POPULATION DYNAMICS AND PEST ASSESSMENT STUDIES OF GRASS GRUB (COSTELYTRA ZEALANDICA (WHITE), MELOLONTHINAE) IN THE NORTH ISLAND OF NEW ZEALAND

A thesis
presented for the degree of
Doctor of Philosophy

University of Canterbury

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Lincoln College
1975

Stages in the life cycle of Costelytra zealandica (White) with pasture damage, caused by the larval stages, in the background.

Top left to top right; adult, eggs and first, second and third instar larvae, pupa, teneral adult and adult in flight.

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ABSTRACT

Studies of the common grass grub (Costelytra zealandica (White)) covered two major aspects, population ecology and pest assessment. The object of these studies was to investigate the feasibility of developing models, predicting population density and estimating the associated losses in pasture productivity, as a basis for the formulation of a pest management programme.

I POPULATION STUDIES

Studies of natural populations of soil insects such as grass grub require the development of accurate and efficient mechanical methods of sampling and extraction as well as a statistically precise and efficient sampling plan.

1. Sampling

a) Mechanical aspects. A split barrelled manually operated corer was developed for sampling grass grub. This implement permitted 175 to 245 samples to be taken daily by one man. Sample sites were drawn randomly within strata by a computer as rectangular co-ordinates and converted to polar co-ordinates, originating from the centre of the 20, 20 x 20 m subplots in each study plot. Sites were then sorted and listed in numerical order based on the polar ordinate. The use of the computer allowed large savings in the time taken to draw and list random co-ordinates and made it possible to randomly draw samples from irregular shaped strata. A large compass wheel enabled sample sites to be located rapidly in the field.

Sampling times were based on beetle flights, seasons and, in the case of pupal sampling, pilot sampling.

Extraction of all the developmental stages of grass grub from soil was accomplished by means of a modified Ladell process which gave a 96 to 98% recovery rate and, dependent on the stage of the insect, a processing rate of between 4.4 and 6.5 man minutes per

sample. The extraction process did not impair the insect's viability.

b) Statistical aspects. The main consideration in the development of a sampling plan was to obtain, for the lowest cost, an estimate of population density with a precision of ± 10% SE. The variance minimizing efficiencies of different methods of stratified sampling and sample allocation were assessed. The different strata used were based on the division of the study plots by; subplots, damaged and undamaged pasture and damaged and undamaged pasture within each subplot. The most efficient method of stratification was influenced by the accuracy with which damaged areas of pasture could be defined and the population level. Although optimal allocation of samples gave spectacular gains in efficiency, the use of this method of sample allocation in the field was not practical.

The adoption of a flexible rather than a rigid sampling plan in which the strata varied with population level and the ease with which pasture damage could be identified, enabled, except at very low population levels, the required level of precision to be attained with the resources available.

Sample size was inversely related to population density but was highest for the egg stage in which grass grub are most aggregated. The sample size required to attain the level of precision sought ranged between 131 sample units, for high third instar larval populations, and 2450 units for low egg populations.

2. Population Dynamics

Over the period of study at Takapau marked changes were not evident in the flight behaviour of female beetles. No parasites, or important invertebrate or vertebrate predators were found at Takapau and the effect of disease organisms on Takapau populations was considered unimportant. In the Waikato region, however, where the native milky disease may infect up to 40% of the third instar grass grub population, disease is considered to be an important factor in population regulation.

Analyses of age specific mortalities within generations indicated that mortality over the autumn and winter was strongly density dependent and above a certain threshold density compensated completely for change in population density. Laboratory experimentation showed that the major contribution to density dependent mortality arose from larval combat which increased as food supply declined. From this observation the hypothesis was proposed that weather conditions which influence the survival of damaged plants and therefore dispersal and aggregation of larvae, influences larval combat. From field data it was found that the autumn threshold for combat mortality over the autumn-winter period was linearly related to pasture production over this period.

At Takapau when summer soil moisture levels were in excess of wilting point larvae were found feeding close to the surface. Under these conditions combat mortality occurred and thus summer mortality was density dependent. When soil moisture levels approached and fell below wilting point summer mortality became linearly related to soil moisture. At Rukuhia as distinct to Takapau highest soil moisture levels under drought conditions were found close to the surface. As a result, larvae did not descend in the soil profile in response to drought and for this reason summer mortality at Rukuhia, under drought conditions, was attributed to the direct effect of the lethally high soil temperatures found near the surface.

It was concluded from these studies that grass grub populations at Takapau fluctuate in response to low summer or high spring soil moisture levels and are regulated in relation to food supply by larval combat. The effect of summer mortality on generation mortality is moderated by the density dependent nature of combat mortality of larvae in autumn-winter. Consequently, mortality in the summer only influences generation mortality if it decreases population density below the threshold density at which autumn-winter combat mortality occurs.

The knowledge gathered from these studies explains why grass grub populations are so difficult to control with transient insecticides and has suggested better ways of using insecticides.

A population model was developed for predicting population changes within and between generations at Takapau. The model was based on the relationships described above and gave encouragingly accurate results. However, it did highlight the need for more accurate definition of the relationships between the major mortalities identified by these studies and the important variables which affect them such as soil moisture, rainfall, pasture production and population density.

II PEST ASSESSMENT STUDIES

A technique which does not involve the use of insecticides was developed for measuring losses in pasture production arising from grass grub damage. This technique is suited to areas of relatively low summer rainfall where the occurrence of grass grub can be located visibly by pasture damage. The method involves the division of a paddock into, and the measurement of herbage production from, three strata; undamaged areas, areas damaged by previous generations and areas damaged solely by the current generation. The sizes of the strata were measured by aerial photography or estimated from a growth curve of the area visibly damaged or, the relationship established between the area of visible damage and insect density. Given the size and herbage production from each stratum the total production for each plot or paddock can be estimated.

Pasture damaged by grass grub showed a deterioration in botanical compostion with an increase in litter and grass weeds and a decrease in white clover. Damaged pasture had a higher percentage of bare ground and in the autumn and winter was poorly utilized by livestock. Seasonal herbage losses were highest in autumn and winter, although the highest loss in monthly herbage production was recorded in late summer. In newly damaged areas seasonal losses in herbage production of 70% and 74% were recorded in autumn and winter respectively whereas the respective losses in the areas damaged by previous generations in autumn and winter were 54 and 34%. Recovery, in terms of pasture production, of damaged areas was complete in late spring.

Observations made in these studies showed that within the strata described the severity of damage caused by low and high populations of grass grub was not significantly different, and that increased losses in pasture production associated with higher populations resulted from an increase in the damaged area rather than an increase in the severity of damage in damaged areas. Based on this information losses in pasture productivity caused by different population levels were estimated from the relationship between population density and the area of visible damage and the mean pasture production from each stratum.

Two models were developed which simulated the growth, over successive generations, in area of visible damage under environmental conditions which favoured the increase or maintenance of grass grub populations. Both models describe the growth in area of damage up to a stage where damage was extensive, ill-defined and impossible to measure. One model is based on the relationships between, the size of individual patches of damage and the factor by which these grow over successive generations, and the rate of appearance of damaged areas in the following generation and proportion of the paddock currently damaged. The other model involves the growth curve of May larval populations under conditions which are favourable for population increase and the relationship between population density and pasture damage. The latter model tended to underestimate the actual growth of the area damaged by approximately 20%.

It was found that the establishment of accurate economic threshold levels for different classes of farms run at different intensities and in different regions is attended by many problems. In the light of current knowledge the translation of losses in herbage production into animal production and economic terms for different farming intensities under different climatic conditions will lead to such grossly inaccurate estimates that the worth of pest assessment studies would be lost. From these studies it was concluded that at best pest assessment studies of grass grub could provide the farmer with information that will allow him to predict population density and the associated losses in herbage production. Given this information the farmer is in the position to make more objective decisions, than would otherwise be possible, on the course of action to follow based on his own socio-economic circumstances.

GENERAL INTRODUCTION

The economic dependence of New Zealand on pastoral agriculture is emphasised by the 80% contribution that this industry makes to the country's total export earnings. The evolution of pastoral farming within New Zealand has been characterised by the introduction of high producing exotic pasture species into land cleared from native vegetation; the application of mineral fertilisers, predominantly phosphatic; the use of white clover (Trifolium repens) as a nitrogen fixer in pastures in association with grass species (particularly ryegrass, Lolium perenne) and the efficient utilisation of pasture in situ by the grazing animal (Levy, 1951).

The establishment of a vigorous soil-plant-animal-soil organic cycle, supplemented by annual inputs of fertilisers and improved methods of grazing has allowed increases in stocking rates and soil fertility to a stage where the more productive pastures may grow in excess of 14,000 kg of dried herbage per ha per annum and support over 25 ewes or 3.75 dairy cows per ha.

The extent and rapidity with which pastoral agriculture has developed in New Zealand can be gauged by the increase in area of sown pastureland from 63,200 ha in 1862 to 8.4 million ha in 1970. Against this background of agricultural development an indigenous melolonthine, Costelytra zealandica (White), has emerged from its native habitat in the native tussock grasslands to become established as the most serious insect pest of our improved pasturelands. Like many other scarabaeids, the larvae of this species which is commonly known as grass grub, feed voraciously on plant roots, undercutting the pasture's rooting system and killing annually large areas of pasture. Under native vegetation, populations are usually sparse and in this environment the species is not regarded as a serious pest (Given, 1968). In improved pastures away from parasites, predators and pathogens, and in the presence of nutritionally superior food plants, populations frequently exceed 400 larvae per m^2 and

cause severe pasture damage (Kelsey, 1970). Unfortunately, the two most productive pasture species, ryegrass and white clover, are susceptible to attack (Radcliffe, 1970).

Prior to 1950, years of severe grass grub damage were legendary and in certain districts dictated farming practices (Flay and Garrett, 1942; Kelsey, 1951). The advent of DDT heralded, for New Zealand, the efficient control of grass grub and other insect pests of pasture. The cheap protection DDT afforded allowed large tracts of hitherto marginal land to be developed. Such was the dependence of New Zealand grasslands on DDT that from 1965 to 1967 more than 5.5 million kg of DDT was mixed with fertiliser for application to pasture (Anon., 1970). With a recognised active soil life against grass grub of three years, enough DDT was used within this period to have enabled, by 1967, 90% of the sown pastureland to be proofed against this insect.

In 1965, the use of DDT was threatened by the discovery of DDT tolerant and resistant strains of grass grub (Elliott and Perrott, 1965; Perrott and Allen, 1968). However, it was the development of an international consciousness of insecticide residues in food products and the imposition of progressively lower tolerance limits set by overseas markets which saw the inevitable banning of DDT on New Zealand dairy farms in 1967 and on sheep farms two years later.

Following the world-wide swing in insecticide usage away from the persistent organochlorine insecticides to the more transient and expensive organophosphates, total reliance for chemical control of grass grub on dairy farms fell on this group of insecticides.

The properties of rapid degradation and/or dissipation which make organophosphate insecticides more acceptable than the persistent organochlorines in terms of the environment, render them less reliable for broadcasting on pasture for grass grub control. With the prohibition of DDT, grass grub control like that of many other insects emerged from a situation where little knowledge of the pest's biology and ecology was required to a

position where the acquisition of this information became fundamental for its efficient control.

The high cost of organophosphate insecticides relative to the value of pasture and the practical limitations of chemical control imposed by the properties of this group of insecticides, has meant, that grass grub control cannot rest solely with insecticides as it did during the DDT era. Post treatment withholding periods for grazing to ensure the safety of livestock limits the proportion of the farm which can be treated at any one time. Problems associated with even aerial distribution on hill country are accentuated with the increased transient nature of these insecticides. Further, the requirement of immediate post treatment rainfall, necessary to wash the insecticides into the soil, is difficult to meet over the optimal period for treatment in late summer - early autumn.

In view of these problems the feasibility of adopting the concept of pest management for grass grub control required evaluation. Pest management studies may be approached through two interconnected routes. The first route is through the development and improvement of control methods. The second route is through ecological investigations and damage assessment studies which lead to modelling the insect's life system so that population density and the associated losses in production can be predicted. The provision of this information allows alternative methods of control or combinations of control methods to be evaluated (Solomon, 1973).

In 1967 in response to a report from a special working party of the National Research Advisory Council of New Zealand, prompted by the then impending ban on DDT, on the present status and future needs of research on pasture insect pests, a co-operative research effort between various research organisations within New Zealand was initiated. The research reported herein was undertaken as a co-operative research programme between the Department of Entomology of Lincoln, University College of Agriculture and the Ministry of Agriculture and Fisheries from

which the author was released from 1968 to 1970 to study full-time for a Ph.D. degree.

The principal object of the studies reported here was to investigate the feasibility of developing models for predicting population density and the associated losses in pasture productivity as a basis for the formulation of a pest management programme for grass grub control.

SECTION I

REVIEW OF LITERATURE

CHAPTER I

LITERATURE REVIEW ON THE BIOLOGY AND ECOLOGY OF GRASS GRUB

I. INTRODUCTION

As early as 1860, Costelytra zealandica (White), more commonly known as grass grub, was recognised as New Zealand's major entomological problem in pasture (Hoy, 1965). Miller (1971) notes that the Maori was familiar with the grass grub beetle and called it "papapapa" and "tutaeruru" and the larvae, by the general term for subterranean larvae, "mɔeone", meaning, to sleep in the ground.

The species' distribution encompasses the three main islands of New Zealand and the Chatham Islands (Hoy, 1963). Given (1968) noted that "apart from dense forest, swamp, unstable sand-dune areas, some very heavy soils and altitudes over 4,500 ft, this insect is almost always present."

