sieves. Plant residues present in soil were weighed, dried and ground (<250 microns) to determine total C and total N. This method was designed by the modification of the washing-flotation method described originally by Ladell (1936) for separating soil fauna (Goh and Villalobos, unpubl.). The following steps were carried out:

- 1) Take the fraction of 2.8-2.00 mm of sieved soil from the experimental unit.
- 2) Weigh and place this soil in 1 mm and 650 µm sieve.
- 3) Wash the soil in a water jet until soil particles are separated from organic residues.
- 4) Collect the organic residues with a cotton gauze in a solution of MgSO₄ (density 1.1).
- 5) Wash in tap water to remove residues of MgSO₄ salt.
- 6) Dry in an oven at 80°C for 24h.
- 7) Grind to pass through a <250µm sieve.
- 8) Measure total C and total N by Dumas combustion as described above.

PASTURE COMPOSITION AT THE BEGINNING OF THE MESOCOSM EXPERIMENT
DESCRIBED IN CHAPTER III.

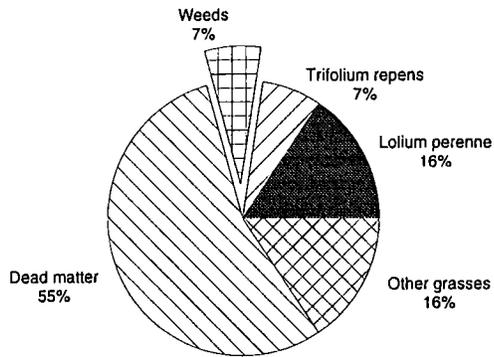
Pasture botanical composition¹ at the beginning of the mesocosm experiment (late March 1992) described in Section 3.2.3 in Chapter III (J. Talbot comm. pers.) in the sites of study at Winchmore Irrigation Research Station. S14 = 39-year-old pasture; A6 = 5-year-old pasture; A1 = 2 year-old pasture.

Site	<i>Lolium perenne</i>	<i>Trifolium repens</i>	Weeds	Dead matter	Other grasses	Total
S14	34.12	15.53	14.77	121.2	34.37	220.01
A6	36.41	64.93	6.37	37.69	0.00	145.40
A1	24.95	132.14	133.44	5.85	0.00	296.41
LSD _(0.05)	35.84	34.40	52.49	9.23	16.29	79.32

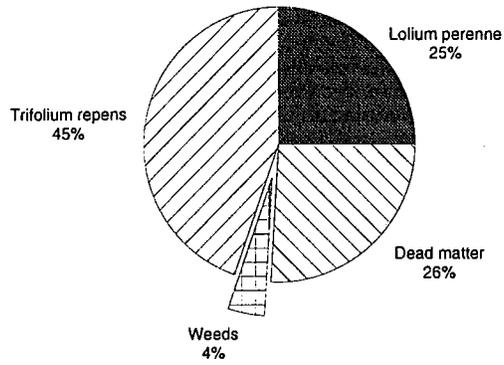
¹values in g dry matter m⁻²

Proportion of the DM of the main species in the botanical composition of the pastures sites studied during the field mesocosm experiment at Winchmore. Data from late March 1992.

S14



A6



A1