Hoy (1965) considered that conservatively the potential loss caused by grass grub damage in the absence of chemical control was approximately 30 million dollars. In spite of this, little detail was known of the species' biology and ecology (Given, 1968; Pottinger, 1968). The swing from the persistent organochlorine to the transient organophosphate insecticides for control of pasture insect pests in New Zealand has highlighted this situation.

The general biology of grass grub has been reviewed by many authors, including Miller (1921, 1945), Dumbleton (1942), Kelsey (1951), Pottinger (1968), Galbreath (1970) and most recently by East (1972).

This chapter reviews the information presented by these authors, augmented by relevant information on other scarabaeids that is pertinent to these studies.

II SYSTEMATIC POSITION

The systematic position of the grass grub as it is accepted today, was recorded by Given (1952, 1960, 1966) and is as follows:

Order	Coleoptera
Family	Scarabaeidae
Tribe	Colpochilini
Costelytra zealandica (White)	

The genus Costelytra which is endemic to New Zealand was erected by Given (1952). Previously C. zealandica had been placed in the genus Odontria (White, 1846).

Morphological descriptions of the adult and mature larvae have been detailed by Given (1952) and Hoy and Given (1952). Given (1966) noted that "the genus Costelytra is not a static one and the range in variation within species and the close alliance of some species indicates that active speciation is in progress." Distinct adult morphological differences in populations of C. zealandica have been recorded (Given, 1952) which suggests that different races exist within the species.

III SEASONAL CYCLE AND BIOLOGY

With the exception of the beetle, all stages in the grass grub's life cycle are completely subterranean in habit. Generally the species is univoltine but under drought conditions (East, 1972) and in high altitude environments (Stewart and Stockdill, 1972) the life cycle may extend over two years.

With the exception of a rather extensive study on flight periodicity conducted throughout New Zealand by Helson (1967), observations on the biology of grass grub have been confined to the South Island and in particular to the Canterbury and Nelson provinces. It might, therefore, be expected that the development

of grass grub in the warmer North Island might precede that published in the literature. In some localities and populations examined, stages within the life cycle may overlap widely (Kelsey, 1968b; Fenemore and Perrott, 1970) and for this reason the following description of the occurrence of the immature stages of grass grub lists only the major periods of occurrence.

Eggs are laid in clusters of 3 to 40 eggs, 7.5 to 17.5 cm deep in the soil (Pottinger, 1968). In the Nelson province eggs are found from the end of October to the beginning of December (Fenemore and Perrott, 1970). Hatching occurs within two to three weeks and larvae appear in December (Kelsey, 1950).

As in most scarabaeids the larvae have three instars (Ritcher, 1958) distinguishable by the width of their head capsules (Kelsey, 1970). First instar larvae are present in the soil from December to January (Pottinger, 1968). Second instar larvae first appear in January and are present in significant numbers until the beginning of April. By April the majority of the population have developed into the third instar. At this stage sex differentiation of larvae is possible (Elliott, 1964).

During May and June the actively feeding larvae lay down large deposits of fat which provides a yellow colouration to the larval abdomen (Perrott, Shortland and Czochanska, 1965). Larval feeding usually decreases from June onward.

Pupae are present in the soil over the spring months of October and November. The sex of pupae may be identified by the presence of a distinct bulge at the posterior end on the ventral surface (Brown, 1966).

The period over which beetles are present in the soil at localities near Nelson varied from 36 to 49 days and dependent on locality and possibly season, this period may extend from mid October to late November (Fenemore and Perrott, 1970). After a post-eclosion period to allow the teneral beetle to mature and the wings to harden, beetles burrow up through the soil profile in preparation for emergence (Miller, 1921). Following emergence and/or flight, beetles burrow back into the soil to shelter during the

day. Under field conditions beetles live for two to three weeks (Fenemore, 1965).

IV BEHAVIOUR OF DIFFERENT STAGES

A knowledge of the behaviour of the major stages in the development of an insect is essential for studying natural populations. The existence of information on the species' behaviour enables population studies to develop more rapidly than in its absence. This knowledge provides an objective basis on which to develop and plan both the mechanical and statistical aspects of census sampling. For the purpose of this review the behaviour of grass grub has been divided into adult, larval and pre-pupal and pupal behaviour.

1. Adult

The major contributions to the literature on adult behaviour of grass grub have been made by Kelsey (1951, 1968a), Fenemore (1965, 1971), Fenemore and Perrott (1970) and Farrell and Wightman (1972). An examination of the work of these authors suggests that the patterns of adult behaviour can best be discussed under three distinct headings: emergence, mating and flight, oviposition and feeding.

a) Emergence, Mating and Flight. The emergence, mating and flight behaviour of grass grub have been studied over a number of seasons in Canterbury (Kelsey, 1968a) and near Nelson (Fenemore and Perrott, 1970; Farrell and Wightman, 1972).

The duration over which 95% of the individuals of a population emerged for the first time near Nelson ranged from 19 to 25 days (Fenemore and Perrott, 1970; Farrell and Wightman, 1972). In Canterbury, beetle activity on the pasture ranged from October to March with peak numbers occurring from November to December (Kelsey, 1968a). Although the peak flight period, in Canterbury, occurs from early November until mid-December, flight is common in January (Kelsey, 1968a). Within the Nelson area

flight extends from November to February with peak flights in different localities ranging from November to December (Fenemore and Perrott, 1970). From a network of light traps over the North and South Islands, Helson* (1967) observed that flights extended from late October to February and confirmed that the peak flight period for most districts occurred during November and/or December.

Flight commences in Canterbury between 7.15 and 8.45 p.m. and few beetles fly as late as 9.20 p.m. (Kelsey, 1951, 1968a). Dawn flights have been observed by Hilgendorf (1902) but this observation has not been substantiated. Flight activity at dusk ranges in duration from 7 to 48 minutes with an average length of 28.5 minutes. Favourable temperatures over the flight period do not extend the duration of flight (Kelsey, 1951, 1968a). Most flight occurs when grass temperatures exceed 9.4 °C and when winds are below 9.6 kph (Kelsey, 1968a). Involuntary flight may occur in high winds when beetles are whipped by the wind from the stems of pasture plants into the air (Miller*, 1921; Kelsey, 1968a).

Emergence appears less sensitive to weather conditions than flight and has been recorded at grass temperatures as low as 2.2 °C and in winds above 57.9 kph. Generally the emergence patterns parallel those of flight (Kelsey, 1968a). Males are known to emerge earlier in the season (Kelsey, 1951) and earlier in the evening than females (Kelsey, 1951, 1968a; Fenemore and Perrott, 1970).

Beetles are sexually mature on emergence, and mating usually occurs, on the ground or pasture, immediately or soon after emergence (Kelsey, 1951; Fenemore and Perrott, 1970; Farrell and Wightman, 1972). Males may couple with females which have only their terminal segments exposed from the soil (Fenemore and Perrott, 1970). Sex ratios of emergent beetles (Kelsey, 1968a) or teneral beetles in the soil usually do not differ significantly from unity (Fenemore and Perrott, 1970).

As with many scarabaeids such as Melolontha melolontha L. (Hauser, 1880), Phyllophaga lanceolata (Say) (Travis, 1939),

Rhopaea magnicornis Blackburn, R. morbillosa Blackburn and R. verreauxi Blanchard (Soo Hoo and Roberts, 1965) mating is mediated by a female produced pheromone (Kelsey, 1966a; Henzell, et al., 1969). The grass grub pheromone is thought to be phenol (Henzell and Lowe, 1970) or a phenolic substance (Osborne and Hoyt, 1970) and Hoyt, Osborne and Mulcock (1971) have shown that a symbiotic bacterium found in the colleterial gland of grass grub is capable of producing a sex attractant chemical.

After emerging on to the pasture females remained stationary and, left undisturbed, are mated and re-enter the soil close to their point of emergence (Fenemore and Perrott, 1970). Mated females seldom fly until after oviposition and providing the vegetative cover is adequate, feed near the point of emergence (Kelsey, 1951). Kelsey (1951) noted that females which remain unmated soon after emergence ascend the stalks of pasture to attract a male and if still unmated fly to and aggregate around feeding sites where mating occurs.

In many seasons female beetles constitute less than 5% of the season's catch (Kelsey, 1968a) but in certain districts this figure may approach 50% (Fenemore and Perrott, 1970). Over the early part of the flight season flight is dominated almost exclusively by males (Kelsey, 1951; Fenemore and Perrott, 1970) but over the latter part the numbers of females increase (Kelsey, 1968a) and may out-number the males.

The slow reinfestation rate along the margins of small areas from which grass grub had been chemically eliminated led Fenemore (1965, 1970) to conclude that the behaviour of grass grub is similar to Phyllopertha horticola (L.) the female of which is mated soon after emergence and re-enters the soil close to her point of emergence to lay 70 to 100% of her eggs. Female Phyllopertha do not feed until body fat is expended and eggs maturated. Unmated females or females which have oviposited appear to feed and mate on bracken feeding sites nearby before returning to pasture (Milne, 1960). This pattern of behaviour is in marked contrast to that of Melolontha melolontha (L.) (Schneider, 1962)

or Amphimallon majalis (Raz), (Evans, 1956). These melolonthids are hypostactically attracted to trees where they maturate their eggs and/or mate and then return to the pasture to oviposit.

b) Oviposition. The more significant aspects of oviposition in relation to population studies include the reproductive potential of the species, its oviposition pattern in terms of egg-numbers and time and the physical factors which influence its oviposition performance. These aspects of behaviour of pasture inhabiting scarabaeids have been the subject of studies by both New Zealand and overseas authors.

Eggs of most species of Melolonthinae are laid singly in cells fashioned by the female (Reinhard, 1944). In this respect the deposition of eggs in clusters by grass grub (Kelsey, 1951) is similar to the oviposition habits found in species of Sericini (Fidler, 1936b).

Compared with many other insects, such as certain Lepidotera, the general reproductive potential of scarabaeids is extremely low. For example A. tasmaniae averages approximately 50 eggs (Carne, 1956), A. majalis between 22 and 59 eggs (Gyrisco, et al., 1954) and P. horticola approximately 12 eggs (Milne and Laughlin, 1956).

The number of eggs recorded by Kelsey (1951) as being laid by grass grub ranged from 3 to 40 with a mean of 22 and a maximum fecundity of 52. In an area near Nelson female beetles laid clusters usually consisting of between 20 and 25 eggs (Fenemore, 1965). Under Canterbury conditions the average number of eggs laid by four populations in the field ranged from 3 to 12 per female. A sample from the same populations held in the laboratory produced a range in, mean egg and mean cluster numbers of 19 to 26 and 1.5 to 1.9 respectively (East, 1972).

Miller (1945) records that oviposition began 7 to 16 days after mating and this is substantiated in part by Kelsey's (1951) minimum post-mating period of 7 days and a 10 day period observed by Fenemore (1971). According to Kelsey (1970) eggs

laid before a 7 day post mating period were not viable and a 4 to 9 day period exists between the oviposition of successive clusters (Kelsey, 1951). Under field conditions near Nelson, eggs were observed in the soil 14 to 18 days after the beginning of emergence but, before primary emergence was completed. Egg numbers increased and reached a peak over a period of 15-25 days (Fenemore and Perrott, 1970).

Studies of the oviposition behaviour of grass grub have failed to establish conclusively that plant species or plant height influence the choice of oviposition sites (Kelsey, 1957, 1968b; Radcliffe and Payne, 1969; Radcliffe and Kain, 1971). Although Kelsey reported that beetles appear to reject bare ground in favour of pasture cover this preference has not been demonstrated in other studies (Radcliffe and Payne 1969; Radcliffe and Kain, 1971). However, Galbreath (1970) observed that beetles caged in a box with bare and grass covered soil exhibited a marked preference for the latter.

Soil moisture appears to influence scarabaeid beetles in their choice of oviposition sites. Maelzer (1961b) noted that Aphodius tasmaniae Hope laid most eggs in soils with a range in moisture levels on the pf scale between 2.8 and 3.2. Female beetles of this species presented with a choice of moisture levels aggregated on soils with moisture levels falling within this range. Oviposition by Phyllophaga implicata (Hn.) is limited by high and low soil moisture levels and under conditions of soil moisture stress oviposition is delayed (Sweetman, 1931). Female A. majalis are known to oviposit deeper in the soil profile in dry than moist conditions (Tashiro, et al., 1969). Galbreath (1970) considers that adult female grass grubs like other scarabaeid species are sensitive to soil moisture. He noted that beetles avoided burrowing into dry soils and under extremes of soil moisture delayed ovipositing.

c) Feeding. The biological importance of adult feeding in grass grub has received little attention. Beetles are known to feed on pasture plants (Farrell and Wightman, 1972) and nearby trees

and shrubs (Kelsey, 1951; Farrell and Wightman, 1972). The importance of beetle feeding has not been ascertained in the field but in laboratory studies beetles fed willow leaves laid 60% more eggs and lived longer than unfed beetles (Farrell and Wightman, 1972).

The fate of beetles feeding on broad-leaved trees over the flight season is unknown. Radcliffe and Payne (1969) demonstrated that female beetles collected from hedge rows and trees were capable of laying eggs and therefore may be an important fraction of the beetle population. The question of whether beetles feeding on the trees move back to the pasture to oviposit forming an oviposition-oogenesis, feeding site, flight syndrome similar to that of Melolontha hippocastani and Melolontha melolontha (Schneider, 1957) and Phyllopertha horticola (Milne, 1959) remains unanswered. Hilgendorf (1902) reported movement of grass grub from broad-leaved feeding sites into the pasture at dawn but this phenomenon was not recorded in studies by Farrell and Wightman (1972). Certainly many beetles die close to the feeding sites as evidenced by the large numbers of dead beetles found at the base of the shrubs or trees. This suggests that beetles after leaving open pasture may spend the rest of their lives feeding on the trees during the night and returning to the pasture or soil at the base of the tree during the day (Kain, unpub.)

2. Larvae

Only factors concerned with larval movement and feeding are reviewed and discussed in this section.

a) Feeding. The feeding habits found in the various subfamilies of Scarabaeidae were summarised by Ritcher (1958) who noted that the larvae of Melolonthinae feed on humus and live plant tissue.

It is clear that grass grub ingest live root material from a wide range of plants and trees (Kelsey, 1951) but what is

not clear is whether the species discriminates between soil and dead and live plant tissue. Power (1968) considered that grass grub larvae ingested soil organic matter in the process of root ingestion. Miller (cited Sutherland, 1971) recorded that grass grub can be reared in humus in the absence of live plant material. Yaacob (1967) reported that grubs eat organic matter and in so doing form part of the chain in the mineralisation process. Larvae held in soil in the presence of roots contained plant material but little soil (Wightman, in press), an indication that larvae prefer roots to soil. Radcliffe (1970) observed that when organic matter in the form of cow dung was added to soil less plant damage occurred. Doubt exists whether this observation was due solely to the better plant growth that this additive stimulated or whether the cow dung acted as an alternative food source.

The attraction of grass grub larvae to grass roots has been clearly established (Galbreath, 1970; East, 1972; Sutherland, 1972). Larvae are more strongly attracted to young rather than old roots and find roots of red clover, lucerne and Lotus pedunculatus more attractive than ryegrass, white clover equally attractive, and dock less attractive (Sutherland, pers. comm.). These observations suggest that grass grub larvae are highly selective in their feeding habits but in the absence of live tissue can exist on soil organic matter.

b) Movement.

(i) Lateral movement. Scarabaeid larvae are capable of burrowing large distances through the soil in search of food (Gyrisco, et al., 1954). Under laboratory conditions and in the absence of plant tissue grass grub larvae are able to move over 6.4 m per month (Kelsey, pers. comm.).

Under field conditions in the presence of food the movement of scarabaeid larvae appears to be very limited. Tashiro, et al., (1969) are of the opinion that the lateral movement of

A. majalis is markedly influenced by the supply and proximity of live roots. The economically important chafer larvae in Britain which include M. melolontha, P. horticola, Amphimallon solstitalis (L.), Hoplia philanthus (Fuess.) and Serica brunnea L. are usually located close to where the eggs were laid as most larval movement is usually vertical (Anon., 1971a). Milne (1963) observes that the average distance traversed by P. horticola from egg to pupae is less than 31 cm.

Field observations indicate that the lateral movement of grass grub larvae does not exceed 61 cm (Fenemore, 1965, 1970). Work by Sutherland (1972) which demonstrated the attraction of grass grub larvae to live plant roots and the possible existence of arrestants in plant roots, the influence of which attenuates larval movement, may explain the reason for the very limited lateral movement of grass grub recorded by Fenemore (1970).

(ii) Vertical movement. The depth at which grass grub larvae are found in the soil profile varies with the stage of development, availability of food and environmental factors such as soil moisture (Kelsey, 1950; Galbreath, 1970; East, 1972).

According to Kelsey (1950) first instar larvae seldom inhabit the upper 5 cm of soil and feed in the vicinity of where the eggs hatch. Second stage larvae are located in the upper 5 cm of soil while third instar larvae are concentrated in the top 2.5 cm (Kelsey, 1950). These observations have been substantiated by East (1972) on non-irrigated pastures in Canterbury, but under irrigated pastures East noted that approximately 40% of the second instar population inhabited the top 2.5 cm. This difference was attributed to the different moisture levels found near the surface during the summer. The findings of Stewart and Stockdill (1972) in a higher rainfall area in Otago show that irrespective of larval maturity, the majority of larvae with the exception of the pre-pupa were present in the top 5 cm and many first instar larvae were found within the top 2.5 cm (Stewart, pers. comm.).

It is well recognised that soil moisture influences the vertical distribution of scarabaeid larvae. Most species, for

example A. majalis (Gambell, 1946; Shorey and Gyrisco, 1960), P. horticola (Milne, 1956), and A. tasmaniae (Maelzer, 1961a), appear to react only to extreme changes in soil moisture, retreating deeper down the soil profile under drought conditions to depths of 10 to 15 cm but ascending rapidly in response to rainfall. Under very dry conditions A. majalis is extremely sensitive to slight changes in moisture differences (Shorey and Gyrisco, 1960). On the other hand Phyllophaga spp. are very sensitive to soil moisture levels and possess a well defined soil moisture preferendum to which they move after rain. This is achieved by altering their vertical distribution in the soil (Granovsky, 1958). Larvae of Sericesthis geminata are known to move rapidly into soil with a 15% moisture content when exposed to moisture gradients ranging from 5% to 30% (Davidson, 1969a).

The reaction of grass grub to changes in soil moisture seems similar to that of A. majalis. At low soil moisture levels (below 10% by weight) second and third instar larvae were located 15 to 18 cm deep in the soil, but in response to 2.5 mm of rain, moved into the top 2.5 cm within 18 hours. At moisture levels above 16% the distribution of grass grub larvae appears independent of moisture (Kelsey, 1970). Galbreath (1970) noted that third instar larvae are able to detect small changes in moisture level and move to avoid moisture stress but become unresponsive to moisture at the near pre-pupal stage. Although the moisture sensing organs present on the antennae of third instar larvae occur on the antennae of first and second instar larvae the later stages were noted to move against moisture gradients in response to food (Galbreath, 1970). It has been suggested by Kelsey (1950) that where moisture is not critical the position of the larvae in the soil profile is influenced by availability of food.

Other factors are known to influence the vertical movement of scarabaeid larvae. Under laboratory conditions Shorey and Gyrisco (1960) found the second instar larvae of A. majalis show a preference for soil temperatures between 17 and 27 ^{o}C. In areas of heavy frost the grubs migrate well below the frost line

but as the ground thaws migrate upward (Gyrisco, et al., 1954). In places where there is heavy sod and a thick covering of snow larvae feed actively in the winter within 2.5 to 5 cm of the surface (Tashiro, et al., 1969).

Miller (1945) considered that grass grub descend in the soil in response to climate to hibernate, and ascend as the temperature in spring rises to feed before pupation. This behaviour is neither consistent with the now generally accepted life history of the pest or observations by Given (1952) of larvae actively feeding in frozen soil.

It is known that larvae of S. geminata are attracted to organic matter and in response to this move close to the surface where higher levels of organic matter are found (Wensler, 1971).

3. Pre-pupae and pupae

The pupal cell of grass grub is constructed by the normal burrowing activity and continued movement of the larvae in the cell which compacts and smooths the walls (Galbreath, 1970). Like A. tasmaniae (Maelzer, 1961a) the grass grub pupal cell is not lined with biological substances (Galbreath, 1970); such as rectal contents (Cumpston, 1941), glandular secretions from the mid-gut (Hayes, 1929) or discharged gut contents (Carne and Chinnick, 1957); which may influence the permeability of the cell to water (Hayes, 1929), as is the case with other scarabaeids.

In the pre-pupal stage the power of locomotion is lost. The abdomen is bent forward and the legs are folded. The only movement is the flexing of the abdomen. At pupation, the larval skin splits at the anterior end and works down to the pupa's posterior (Galbreath, 1970).

V SPATIAL DISTRIBUTION

The spatial distribution of an insect will markedly influence sampling. For example, sample size, and the design of

a sampling programme are factors which are affected by the spatial distribution of the insect concerned.

The aggregated distribution of scarabaeid larval populations is well known (Carne, 1956) and has been recorded for such species as A. tasmaniae (Carne, 1956), A. majalis (Burrage and Gyrisco, 1954a, b), P. horticola (Milne, 1963), Popillia japonica Newm. (Fleming and Baker, 1936) and R. morbillosa and other scarabaeids commonly found in pasture on the northern tablelands of New South Wales (Davidson and Roberts, 1968a). The factors responsible for aggregation in scarabaeid populations include:

- Mating and oviposition behaviour; where beetles are mated soon after emergence and oviposit close by (Milne, 1959) or are attracted to trees or fence lines where they mate and oviposit (Carne, 1956; Tashiro, et al., 1969).
- Oviposition site preferences of dispersing females.
- Limited lateral movement of larvae (Milne, 1959) and the congregation of larvae at available sources of food (Tashiro, et al., 1969).
- Differential mortalities such as has been observed in P. horticola populations between wet and dry areas (Milne, 1963).

Aggregation in the immature stages of Phyllophaga fusca (Froel.) and P. anxia (Lec.) is most pronounced at the egg and first instar larval stages but decreases to the third instar stage and then increases slightly from there to the beetle stage (Guppy and Harcourt, 1970). This increase in randomness reflects the dispersal of larvae from the oviposition site, the action of density dependent mortalities and an increase in bird and mammalian predation as the larval stage matures and feeds closer to the surface.

Kain and Atkinson (1970) noted that the mating, oviposition and larval feeding behaviour of grass grub causes its populations to be distributed in discrete dense aggregates or colonies.

VI LIFE SYSTEM OF GRASS GRUB

In this section factors which may influence the determination of grass grub abundance are reviewed and where possible their importance in the grass grub life system is assessed.

1. The Physical Environment

(a) Moisture. It is generally accepted that soil moisture has a profound influence on the survival of most soil animals.

Excessive soil moisture conditions are known to cause high mortalities in field populations of P. horticola (Milne, 1963) and A. majalis (Tashiro, et al., 1969). Third instar larvae of A. tasmaniae drown at high soil moisture levels and become predisposed to attack by disease (Carne, 1956; Maelzer, 1961a).

Maelzer (1961a) noted that low soil moisture levels inhibit the emergence of A. tasmaniae first instar larvae to feed and when such conditions persist long enough larvae starved. Under conditions of adequate moisture the weight of the pre-pupal stage of A. tasmaniae, which is well correlated with fecundity, was heavier than under less favourable conditions (Maelzer, 1961a). Water loss in the pre-pupa of A. tasmaniae has been found to retard pupation and increase mortality at pupation, 10 to 40 days after treatment.

Davidson, et al., (1972b) observed that survival was optimal for the first instar larvae of Sericesthis nigrolineata at a moisture level range between plant wilting point and saturation point but decreased as moisture levels approached either extreme. This pattern of survival gave a soil moisture-survival response curve with an upper and lower threshold range.

Wightman (unpub.) observed a similarly shaped moisture response curve with grass grub larvae. Under laboratory conditions the optimum range of moisture for the survival of grass grub in a mixture of soil and sheep dung fell between 25 and 33% (2.4 to 3.0 bars.) which was below field capacity but above wilting point for the mixture (Wightman, 1972).

From scant field data Farrell (1972a) considered that adverse climatic conditions such as summer droughts which dry out the soil induce larval mortality. Subjected to these conditions larvae move deep into the soil out of the root zone and hence, starve.

Galbreath (1970) studied the effect of soil moisture on all the stages of grass grub over short periods. He found that eggs could develop in a humid atmosphere and contrary to Kelsey's (1951) findings, did not need to be in contact with water at any stage in their development. Galbreath (1970) confirmed Kelsey's (1951) observation that eggs are able to survive both desiccation and flooding. Second and third instar larvae and beetles were found by Galbreath to be extremely resistant to desiccation or flooding, particularly in the second instar stage.

Wightman (1972) observed that low soil moisture levels induce aestivation and thereby reduce larval feeding. This restriction on larval feeding may be reflected in adult fecundity. East (1972) observed that drought conditions cause a large fraction of the grass grub population to enter a two-year life cycle and thereby reduced generation fecundity. Trought (pers. comm.) has observed that second and third instar larvae drowned when subjected to waterlogged soil for a period of 6 to 9 days, while on the low lying areas of Manawatu, grass grub larvae are found concentrated on the freer draining rises (Cumber and Cowie, 1954).

Galbreath observed that pupae are most susceptible to desiccation but noted that their cells are usually located relatively deep in the soil profile. For this reason pupae are not likely to suffer from low soil moisture levels in spring. Conversely he noted that pupae were protected from drowning by the air space in the pupal cell and their dorsal ridges on which they rest. These ridges reduce the contact the pupa has with soil water.

(b) Temperature. Low temperatures during the summer when the young larvae of M. melolontha and M. hippocastani are present in the soil are thought to be the decisive factor limiting populations (Ritcher, 1966). The cold hardiness

of certain scarabaeids to low temperatures is demonstrated by the ability of A. majalis to recover after being frozen solid (Tashiro, et al., 1969). Observations of grass grub actively feeding in frozen soil suggests that grass grub are also tolerant of low soil temperatures (Given, 1952). Stewart and Stockdill (1972) contend that one of the factors extending the life cycle of grass grub populations from one to two years in the southern regions of New Zealand and thereby limiting the insect's annual reproductive potential are low temperatures associated with increasing altitude and latitude.

In dry soil (5.25%; 70 bars) eggs died in less than 8 days at 16.8 °C while the optimal constant temperature for rearing larvae under moist conditions is between 17.5 and 20.0 °C (Wightman, 1972). Davidson, et al., (1972b) have demonstrated the interaction between soil moisture and temperature in influencing the survival of first instar Sericesthis nigrolineata. These authors found that the thermal death point fell as soil moisture levels decreased.

2. Biotic Environment

Diseases (Carne, 1956) and predation (Raw, 1951) are known to cause large reductions in scarabaeid larval populations but less is known of the importance of parasites. Bacterial, fungal, rickettsial, viral and protozoan diseases are all known to occur in scarabaeids.

(a) Diseases

(i) Bacteria. The "milky diseases" are recognised as causing important mortalities in certain scarabaeid species (Dumbleton, 1942; De Bach, 1964; Tashiro, et al., 1969).

Dumbleton (1945a) recorded a native milky disease of grass grub which he described as "an undetermined species of Bacillus close to Bacillus popilliae", near Nelson and Seddon, where the incidence of diseased larvae reached about 38%. Since then, it has been found widely in New Zealand and while levels of infection under field conditions may rise as high as 57%, this is uncommon and the general level of diseased larvae in May, which seems to be the peak time of occurrence, is seldom above 5% (Hoy, 1955).

Milky disease (probably native spp.) are known to attack both Pyronota festiva Fabr. and Heteronychus arator Fabr. (Helson, 1965).

B. popilliae was introduced into mid Canterbury in 1948. The annual incidence of diseased larvae at the liberation sites over the preceding eight years ranged from 7 to 24% (Kelsey, 1966b). It is as yet very localised and is confined to within half a chain of the liberation sites (Kelsey, 1966b).

(ii) Fungi. The cosmopolitan green muscardine fungus Metarrhizium anisopliae (Metsch.) attacks many species of scarabaeids (e.g. Carne, 1956; Tashiro, et al., 1969). Latch (1965) isolated it from three native scarabaeids, grass grub, Pyronota festiva (Fabr.) and Pericoptus truncatus (Fabr.). Grass grub is also attacked by the white muscardine fungus Beauveria bassiana (Balsamo) (Helson, 1965) and a species of Cordyceps which was thought by Brown (1966) to be the cosmopolitan species C. entomorrhiza (Dicks).

Neither the geographic distribution, nor the levels of infection that entomophagous fungi cause in grass grub populations has been assessed. Their incidence in the Canterbury populations of grass grub studied by East (1972) did not exceed 2%. From this report and other scant information available it seems likely that their incidence is generally very low. This conclusion is consistent with Latch's (1965) observation, that "the soil temperatures in New Zealand are seldom high enough for M. anisopliae to maintain optimal growth and it is therefore doubtful that the species could be used for control purposes."

(iii) Rickettsia. Rickettsial diseases of scarabaeids have been recorded in Popillia japonica, possibly Oryctes rhinoceros (L) (De Bach, 1964) and A. majalis (Tashiro, et al., 1969). Rickettsia-like organisms have been isolated from grass grub larvae from the Otago and Canterbury provinces with clinical symptoms similar to "milky diseased larvae" (Moore, pers. comm.).

(iv) Virus. An irridescent blue virus infecting third instar grass grub has recently been found in a locality in South Canterbury (Moore, pers. comm.).

(v) Protozoa. An undescribed species of Micrococcus has been isolated from grass grub by Helson (1965).

(b) Parasites. Three tachinid parasites of scarabaeids have been recorded. Given (1945) identified Avibrissina brevipalpis Malloch and Neotachina laticornis Malloch from the larvae of Pyronota inconstans Brookes collected in the Nelson district. From the same general area Procissio cana Hutton has been reared from Chlorochiton species. Thomas (1963) recorded grass grub parasitised by P. cana in the Nelson district but noted that the incidence of parasitism was only in the order of 3%. The recognised distribution of P. cana has since been extended to include areas in Otago and Southland where parasitised Odontria halli Brown, O. striata White as well as grass grub have been collected (Brown, 1966). The incidence of parasitised grass grub recorded by Brown (1966) ranged from 25 to about 50%. The recorded habitats of P. cana indicates that the importance of this parasite in controlling grass grub populations is limited to localised areas where native vegetation verges improved pastureland rather than in the open improved grasslands.

Over 14 thynnid parasites have been introduced into New Zealand from Australia and South America to control grass grub, unfortunately all have failed to establish (Given, 1967).

A nematode, Mermis sp. is found in localised areas near Ashburton and here is responsible for high larval mortalities of grass grub (Hoy, 1955). Neoaplectana glaseri Steiner, a parasitic nematode of P. japonica introduced into New Zealand from America in 1945 established but is confined to its liberation sites (Hoy, 1955). Hoy (1955) noted that a combination of environmental factors in relation to the grass grub's life history seem to be responsible for the nematode's limited success (Hoy, 1955).

(c) Predators.

(i) Invertebrate. Two asilid larvae Itamus varius and Sarapagon antipodes have been recorded by Miller (1924) to be larval predators of grass grub. It is doubtful that these predators destroy many larvae as Kelsey (1951) noted that asilids require only one to two third instar larvae to reach maturity and East (1972) reported that the consumption rate of grass grub by asilids is very low. The larva of a tabanid (Ectenopsis lutulenta Hutton) is capable of killing third instar grass grub but from studies of its consumption rate and density the species seems to be unimportant (East, 1972).

Two adult carabids Mecodema crennicolle and Metaglymma tibiale feed voraciously on grass grub larvae (Brown, 1966). Brown (1966) and Kelsey (1951) considered that carabid larvae destroy many grass grub before they reach maturity. A staphilinid beetle (Leptacinus labralis (Brn)), a potential predator of first instar larvae, builds up to high numbers under Canterbury pastures (East, 1972). East (1972) observed, however, that its peak density occurs in February when grass grub exceeds its host size range. The species usually frequents the top 5.0 cm of the soil profile and therefore does not come in contact with first instar larvae which are usually found deeper in the soil. Species of elaterid larvae commonly found in pasture have been reported as being potential predators of undetermined importance on all stages of grass grub larvae (East, 1972).

The high density of the centipede Zelanion morbosus (Hutton), which under laboratory conditions has a high consumption rate of eggs and first instar larvae of grass grub, may occur at densities in the field of up to 45 per m^2, but its performance in the laboratory does not match that in the field (East, 1972).

East (1972) concluded, from his studies of potential invertebrate predators, that invertebrate predation was not a significant factor in the population dynamics, of grass grub in Canterbury.

(ii) Vertebrate predators. Three exotic species of birds may be considered important predators of grass grub in uncultivated pastureland, namely the starling (Sturnus vulgaris), the white and black magpies (Gymnorhina hypoleuca and G. tibicen) and rooks (Corvus frugilegus).

Starlings are known predators of scarabaeid larvae throughout the world (Raw, 1951; Carne and Chinnick, 1957; Ritcher, 1958; Tashiro et al., 1969; Guppy and Harcourt, 1970) and are now widely distributed throughout New Zealand (Falla, Gibson and Turbott, 1966). Many authors have noted starlings feeding on grass grub (E.G. Cockayne, 1911; Miller 1924; Dumbleton, 1942) but only recently has any definitive and quantitative studies on starling predation been carried out. East (1972) found that from March to July, on irrigated pastures, starling predation can reduce grass grub populations by 40 to 60% and as a result grass grub densities in early autumn did not exceed the density threshold for severe pasture damage. Irrigation is known to facilitate starling predation by keeping the soil soft during the summer and autumn and localizing the larvae within the top 3.0 cm.

Kelsey (1951) and Oliver (1955) have recorded that magpies fed on grass grub larvae and it is known that grass grub larvae form an important source of food for rooks in Hawkes Bay over the autumn and winter while the adult beetle makes a significant contribution to their spring diet (Purchas, pers. comm.).

Adult grass grub are also known to be eaten by the hedgehog Erinaceous europaeus (Brockie, 1958).

Apart from isolated instances where starling predation may be important vertebrate predation like invertebrate predation does not appear to be an important factor in the control of grass grub.

(d) Combat Mortality. In overcrowded situations certain species of scarabaeid regulate their densities through combat mortality (Kelsey and Hoy, 1950; Carne, 1956; Soo Hoo, 1968; Chadwick, 1970).

Soo Hoo (1968) describes the behaviour of S. germinata. "Larvae construct or attempt to construct earthen cells to isolate

themselves from their neighbours. If the walls of the cell collapsed with the movement of the insect the resident attacked the intruder." The attacking larvae orally exude a black fluid which injected into the blowfly Lucilia cuprina (Wiedemann) caused death. It is known that R. magnicornis when excited also regurgitates black liquid and attacks other larvae (Chadwick, 1970).

Carne (1956) found that combat mortality of the surface feeding scarabaeid A. tasmaniae, foraging on the pasture in an area where dispersal was restricted, accounted for 80% of the population. Density thresholds for mortality in this species changed as larvae grew and their food requirements increased forcing them to forage over a larger area.

Kelsey (1951) and Wightman (1972) noted that combat mortality occurs when larvae are confined to containers in the laboratory and Wightman has recorded, under laboratory conditions, optimal densities for survival. The degree of combat mortality seems inversely related to food supply and soil moisture (Kelsey, 1951). Injuries sustained in combat between grass grub involved punctures to the legs and body and under food stress it is not uncommon to observe larvae consuming their dead (Kelsey, 1951). Under field conditions combat mortality can account for 94% of a population (Kelsey, 1951).

(e) Grazing Animal-Plant-Insect Relationships. Only recently has attention been directed to studying the influence of the insect-plant-grazing animal interaction on pasture insect populations. Davidson, et al., (1970b) constructed a flow diagram of the pasture scarabaeids ecosystem in the New England region of Australia. Adopting a systems approach these authors have started to quantify in the laboratory and validate in the field the relationships between different factors in the ecosystem and insect density in an attempt to predict long term changes in the abundance of scarabaeids and the relationship between larval density and losses in pasture productivity.

There are three basic relationships to be considered in the animal-plant-insect relationship, namely, the insect-plant,

animal-insect and animal-plant relationships.

In considering these relationships it is important to recognise that pasture is a dynamic association of plants, the composition of which may change in response to season, management and insect damage. Such changes may induce changes in insect survival and fecundity by altering the quality and quantity of the pests' food (Farrell, 1973). It is known that grass grub are sensitive to changes in food plants and certain plants such as lucerne (Kain and Atkinson, 1970; Farrell and Sweney, 1972) and Lotus pedunculatus (Farrell and Sweney, 1972) adversely affect the survival of grass grub larvae. On the other hand larval survival of grass grub larvae is favoured by white clover (Kain, unpub.).

Sucrose has been identified as a phagostimulant for grass grub larvae (Sutherland, 1971) and carbohydrate reserves of grass roots, which are high in sucrose, have been shown to be affected by such factors as the rate of plant growth (Davidson, 1969b) root pruning and defoliation (Sullivan and Sprague, 1953) and plant age (Dudzinski unpub. cited Wensler, et al., 1971a). Therefore management of the grazing animal or other management factors may influence grass grub by altering the botanical composition of the pasture or altering the composition of roots. Heavier or more frequent grazing is also known to decrease the root biomass (Troughton, 1957) and therefore the grass grub's food source.

The effect of grass grub damage on the host plant will influence the insects' food supply. The extent to which this occurs will be determined by the ability of the damaged plant to survive and to grow in a competitive association. Many authors have noted that vigorously growing plants are less affected by scarabaeid damage than those that are less vigorous (e.g. Graber, et al., 1931; Carne and Chinnick, 1957; Radcliffe, 1970). Factors which affect plant vigour such as soil fertility (Graber, et al., 1931; Radcliffe, 1970) soil moisture (Graber, et al., 1931; Radcliffe, 1970) and frequency of defoliation (Graber, et al., 1931) will undoubtedly influence scarabaeid damage. Certain plants are known to be more tolerant to the root pruning of grass grub than others. Radcliffe (1970) observed that white clover appeared to be preferentially damaged and ranked the given plants in the following

order of decreasing susceptibility to grass grub damage; white clover, brown top, ryegrass and Yorkshire fog.

The grazing animal can reduce grass grub survival directly through treading (Green, 1920; Kelsey, 1951; East, 1972). East (1972) noted that under moist conditions stock treading caused large mortalities where the rooting system of pasture had been destroyed by the feeding activities of grass grub.

Soil compacted by animal treading is not infested by the various pasture chafers in England (Anon, 1971a). The reason for this is not clear but it may stem from a decrease in gaseous diffusion caused by increased soil compaction (Edmond, 1958) which adversely affects the survival of scarabaeid larvae.

Other workers have reported relationships between the density of scarabaeid larvae and stocking rate. Roberts (1970) noted that certain pasture scarabaeids favoured low stocked paddocks and that stocking rates above 8 sheep per ha depressed the scarabaeid biomass. Roberts and George (1972) have recorded that not only stocking rate but sheep breed and lambing time, factors which affect pasture management, also influences the scarabaeid biomass. A similar trend was noted for Adoryphorus couloni where stocking rates above 8.75 wethers per ha reduced population densities by over 80% (Douglas, 1972). Under lax grazing, pasture maintained a dense dry cover over the summer which insulated the top soil and helped to conserve moisture thereby producing ideal conditions for the development of A. couloni larvae.

From what is known of the animal-plant-insect relationship of pasture scarabaeids it would seem important when conducting population studies on insect pests of pasture to control or monitor the major variables which affect these relationships, such as stocking rate, botanical composition of pasture and pasture productivity.

CHAPTER II

LITERATURE REVIEW ON POPULATION DYNAMICS AND STATISTICAL ASPECTS OF SAMPLING

The review of literature in this chapter of the thesis is divided into two sections. In the first section the theoretical aspects of population density determination, the life system concept in population studies and the approaches to studying population dynamics and methods of analyses are discussed. In the second section attention is confined to problems of sampling invertebrate populations.

I. POPULATION ECOLOGY

(1) Population Theory

The theories of insect abundance and distribution were studied to provide a background and framework for studying the population dynamics of grass grub.

(a) Theories. It is perhaps indicative of the confusion that has existed that so many authors have reviewed the theories on the abundance and distribution of animals (Solomon, 1949, 1957, 1964; Thompson, 1956; Richards, 1961; Clark, et al., 1967; Richards and Southwood, 1968; Wilson, 1968). Richards (1961) reported that authors were far from reaching agreement on general theoretical principles and wondered whether some authors were more interested in proving their opponents wrong than providing evidence for alternative hypotheses.

Perhaps the best subdivision of the contributions to theoretical population ecology is that provided by Clark, et al., (1967). These authors divided population theories into four.

- Those in which density plays a key role in the determination of population numbers by operating as a regulatory mechanism (e.g. Nicholson, 1933, 1954);
- Those in which density processes are regarded as minor or secondary and play no part in determining the abundance of a species (e.g. Andrewartha and Birch, 1954);
- Those that accept a middle of the road course between the views of Nicholson and Andrewartha and Birch, and
- Those which place emphasis on genetic factors (e.g. Chitty, 1960, 1965).

(b) Mechanisms of Regulation. The acceptance of density dependent processes as an important phenomenon in population regulation is widely held (e.g. Solomon, 1949, 1957, 1964; Richards, 1961; Klomp, 1968; Richards and Southwood, 1968; Varley and Gradwell, 1970; Clark et al., 1967) although, dissension on this point still exists (Birch, 1962; Ehrlich and Birch, 1967).

The extent to which density dependent processes operate may change in relation to the relative position the species is in, in its habitat range. For example it is thought that in the fringe areas of the species distribution compared with the more favourable areas density dependent processes play a more limited role in population regulation (Richards, 1961; Richards and Southwood, 1968; Huffaker and Messenger, 1964; Nicholson, 1958; Bateman, 1968; Reynoldson, 1958). In fact Watt (1968) considers that factors such as climate may never allow the population to become dense enough to permit density dependent processes to operate. Density dependent mechanisms may include intraspecific competition, the action of certain predators, parasites and pathogens, density induced emigration, territorial behaviour and genetic polymorphism (Clark, et al., 1967).

Solomon (1957) pointed out that food shortage not induced by insects cannot be regarded as a density dependent process. Although food determines the upper limit of population increases, it is considered that conditions of food shortage limiting population increases are uncommon (Thompson, 1956; Milne, 1957a). Food shortage is known to induce, combat mortality in Aphodius howitti (Carne, 1956), dispersal of Choristoneura fumiferana (Morris, 1963a) while conditions of food quality as well as overcrowding are factors which induce migration in aphids, locusts (Kennedy, 1961) and the leaf hopper Circulifer tenellus (Huffaker and Messenger, 1964).

It is now considered that genetic shifts which occur at high population densities (Chitty, 1960) are a possible factor in population regulation (Clark, et al., 1967). Franz (1949) hypothesised that outbreaks and collapses of certain insects in Europe resulted from favourable conditions permitting increases in less fit genotypes which are more prone to destruction by unfavourable conditions. Wellington (1960, 1964) has observed a loss in insect viability at high population densities and Baltensweiler (1968) pointed out that Chitty's (1965) theory of polymorphic types presents the most logical explanation of cyclic fluctuations in the grey larch tortrix.

Many workers have been able to account for the fluctuations in insect numbers in terms of climatic conditions (Davidson and Andrewartha, 1948; Madge, 1956; Maelzer, 1961a, 1964; Bateman, 1968). The concept of "climatic release" resulting from periods of favourable climatic conditions suitable for population increases has been adopted by Morris (1963a) to explain outbreaks of spruce budworm. On the other hand the influence of weather may not be so direct. For example, the effect of weather may be density linked as is the case with certain insect pathogens (De Bach, 1958) or where a density induced genetic shift increases the severity of climatic factors (Chitty, 1960). In certain instances populations may so modify their environment that the

effects of adverse climatic conditions are exaggerated (Wilson, 1968). The interaction of weather, food and dispersal is not well understood.

Wilson (1968) considered that the basic mechanisms are probably few but their variations innumerable and observed that phytophagous insects probably have several mechanisms of population regulation operating at different densities. He concluded that the principal factors involved in ascending order of importance might be: unfavourable weather or climate and polyphagous natural enemies; specialized entomophages; dispersal, migration and disease and food shortage.

(c) Conclusion. The rift between the proponents of biotic versus the physical environment schools in population regulation is largely "semantic" (Varley, 1947) and seems to arise from inadequate analyses (Wilson, 1968). Clark et al., (1967) after reviewing the supporting evidence concluded that the population theories were really "different ways of evaluating the same things conditioned by experience, preference, aptitude, but mention of the fact is necessary because of the uncompromising way in which leading theorists have adhered to their particular view points".

The study of population theories provides a worthwhile background for population studies but does not provide a useful framework or guide for studying natural populations.

(2) Life System

A brief description of the life system concept as proposed by Clark et al., (1967) is given here since conceptually it presents an extremely useful but unrestrictive frame-work for studying population ecology in the field and therefore for rationalising population theory at a practical level.

Clark, et al., (1967) have defined the life system as "that part of an ecosystem which determines the existence, abundance and evolution of a particular population".

The two components of a life system, the subject population and its effective environment are known as co-determinants. The interaction of these co-determinants of abundance affect ecological processes which control primary events (demographic characteristics of a population) and secondary events. The latter govern the extent to which primary events operate by altering food supply or acting directly on an individual.

Based on empirical evidence and logical deduction, Clark, et al., (1967) have proposed a general working hypothesis on population abundance. They claim that animal abundance depends on environmental agencies and species characteristics. These interact to determine when and how the regulation of a population is possible. This interaction sets the levels between which numbers fluctuate and controls the operation of processes related to population density by negative feed back. These processes act probablistically or automatically as regulating mechanisms to limit numerical increase.

The extent to which regulating mechanisms operate in a life system is dependent on the "innate ability" of the population to increase and the extent to which this is counteracted by density independent subtractive influences. In some life systems regulating factors may only function intermittently. Further, in different life systems of the same species the frequency and intensity with which regulating mechanisms operate may differ (Clark, et al., 1967).

(3) Population Dynamics Studies

(a) Objects. The ultimate object of most studies on population dynamics of pest species by economic entomologists is the provision of information which will permit pest management by the manipulation of the pests' life system, to prevent rapid population increases (i.e. outbreaks), or to lower the general population level (Southwood and Way, 1970). Many authors have urged that long term population research be undertaken in order that control strategies may be more objectively based (Chant, 1964; Le Roux, 1964b; Morris, 1963a,b; Pottinger, 1967; Clark, et al., 1967).

The success of insect population management involves the effective integration of what is learned about the species' characteristics and the effect of ecological processes and environmental processes on the life system (Clark, et al., 1967) as well as the accurate determination of economic threshold levels (Way, 1973). Clark, et al., (1967) consider that it is desirable to be able to mathematically model data of population studies since mathematical models give the most useful summaries of quantitative studies. Compared with other forms of synthesis, models can provide a quantitative means of showing how much is understood of the life system (Morris, 1963a; Watt, 1961, 1962). Models can be used in conjunction with experimentation in elucidating ecological principles (Varley and Gradwell, 1963; Solomon, 1964) and are invaluable for optimising pest management strategies (Watt, 1963c, 1964a).

(b) Methods of Study. Essentially there are two distinct but complementary approaches to the study of population dynamics; life table and key factor, and process studies.

(i) Life Tables and Key Factor Studies

This approach involves the collection and analyses of field data leading to graphic or mathematical models.

Multifactor studies of natural insect populations initiated in Canada by Morris and Miller (1954) have shown that the demographic life table provides a useful basic framework for intensive population studies of insects (Morris, 1963a). The historical development of the life table has been well reviewed by Deevey (1947) and its modification and use for studying insect populations discussed in detail by Morris and Miller (1954), Southwood (1966), Harcourt (1969) and Varley and Gradwell (1970). For this reason these aspects will not be considered here.

Harcourt (1969) noted that life tables are not an end in themselves but a systematic summary of age interval survival which can easily be interpreted in relation to population growth.

A single life table provides little information on factors affecting the species and it is only by replication in time and space that the full value of the data can be realised (Morris and Miller, 1954).

Ives (1964) drew attention to the many problems that may be encountered in life table studies. These include the large input in time and effort involved in taking sufficiently large samples to attain estimates of the mean population density with acceptable limits of statistical precision. He noted that this was a particular problem for soil inhabiting insects. Other factors such as overlapping generations and emigration or immigration are also known to create problems in life table studies (Southwood, 1966).

(ii) Process Studies

This approach is exemplified by Hollings' (1963) work with component analysis in which particular ecological processes such as predation are determined or, by Davidson's et al., (1970b) studies on the effect of soil moisture and temperature on scarabaeid populations.

Ideally, process studies should provide information in the form of mathematical submodels which may be fitted into an overall mathematical model of the life system (Watt, 1968; Clark, et al., 1967).

Holling (1963, 1968) pointed out that the movement of populations into the laboratory allows simple and realistic relationships between population processes to be established which cannot be accomplished without extensive field experimentation. Theoretically component analysis of major population processes should precede detailed life table studies (Holling, In Le Roux, 1963). Richards and Southwood (1968) noted the complementary nature of the two approaches and Clark, et al., (1967) observed the soundness of developing life table or key factor studies and process work together since one lends direction to the other. Clark, et al., (1967) noted that a knowledge of the principles obtained from laboratory experimentation may determine

data requirements for the discovery of regulatory mechanisms and better methods for manipulating pests. Morris (1969) and Varley and Gradwell (1970) considered that without accompanying experimental work on component analysis of casual pathways and mode of operation of ecological processes, advancement in modelling natural populations will be restricted. Likewise, Holling (In Le Roux, 1963) pointed out that life table studies have a potential which is unlikely to be utilised unless component analysis of population processes leading to the development of realistic submodels are developed.

(4) Interpretation of Mortality Data

An excellent discussion on the interpretation of mortality data has been presented by Morris (1957) in which he reviews ways of expressing data and examines the importance of constant compared with variable mortalities, the sequence in which mortalities operate and the implication of mortalities which operate contemporaneously (occurring within the same age interval).

Morris (1957) noted that mortalities which are constant from generation to generation irrespective of their size do not contribute in themselves a great deal to population change. Rather, population fluctuations result from usually small but highly variable mortalities the influence of which increases greatly when population mortality is high. As total mortality increases over the life cycle a change in percentage mortality of a variable mortality operating late in the life cycle is likely to be more influential in determining the population trend index (I) than the same mortality occurring at an earlier point (Watt, 1963a; Le Roux, 1963).

Morris (1957) found that generation mortalities for spruce budworm varying from endemic to epidemic levels were remarkably similar and ranged from 95 to 98%. When it is considered that for this insect the constant mortality rate (C.M.R.) defined as the generation mortality required to maintain I at unity is 99% it can be appreciated that very low additional mortality will cause marked changes in I. This sensitivity may allow a very

low but variable contemporaneous mortality to increase generation mortality above the C.M.R.

Mortalities that are primary determiners of I are known as key factors. Many key factors have been identified for a wide range of insects (Harcourt, 1963b, 1969; Morris, 1963a, 1963b; Le Roux, et al., 1963; Varley and Gradwell, 1968) although some authors hold that in certain life systems, key factors may not exist (Ives, In Le Roux, 1963).

In situations where large reductions in populations caused by large constant mortality factors occur before the appearance of the damaging stage of the insect, constant mortalities while not being ecologically important in that they do not determine population trends, may be economically important (Harcourt, 1963a).

There are four types of mortality: direct density dependent mortality, inverse density dependent mortality, delayed density dependent mortality and density independent mortality (Varley and Gradwell, 1970; Solomon, 1964). With the exception of delayed density dependent mortality these mortalities can be defined mathematically as well as verbally. The actions of these mortalities evoke different population responses.

In general it is recognised that direct dependent mortalities regulate populations around an equilibrium level (Varley and Gradwell, 1970; Solomon, 1964; Richards and Southwood, 1968). Initially, overcompensating density dependent factors will induce instability with successive generations alternating between high and low. With time the size of these fluctuations around the equilibrium will diminish. Inverse density dependent mortalities on the other hand increase instability and detract from the stabilising influence of direct density dependent mortality. Most inverse density dependent factors are inverse throughout part of the population range and direct over the remainder. An exception to this appears to be the action of the non specific parasites of the winter moth (Varley and Gradwell, 1970). Delayed density dependent factors create instability in populations.

Density dependent mortalities of this type may result from specific and non specific parasites which lag behind the host numbers or the effect of lower food quality and quantity, the effects of which do not appear until the next generation (Varley and Gradwell, 1970).

Density independent mortalities fall into two classes, constant and variable mortalities. The impact of both on population levels have been discussed earlier in this section.

Solomon (1964) presented rules for describing the effective killing power of different combinations of similar and different mortalities operating in sequence.

- Survival from a succession of independent mortalities is not affected by the order in which they operate.
- Survival from two density dependent mortalities is lower when the more powerful mortality operates first.
- Survival from density dependent and density independent mortality is lowest when the density independent factor operates first.
- Survival is highest when a density independent mortality operates before a constant mortality; and mortality is greater when a density dependent factor operates before a constant mortality.

Since density dependent processess are considered to be regulatory, it is unlikely that a key factor which is essentially a disturbing factor and which induces departures from the equilibrium would be density dependent. Density dependent factors can however induce disturbances if they work in a delayed manner (Southwood, 1967) and therefore be a potential key factor. Harcourt (1971) for example found that the key factor for the Colorado potato beetle Leptinotarsa decemineata was dispersal which acted in a density dependent manner.

(5) Analyses of Population Data

An appreciation of the advantages and limitations of different methods for analysing population data is necessary so that they can be interpreted in the most objective manner. Analytical methods for population data include survivorship curve analysis, survival analysis, key factor analysis and mortality analysis.

(a) Survivorship Curves. Perhaps the simplest way of presenting changes in population survival with time within a generation is by the construction of a survivorship curve. Graphed in this manner the survival pattern is easily observed and reveals periods of population stability and instability (Morris, 1955).

Ito (1959) pointed out that the study of survivorship curves can be a helpful aid in the study of insect epidemiology. The characteristics of different basic shaped survivorship curves have been described by Pearl and Miner (1935), Deevey (1947), Slobodkin (1962) and Ito (1959). Deevey (1947) recognised the following three basic shapes for survivorship curves plotted on a semi-log scale; convex (type 1), a diagonally straight line (type 2) and concave (type 3). On the other hand Slobodkin (1962) using an arithmetic scale recognised four curve shapes; convex, where mortalities act most heavily on older animals; a diagonally straight line, where a constant number die per unit time; concave (or diagonally straight line if plotted on a semi-log scale), where mortality rate is constant which is Deevey's type 2 curve; and concave, where mortality is heaviest on young animals.

Ito (1959) studied semi-log survivorship curves of 16 holometabolous insects and noted two common features. First, the shape of the curves were consistent with Deevey's type 2 and 3 curves as a result of the relatively large number of eggs laid and except in certain orders of insects a general lack of maternal care. An increase in maternal care produced a change in curve shape from concave to convex. Second, the curves were

staircased in shape and consisted of three steps corresponding to periods of decrease. These periods occurred from the egg to the first larval stage, from the last larval stage to the pupa and over the senescent adult period. The second period of decrease resulted from the activities of insect parasites and infectious diseases.

Murai (1967) derived a mathematical function embodying a parameter he called environmental capacity and showed that all of the three types of survivorship curves described by Deevey (1947) could be obtained by changing the value of this parameter.

(b) Survival Analysis. Watt (1961, 1963d) proposed a method for analysing life table data and developing population models based on the following equation:

$$\underline{I} = \frac{P(n+1)}{P(n)} = \underline{SE}\ \underline{SL}\ \underline{SP}\ \underline{SA}\ \underline{P_{+}^{o}}\ \underline{F}\ \underline{R}$$

where, $\underline{I}$ is the population trend index (Balch and Bird, 1944),

$\underline{P(n)}$ = the population level in generation $\underline{n}$,

$\underline{SE}$ = the survival of eggs,

$\underline{SL}$ = the survival of larvae,

$\underline{SP}$ = the survival of pupae,

$\underline{SA}$ = the adult survival,

$\underline{P_{+}^{o}}$ = the proportion of adults which are female,

$\underline{F}$ = mean fecundity per female,

$\underline{R}$ = proportion of potential eggs that are actually laid

For each age interval survival, survival is given by

$$\underline{S} = \underline{N_2}/\underline{N_1}$$

where $\underline{N_1}$ is the number alive at the beginning of the age interval and $\underline{N_2}$ is the number at the end of the age interval.

Although Watt (1961) recommended that $\underline{I}$ should be determined on the adult population the proposal of Morris (1963a) that $\underline{I}$ be assessed on eggs rather than adults since the egg stage is more uniform in quality has been generally adopted.

Determination of the key age interval or factor is obtained from the above equation after transformation to a log scale to reduce the variance and provide linearity. This is done either by simple correlation analyses (e.g. Harcourt, 1963b; Morris, 1963b; Paradis and le Roux, 1962; and Pottinger and Le Roux, 1971) or by multiple correlation analyses (Mott, 1966). The key age interval is identified as that interval which contributes the highest proportion of the variance of $\underline{I}$ (Morris, 1963a; Mott, 1966). Once this is established studies may be confined to this stage. From multiple correlation analysis, variance-covariance matrices can be drawn up from which the joint action of age interval survivals on $\underline{I}$ and the important sources of variance and covariances can be examined. Unlike the simple regression analyses this method recognises that the components of $\underline{I}$ may vary together. From variance-covariance matrices it is possible to obtain clues as to the causal pathways of mortality factors from the size of the covariances and whether the covariances are positive or negative (Mott, 1966). Mott (1966) cited an example from studies on the gypsy moth where the use of a simple regression analysis highlighted the wrong age interval as that making the principal contribution to the variation in $\underline{I}$. An examination of the variance and covariances showed that the proportion of the total variance of $\underline{I}$ contributed by the incorrectly identified age interval was very low. This situation arose because the wrongly identified component was highly correlated with the more important mortality.

(c) Key Factor Analysis. This method of analysis was designed to "detect key factors determining the rate of population change regardless of density responses" (Morris and Royama, 1969) and "as a useful lead to more useful studies not as a substitute for them" (Morris, 1959). In particular the method was developed to allow changes in population to be predicted where only few estimates of population density per generation are available (Morris, 1959, 1963b, 1969). The equation for analysis takes the following form:

$$\log P(n+1) = \log F + \log P(n)pg + b\ (w - \bar{w})$$

where $P(n)$ is the number in the present generation n; $\log F$, the intercept in the equation, the antilog of which is referred to as the effective rate of increase; p, the proportion parasitised; g, the proportion killed by predators; and $b\ (w - \bar{w})$, an index of weather which is usually taken as deviations around the weather index value ($\bar{w}$) taken where deviations from the regression of $\log P(n+1)$ on $\log P(n)pg$ are zero.

In its simplest form the equation consists of two terms as for example in the black budworm analysis where the numbers in the next generation were predicted from percentage parasitism in the current generation (Morris, 1963b). Southwood (1966) stressed that this is the key factor for prediction not necessarily the principal factor governing fluctuations. Varley and Gradwell (1965) have pointed out that a key factor may differ for the same population where the trend index is based on different stages and suggested, where possible, that the key factor analysis should be aimed at predicting the damaging stage. Briefly the steps in the key factor analysis consist of regressing $\log P(n+1)$ on $\log P(n)$. A low coefficient of determination (r^2) suggests that the population fluctuates highly from generation to generation and describes the net rate of reproduction. The simple regression coefficient reflects the total amount of density dependence recognised in the system. Suspected density dependent factors are then incorporated in the independent variable. Once the slope has reached unity all the density dependent factors have been accounted for.

Providing the regression is close to one and the residual variance is low, that is the coefficient of determination is high, the equation provides an adequate prediction of the population size in the following generation. Morris (1963b) considers that, with expansion and more detailed work, these types of models will provide an empirical yet biological framework in which to build up explanatory and realistic models and thereby enable the precise mode of action of the recognised key factors to

be studied. Southwood (1966) considers the method is not suited to studying populations with short population cycles and to avoid spurious results should be confined to populations which have more protracted population cycles.

The method of analysis has come under criticism on the grounds that the relationship of log P(n+1) on log P(n) may not be linear (Varley and Gradwell, 1968, 1970; Salt, 1966), that the method has in certain instances failed to detect density dependence when it has been present (Luck, 1971) and conversely, indicated density dependence in systems where it was absent (Eberhardt, 1970; Maelzer, 1970). Further criticism has revolved around the ability or rather the inability of the method to detect total density dependence in the system whenever the system contains widely variable mortality due either to density independence or delayed density dependence factors (Varley and Gradwell, 1970). Hassell and Huffaker (1969) working with artificial models which included mortalities other than density dependent factors, found the method did not give meaningful results.

Solomon (1964) noted that it may be more valid to analyse for key factors in periods of increase and periods of decrease in order to distinguish, the movement towards an equilibrium, a change in equilibrium and density independent changes. Campbell (1967) found that in sparse and dense populations of the gypsy moth the importance of specific age interval survival rates on population trend changed. In dense populations variation in survival rates over the fourth to fifth instar stage contributed the greatest variation in I. In the sparse populations the first to third instar age interval was better correlated with I but both age intervals were important in the determination of I.

Solomon (1968) considers that inspite of this, the relationship of log P(n+1) on log P(n) should not be abandoned since the final confirmation as to how factors operate must rest with further studies aimed at understanding the biological processes involved. Benson (1973) Also considers that the method may still be useful for the simple prediction of population change.

(d) Mortality Analysis. Varley and Gradwell (1960, 1963, 1968) found it convenient to record population changes on a log basis and to assess the killing power (k value) of each mortality obtained from life table studies by the difference between the log density of the population before and after the event. Where these mortalities operate separately and in succession then:

$$K = k_0 + k_1 + k_2 + k_3 + k_4 \; - \; - \; - \; k_i$$

where K is the total killing power present in a generation and equal to the sum of all the individual killing powers (k_i). Key factor determination is made by visual correlation by graphing all the k_i values against K.

Age interval mortalities may be examined for their mode of action by regressing the k_i values against the log density of the population at the beginning of the interval (Varley and Gradwell, 1968). Since the two variables are not independent a significant but spurious regression could arise from sampling errors. A proof of density dependence for each interval is therefore needed and can be obtained by regressing initial upon final and final upon initial log densities. Where both slopes are significant from unity (Varley and Gradwell, 1968; Watt, 1964b) and are on the same side of the slope of unity (Varley and Gradwell, 1968; Luck, 1971) density dependence is assumed if the regression coefficient of k_i on log initial density is significantly different from zero. (Varley and Gradwell, 1968; Watt, 1964b). A coefficient of>unity indicates overcompensating density dependence, less than unity undercompensating density dependence and unity compensating density dependence.

Benson (1973) reviewed in detail the limitations and the problems in using this analysis. He noted that these are most acute when density dependent mortality is near perfectly density dependent and in these instances suggested that density dependence should be investigated experimentally.

Sequential plotting of k_i values against log density can provide further information on the operation of mortality factors.

For instance delayed density dependent factors give circular or spiral type graphs, density independent factors irregular or zigzag patterns and density dependence both direct and inverse, a narrow band of points. While these graphic methods allow the detection of delayed density dependence, a method which satisfactorily measures delayed density dependence has not been developed (Varley and Gradwell, 1970).

(6) Models

There are essentially two types of population models that have been developed in population studies of insects, namely those that predict changes in population density, and those that predict population levels (Varley and Gradwell, 1970).

Models of the former type are developed to predict changes in population density from generation to generation and are of interest in economic entomology where predictions can be related to economic threshold levels (Morris, 1963b; Varley and Gradwell, 1970). The simplest of these kinds of models has been discussed under the section on key factor analysis and may consist of as few as two terms (Morris, 1963b). Equally simple models have been developed by identifying from life table studies the key age interval and using the simple regression equation between log survival within the key age interval and log I (Morris, 1963b; Harcourt, 1963a,b; Le Roux *et al.*, 1963; Pottinger and Le Roux 1971). Such simple and empirical models can be remarkably precise for the conditions under which they were derived.

A series of submodels may be constructed for each age interval survival in the life table to provide an overall population model. The purpose of these models is to determine the effect of age interval survival and key factor manipulation on population changes. Watt (1961) has proposed two main methods for modelling survival, the inductive and deductive-inductive methods. Although quite complicated inductive models involving submodels of all age interval survivals within a generation have been developed from life table studies, they are empirical and have little biological meaning, (e.g. Morris, 1963a).

They are largely constructed by multi-variate analysis which may give rise to spurious relationships (Watt, 1962; Varley and Gradwell, 1970) particularly when meteorological variables are concerned (McFadden, 1963). To date these overall population models have accounted for less than 50% of the variance (Morris, 1963a; Embree, 1965; Campbell, 1967) and Morris (1969) observed that no really adequate empirically based model has been produced. The production of the empirical models gives no indication of causal relationship (Varley and Gradwell, 1970) and has led Watt (In Le Roux, 1963) to state that extrapolation of such models outside the study areas, is unwise. Watt (1961) has pointed out that explanatory models can only be developed by the deductive-inductive method, an approach which is almost identical to that adopted by Holling (1963) in his studies on component analysis of population processes. In the deductive-inductive approach the relationship of the important variable and survival are graphed. An equation which best expresses this relationship is then selected in the logical tree manner described by Watt (1961, 1969). The equation selected is then integrated and transformed to a form suitable for testing against the observed results. A new equation is selected if the previous one was inadequate until a suitable equation is found. Another factor is then chosen and the process continues until all the important factors have been identified and described. These sub-submodels are then integrated into a submodel and tested in a similar way. In this manner each submodel of the model is built up. The construction of this type of model is not only predictive but biologically realistic and therefore offers an insight into population dynamics (Watt, 1961, 1969; Southwood, 1966).

A model for predicting population levels of the winter moth has been developed by Varley and Gradwell (1968). This type of model was developed to explain observed changes in populations which occur about an equilibrium level and is of use for predicting the long term consequences of introduceing additional mortality factors into a life system (Varley and

Gradwell, 1970). Varley and Gradwell (1968) have developed their simple model using submodels based on the density relationships of the mortality processes described in the previous section. Their model of the winter moth accounts for 87% of the generation to generation variance. In this life system the key factor was shown to be the disappearance of the hatching larvae before their establishment in the buds. Like other authors in the field Varley and Gradwell (1970) consider that the factor limiting realistic mathematical models of population behaviour is the lack of knowledge about the behaviour of parasites and predators. If density independent factors are modelled as well as density dependent factors this model would also predict changes in populations from one generation to another.

(7) Concluding Discussion in Relation to the Proposed Plan of Study

At the time these studies were commenced there was no very detailed information on such aspects of grass grub behaviour as emergence, mating, oviposition and dispersal and little information on the general biology and ecology of the pest.

In view of this it was considered that the soundest approach to studying the population dynamics of grass grub was as follows:

Adopt the age specific life table approach.

In the absence of adequate biological and ecological information this comprehensive and systematic approach to population dynamics studies is more certain to lead to the identification of the key age intervals or key factors than a less comprehensive approach. The systematic recording of age interval survivals enables the development of age specific models of survival leading to the construction of overall population models. These models can provide a framework for studying the effect of mortality manipulation on population levels and therefore in conjunction with injury threshold levels of pastures provide a sound basis for pest management studies. Watt (1961) noted that even if it proves impossible to develop

reliable and comprehensive population models for insect pests the exercise is worthwhile since it will provide a better understanding of the pest's life system and this knowledge should lead to improved methods of control.

The alternative approach was to study the biology and general ecology in both the laboratory and the field and select by intuition, tempered by observations and results from experimentation, possible key factors. The predictive value of these factors in relation to population change could then be tested as per Morris (1963b). While this key factor analysis approach may lead to models which are capable of predicting changes in population density from one generation to the next it does not provide a framework in which to test pest management programmes.

The acceptance of a life table approach to population studies presupposes that it is feasible with available resources to obtain estimates of population density for the important developmental stages of the insect with the required degree of accuracy and statistical precision. A sound knowledge of adult behaviour is essential to provide guidance in such matters as, location of sampling units, the selection of study plots and the manner in which dispersal is to be monitored. Detailed information on other facets of the insect's biology will undoubtedly assist in organising and interpreting life table information (Morris, 1963a; Pottinger, 1967). In view of the importance of information on the insect's biology for life table studies, studies on the biology of the adult grass grub were undertaken. These are not described in the thesis.

It was decided to limit intensive population life table studies to two sites. This action was prompted by Morris (1963a) who concluded from his massive studies on spruce budworm that, it may have been more profitable to study factors and processes which influence temporal differences intensively on a few plots and to study extensively on a range of different plots, factors and processes that determined spatial differences. Varley and Gradwell (1970) confined their studies on the winter moth, the female of which is flightless, to five separate oak trees in a

mixed woodland. In these studies the authors were able to separate temporal from spatial differences. From their studies Varley and Gradwell (1968) were able to construct an accurate model to explain why observed changes in population numbers took place around an average level.

Use laboratory studies in conjunction with life table studies to rapidly identify potentially important abiotic and biotic mortality factors.

In the case of abiotic factors, laboratory studies will enable important factors and their critical ranges to be defined and the causal pathways through which these factors operate to be elucidated. Morris (1963a) noted that in practice it is impossible to measure all variables which might influence populations. In spite of the large resources involved in the spruce budworm studies it was found that the principal factor limiting the value of the predictive models was the failure to measure the pertinent independent variables or to measure them in the best possible way. The early identification of mortality factors and the determination of their critical levels (e.g. soil temperature) will eliminate the measurement of unimportant variables or limit the measurement of the important variables to seasons when they are approaching their critical range.

Carry out ecological process studies to determine the relationship between survival and different mortality factors.

Process studies in conjunction with field data should lead to the development of realistic submodels for each mortality factor. Without process studies the development of accurate population models is impossible (Holling, 1963; Clark et al., 1967). This is evidenced by the failure of empirically derived population models (Morris, 1963a).

Finally, integrate the age interval submodels into an overall population model.

II PROBLEMS OF SAMPLING INVERTEBRATE POPULATIONS

Usually, the main object of census sampling invertebrate populations is to obtain as accurate and precise an estimate of population density as possible with given resources, or a predetermined level of precision for the lowest possible cost. An examination of the literature concerned with sampling relative sessile invertebrates suggests that efficient sampling programmes may be developed in the following sequence. Initially the variance-cost minimizing properties of different sized sample units are assessed and the most efficient unit chosen. Sampling times are then chosen which give reliable estimates of population density and as much incidental information as possible on the causes of mortality, in an effort to minimize the number of samplings required. Spatial distribution and the possible problems arising from this such as the use of transformations are assessed prior to examining the efficiency of different sampling designs. Once the most efficient sampling plan is known, sample size is estimated for different developmental stages over a wide range of population density and the sampling costs estimated in order to ascertain whether it is feasible to undertake the proposed studies with the available resources. The problems involved in these studies and the ways that these may be overcome are considered in this section of the literature review.

(1) Selection of the Sampling Unit

When sampling from a random distributed population the efficiency of any sample unit size in reducing variance is equal (Finney, 1946; Taylor, 1953; Waters and Henson, 1959). With overdispersed populations the smaller sample unit reduces the total area or volume required to be sampled for equal precision (Taylor, 1953; Finney, 1946).

Different sized soil sample units have been evaluated for sampling, the larvae of two scarabaeids (Fleming and Baker, 1936; Burrage and Gyrisco, 1954a) and wireworms (Yates and Finney, 1942; Finney, 1946). Since the distribution of the scarabaeids

are aggregated, like that of grass grub (Kain and Atkinson, 1970), these studies found that, for a given area or volume of soil sampled the variance was minimized with smaller sample units. With wireworm Yates and Finney (1942) noted that 10 and 15 cm diameter soil cores were equally efficient at low population densities but the smaller was more efficient at higher densities. The reason for this is that, as the density of wireworm populations increases the apparent distribution of the insect changes from random to being overdispersed (Waters and Henson, 1959).

In practice, the logical criterion governing the selection of the optimal sized sample unit is a compromise between the sample unit size which minimizes variance and the sample size which minimizes cost (Lyons, 1964; Finney, 1946). Usually the cost involved in taking and extracting insects from a large number of small samples compared with a small number of large samples may more than offset the variance minimizing qualities of the smaller sample unit. The comparisons in the efficiencies of different sized units are calculated and then reduced to a common base, which is usually the size of the smallest sample unit. The net cost for equal precision is given by $\underline{Cu}\ \underline{Su}^2$ where $\underline{Cu}$ is the cost and $\underline{Su}^2$ the variance per unit reduced to a common basis (Finney, 1946; Lyons, 1964).

(2) Timing of Sampling

If reliable estimates of population densities and age specific mortalities are to be obtained with minimum effort, then the timing of sampling is particularly important. This aspect of studying natural insect populations has been reviewed in detail by Morris (1955, 1960). Generally it is desirable to sample in periods when insect numbers are relatively stable. Where possible periods in which high mortalities occur should be avoided, as day to day declinations in numbers may be significant and thereby provide outdated estimates by the time samples are examined. In instances where a dynamic period of the life cycle is sampled the sampling period should be short and development and mortality in samples arrested by cool storage. Sampling times may be

dictated by the presence of stages which are easily detected, relatively immobile and in the case of surface feeding phytophages firmly attached to the sample.

A further important consideration in selecting sampling periods is the amount of incidental information that it is possible to obtain on the magnitude and causes of mortality. Use has been made of egg shells and pupal cases to provide data on mortalities caused by parasitism, predation and disease (Morris, 1955; Le Roux and Reimer, 1959; Hudon and Le Roux, 1961). Unfortunately the rapid degradation of insect cadavers in the soil greatly restricts the flow of this information for insects inhabiting moist soil.

In practice the selection of sampling intervals will be determined by the insect's habits and life history and the peculiarities of the habitat unit. Such methods as pilot sampling (Morris, 1955), the use of indicator populations in outdoor cages (Harcourt, 1961a) and the relationship between the velocity of development and accumulated day degrees (Harcourt, 1962) have been used in planning sampling intervals.

In general the timing of insect sampling becomes increasingly difficult with multivoltine species the more generations they have. This usually leads to an increase in the overlapping of developmental stages and generations. For such insects, methods for estimating the number of individuals entering each developmental stage have been developed (Southwood, 1966). As grass grub in the study areas is a univoltine species in which overlap between stages is not very pronounced, further discussion on this problem is not warranted here.

(3) Spatial Distribution

The spatial distribution of the insect within its habitat will influence such factors as the use of transformations for statistical analyses and sampling patterns.

(a) Frequency Distributions

Frequency distributions are a product of the spatial distribution of the variable concerned, in this case insects. Because most parametric statistical tests assume that the variable is normally distributed the frequency distribution of the insect being studied requires examination.

Generally speaking frequency distributions of animals fall into three categories: those that are dispersed at random, where the variance is equal to the mean; those that are over-dispersed, aggregated or contagious, where the variance exceeds the mean; and those that are under-dispersed or uniformly dispersed where the variance is less than the mean. Under-dispersed or uniform distributions are rarely found in natural animal populations (Iwao, 1970).

(i) Random Distribution. When insects are randomly distributed the resulting frequency distribution is adequately fitted by the Poisson series (Waters and Henson, 1959) which occupies a central position between over- and under-dispersed frequency distributions. The distribution is defined by one parameter, the mean, since the expected variance of the Poisson equals the mean. In fact animals are rarely distributed randomly even in a seemingly homogenous habitat (Waters and Henson, 1959; Iwao, 1970). In many cases random distributions are recorded at low mean densities. This seems to arise from a statistical deficiency, in that frequency distributions may not detect aggregation at low densities (Iwao, 1970). Similarly, smaller sample units induce a similar effect (Waters and Henson, 1959).

(ii) Over-dispersed Distribution Models. Over-dispersed frequency distributions are most frequently encountered in animal populations, and result from several factors. These include: mutual attraction between individuals; sexual attraction; heterogeneity in the habitat in relation to the animals' preferences and survival rates; reproductive behaviour such as a mass deposition of eggs or care of the young, and a limited ability for dispersal (Southwood, 1966; Bliss and

Calhoun, 1954; Iwao, 1970). Cassie (1962) considers that of all the over-dispersed frequency distribution models that are available, those which are most applicable in an ecological context are, in order of ascending skewness, the Thomas (Thomas, 1949), Neyman type A (Neyman, 1939), Polya Aeppli (Polya, 1931), negative binomial (Anscombe, 1949) and the discrete lognormal (Fisher, et al., 1943). Of these the negative binomial has proved particularly applicable for insect counts and since it was first derived by Anscombe (1949) has been used extensively. For this reason the negative binomial is the only over-dispersed frequency distribution model considered in this review.

The series is defined by two parameters, the mean ($\bar{x}$) and a positive exponent k and is expressed by the expansion of $(q - p)^{-k}$ where $p = \bar{x}/k$ and $q = 1 + p$. With increasing randomness k tends to infinity to give the Poisson series whereas with increased aggregation k tends to zero to give the logarithmic series. If only units with insects are included the logarithmic series results. The variance of negative binomial distribution is given by $s^2 = \bar{x}^2/k$ (Anscombe, 1949). The parameter k can be calculated by different methods (Anscombe, 1949; Bliss, 1958; De Bauche, 1962; Legay, 1963; Katti and Gurland, 1962), the efficiencies of which vary depending on the size of the mean (Anscombe, 1950). The simplest estimate in which

$$k = \frac{\bar{x}^2/s^2 - \bar{x}}{s^2 - \bar{x}}$$

is efficient only at low means and is therefore unsuitable for general use (Anscombe, 1950). A fully efficient estimate of k (Bliss and Calhoun, 1954) is the maximum likelihood estimate which is calculated by iteration in the following equation

$$N \ln \left(1 + \frac{\bar{x}}{k}\right) = \sum \left(\frac{Ax}{k + x}\right)$$

in which ln is natural logs, Ax is the sum of sampling units containing more than x individuals (e.g. $A6 = f7 + f8 + f9$) and

N the total number of samples. Shenton and Wallington (1962) have demonstrated that even the maximum likelihood estimation of k will be biased if the mean is small and k large. Tests of adequacy of the negative binomial as a model of the observed data are given in Bliss and Calhoun (1954) and Southwood (1966). There are three basic tests, the chi-square and the u and t tests which are the more efficient tests. Evans (1953) has provided a method based on the k and mean of the distribution which out of the latter two tests is the most efficient for the data concerned.

For many insects, a k can be estimated which is common for the species over a wide range of population densities and is therefore useful in transforming data (Anscombe, 1948), developing sampling programmes (Wald, 1945; Waters and Henson, 1959; Oakland, 1950; Morris, 1954) or estimating the number of samples required for a given level of precision (Rojas, 1964). Common ks can be estimated, by extending the maximum likelihood procedure as outlined by Bliss and Calhoun (1954), or by a moment estimate of k based on the slope of a regression line (Bliss and Owen, 1958). Alternatively a common k can be computed graphically but in more critical cases a weighted regression estimate is needed. (Bliss, 1958; Bliss and Owen, 1958).

The fitting of discrete mathematical distribution models gives little insight into the underlying causes of the observed distributions (Bliss, 1958). It is known, for instance, that an observed distribution can be approximated by more than one model, that the distribution of the same species may vary from occasion to occasion, and that the same distribution models can be derived from different basic assumptions (Iwao, 1970) or be an artifact of the sample unit size (Waters and Henson, 1959). In the absence of other biological observations the fitting of frequency distribution models is an empirical exercise which throws little light on the underlying processes involved but is useful in the planning of sampling programmes and selecting transformations (Waters and Henson, 1959).

(b) Transformation

Since insect populations are usually aggregated the following assumptions on which statistical tests of significance are based are usually not fulfilled.

- Sample counts are normally distributed
- The variance is independent of the mean.
- Separate variance estimates are independent i.e. additive (Sokal and Rohlf, 1969).

Where these conditions are not met, transforming the raw data to a new scale may overcome these problems. Of the above assumptions the second is more important that the former (Hayman and Lowe, 1962) and if satisfied improves the validity of the latter (Bliss and Owen, 1958).

Many transformations for insect counts have been proposed. These include: $\sqrt{x}$ or, where numbers are low and zeros occur in the data $\sqrt{x + .5}$ (Bartlett, 1937); $\log (x + 1)$ (Williams, 1937) and $\log (x + k/2)$ (Anscombe, 1948) in which k is the dispersion parameter of the negative binomial. Beall (1942) outlined methods with which a transformation could be worked out for a specific problem. More recently Taylor (1961) proposed his system for deriving suitable transformations based on the power relationship which exists between the mean and variance. The relationship can be expressed as $s^2 = ax^{-b}$, where a and b are constants derived from regressing the log variance on log mean ($\log s^2 = \log a + b \log \bar{x}$) in which a is the intercept at zero $\log s^2$ and b the regression slope. The intercept a is largely a sampling factor while b the slope, appears to be an index of aggregation. Data is transformed to x^p where x is the raw variable and $p = 1 - \frac{1}{2}b$ where b is the regression coefficient.

Another scheme for working out transformations has been put forward by Iwao and Kuno (1968). Their choice of suitable transformations are derived from the intercepts of the linear regression of mean crowding on the mean. Mean crowding (m^*) is defined as the mean number of individuals per individual in the

same quadrat and is given by $\overset{*}{m} = \bar{x} + (s^2/\bar{x} - 1)$, where $\bar{x}$ is the mean and s^2 the variance. Over a diverse range of theoretical and biological distributions, mean crowding is linearly related to the mean as follows:

$$\overset{*}{m} = a + bm$$

where a is the intercept which reflects the contagiousness inherent in the species and is termed the "index of basic contagion". The regression coefficient (b) is termed the"density contagiousness coefficient" since it indicates the manner in which individuals distribute themselves in the habitat with changes in density. These two parameters measure the dual nature of aggregation (Iwao, 1968).

Southwood (1966) considers that in practice where errors are fairly large it is sufficient to use a square root transformation for slightly aggregated populations and a logarithmic one for populations which are markedly aggregated. Unfortunately, transformation of data in life table studies can lead to problems (Southwood, 1966). The apparent distribution changes, with density (Waters, 1959), stage of development (Guppy and Harcourt, 1970) and sample unit size (Waters and Henson, 1959; Lyons, 1964) may necessitate the use of many different transformations. The comparison of means based on different transformations is difficult and necessitates transforming the transformed mean back to the original scale and correcting for bias (Finney, 1941b; Neyman and Scott, 1960). In other instances no common transformation is adequate to transform data to normality unless sample units are independently pooled (Andersen, 1965).

Theoretically data from over-dispersed populations should be transformed (Beall, 1942; Snedecor and Cochran, 1959). However, the consensus of opinion concerning transformations is that moderate departures from normality do not induce gross errors in the significance levels of the f and t tests (Hey, 1938; Bartlett, 1935; Pearson, 1931; Le Roux and Reimer, 1959;

Wadley, 1967), and generally, transformations are not considered necessary unless the need for them is extreme (Wadley, 1967; Scheffe, 1959). Cochran (1963) noted that good sampling practice may tend to make the normal approximation more valid. The removal of extremes by stratification from the main body of population reduces skewness and therefore improves the normal approximation.

The question which arises is what does moderate departures from normality mean? From recent work by Abrahamsen and Strand (1970) on the application of parametric statistics to counts of enchytraeid worms, which have a very marked positively skewed distribution, the confidence intervals did not cover the true mean as often as expected but at means above 10 per sample this was scarcely significant. Even at levels as low as one per sample this did not change greatly with the 95% confidence limit being closer to the 90% level. While log transformation gave a slight improvement in the *ANOVA, the ANOVA was found robust against type 1 errors which led Abrahamsen and Strand (1970) to conclude that "transformation for this purpose seems unnecessary".

In many cases all that is required from a sample is an estimate of the mean population and its confidence limit. It is known that the arithmetic mean of a sample can be used as an unbiased estimate of the mean density. The central limit theorem states that the distribution of means of large samples from any population tends to normality with a standard deviation of S/N where S is the population standard deviation and N the sample size (Cochran, 1963). The question that arises is how large must N be before the normal approximation for the estimate of confidence limits is accurate enough with highly skewed distributions. In order to estimate the number of samples required (SN) Cochran (1963) recommends the use of the equation

$$SN = 25\, G1^2$$

*ANOVA = Analysis of variance

where G1 is Fisher's measure of skewness. This rule is designed so that the 95% confidence probability will be wrong less than 6% of the time.

This approach to the problem has been adopted by Lyons (1964) with his work on the sawflies Neodiprion swainei and N. sertifer but unfortunately a problem which confronts the use of Cochran's equation is that it often gives impractically high numbers of sample units (Abrahamsen and Strang, 1970).

In view of the problems in life table work associated with transformations there appears little justification for transforming data providing that the sample size is large enough so that the central limit theorem is applicable. If adequate transformations are available and are not difficult to use their use is probably worthwhile for investigating the efficiencies of different sampling plans but not for estimating means of populations and their confidence limits. Such an approach to the problem has been adopted by Harcourt (e.g. 1961a, 1962).

(c) Sampling Pattern

The essentials of sampling are that sampling should be representative in order to obtain as accurate a picture as possible; it should be devoid of bias and should be random (Wadley, 1967). Morris (1960) pointed out that "very little insect sampling is truly random. In most cases we do not know how to randomise in sampling, especially for mobile species" and concluded at best sampling plans might be called unbiased.

Randomness does not lead to increases in efficiency but is a basic assumption on which error estimates are based. Cassie (1962) observed that for sampling plankton populations simple random sampling compared with systematic sampling was less efficient as the latter provides a more representative sample. Unfortunately little is known on the theory of error estimation of systematic sampling. A compromise between the error estimating properties of random sampling and the enhanced

representivity of systematic sampling is stratified random sampling. For this method of sampling the habitat is divided into separate strata from which samples are drawn at random.

With stratification the variation between strata means is eliminated from the sampling error and the variance of the population mean arises solely from the sampling units within the strata (Sampford, 1962; Cochran, 1963). It therefore follows that an increase in precision from stratification will result when the variance within individual strata is less than the overall variance of the population. To this end populations should be divided into as homogeneous subgroups as possible. Biological knowledge of the species being studied and previous sampling data can lead to more objective stratification and therefore to larger gains in precision. For example, Prebble (1943) and Stark and Dahlsten (1961) in their studies were able to delete areas where predictably few insects were found. Abrahamsen (1969) on the other hand found that where the size of the strata coincided with the size of the aggregates or colonies large increases in precision were obtained for the appropriately sized stratum.

Where there is no clear-cut criteria for delineating strata divisions it is usual to divide the habitat into equally sized strata and allocate the samples proportionately between them. Under these circumstances although stratification does not lead to large gains in precision (Cochran, 1963; Snedecor and Cochran, 1959) it is better than simple random as it improves sample representivity (Finney, 1946; Healy, 1962; Lyons, 1964; Abrahamsen, 1969).

There are two methods of allocating the number of sample units between strata. Proportional allocation where the sampling fraction is the same in every strata and optimum allocation where the sample fraction is allocated in such a fashion as to minimize

the cost of obtaining a specific level of precision or maximize precision for a given cost (Cochran, 1963; Hansen, et al., 1953; Sampford, 1962).

Where costs of sampling are similar the allocation of samples per stratum is based on the proportion that the stratum standard deviation contributes to the overall standard deviation. The gains in precision resulting from optimum allocation compared with proportional allocation arise from the elimination of the influence of differences among strata standard deviations (Cochran, 1963). Optimum allocation of samples is more applicable to situations where data from previous samplings or pilot samplings are on hand for allocating the number of sample units to each stratum (Cochran, 1963). Sampford (1962) noted that maximum gains in precision from optimal allocation arise when the number of strata are small, possibly no more than six, with widely differing means.

Cochran (1963) has formulated three general rules governing the number of sample units to be taken from each stratum. He suggests that a large sample should be taken when the stratum is larger, when the stratum is more internally variable and when sampling within a stratum is cheaper.

Although at least two samples are required per strata in order that the within strata variance can be estimated Cochran considers that to obtain a reliable estimate of the stratum mean and variance six samples should be taken from each stratum.

In preliminary sampling, small sample units carefully stratified allow the development of the most efficient sampling designs (Morris, 1960). Once the preliminary data has been collected major variance components are detected by ANOVA and the different methods of allocating sampling resources, as dealt with by Cochran (1963), Hansen et al., (1953) and Sampford (1962) may be used in designing optimal sampling plans (Morris, 1955; Le Roux and Reimer, 1959; Harcourt, 1961a; Pottinger and Le Roux, 1971).

(4) Sample Size

A level of statistical precision of 10% standard error of the estimated population mean was arbitrarily set by Morris (1955) for population studies of the spruce budworm and has been generally adopted as a reasonable level of precision for most life table studies (Le Roux and Reimer, 1959; Harcourt, 1961a, 1962; Pottinger and Le Roux, 1971).

The method for estimating the number of samples required for a given level of precision is

$$N = \frac{ts^2}{D\bar{x}}$$

where N is the number of samples, s is the standard deviation, $\bar{x}$ is the sample mean, D is the required level of precision expressed as a decimal and t is set for a given probability.

In cases where a common k, the dispersion parameter of the negative binomial, is known the sample size (N) can be estimated from the below equation of Rojas' (1964).

$$N = \frac{\frac{1}{\bar{x}} + \frac{1}{k}}{D^2}$$

This formula is identical to that proposed by Kuno, et al., (1963).

A more general method for estimating sample size has been published by Iwao and Kuno (1968, 1970) based on the linear relationship between mean crowding and mean density.

(5) Sequential Sampling

A sequential sampling procedure enables sampling to stop as soon as enough data has been gathered to allow a decision to be reached on population density. Wald (1945) described a method of sequential sampling developed for quality control in industry which has since been adopted by biologists (Oakland, 1950; Waters, 1955;

Morris, 1954; Ives, 1954). The method serves to classify populations. In constructing a sequential sampling plan of the Wald (1945) type, insect population levels or classes must be related to infestation classes which in the case of phytophagous insects are usually based on the relationship of insect density to damage. For very low and high populations few samples are needed to classify populations. The method for constructing sequential sampling plans of this type is outlined by Waters (1955) and Southwood (1966).

Other methods of sequential sampling have recently been developed by Kuno (1969) and Green (1970) and are based respectively, on both the intercept (a) and slope (b) of the linear relationships between mean crowding and mean population density (Iwao, 1968) and the variance and mean population density (Taylor, 1961). These methods are designed to provide estimates of population density with fixed levels of precision. The relationship between the cumulative total insect count for Kuno's method is given by

$$\underline{Tn} = \frac{\underline{a} + 1}{\underline{D}^2 - \frac{\underline{b}-1}{\underline{n}}}$$

where $\underline{Tn}$ is the cumulative total counts and $\underline{n}$ is the number of sample units, $\underline{a}$ and $\underline{b}$ the intercept and slope of the mean crowding - mean population density relationship and $\underline{D}$ the level of precision as a ratio of the standard error and mean. The plotting of $\underline{Tn}$ on $\underline{n}$ provides the stop line.

Similarly the stop line for Green's method is given by

$$\log \underline{Tn} = \log \frac{\underline{D}^2/\underline{a}}{\underline{b} - 2} + \frac{\underline{b} - 1}{\underline{b} - 2} \cdot \log \underline{n}$$

where $\underline{a}$ and $\underline{b}$ in this case are the intercept and regression coefficient of log variance on log mean relationship. Unlike Kuno's (1969) stop line, this equation gives a straight stop line varying from horizontal for the Poisson distribution to negative and positive slopes depending on the degree of over-dispersion.

The advantage of Kuno's (1969) and Green's (1970) sequential sampling methods compared with Wald's type is in the

case of certain insects a common $\underline{k}$ is not a stable parameter and for other insects does not exist (Bliss and Owen, 1958; Berthet and Gerard, 1965). Perhaps a greater advantage is that these methods give estimates of the mean density whereas Wald's type of sequential sampling plan serves only to classify populations.

CHAPTER III

LITERATURE REVIEW ON PEST ASSESSMENT STUDIES OF PASTURE INSECTS

I. INTRODUCTION

In the main, attention is confined in this review to problems associated with the establishment of economic injury threshold levels for pasture insects. The economic threshold level for injury has been defined as "the lowest population density that will cause economic damage" (Stern, et al., 1959). Economic damage is the amount of injury which will justify the cost of artificial control measures. As a consequence, this level may vary in response to differences in management, levels of production and changes in costs and prices. The establishment of economic injury levels is recognised as one of the prime prerequisites of any objectively based agricultural pest control programme; no matter whether the programme concerned involves control by an integrated pest management programme (Huffaker, 1970) or solely with insecticides (Smith, 1970). Economic injury levels are considered essential for assessing the importance of the components of pest control programmes such as the value of, natural enemies, pesticides and cultural practices in maintaining pest populations at sub-economic levels (Stern, et al., 1959).

Initially the establishment of accurate economic threshold levels for pasture pests requires the relationship between insect density and pasture productivity to be defined and then losses in pasture productivity to be estimated in terms of animal production, in order that these can be estimated in economic terms.

Pastures, as distinct from crops, are grown as feed for livestock and are "seasonally dynamic and competitive associations of plants, the balance of which is maintained by the grazing animal". (Douglas, pers. comm.). Whitehead (1966) noted that "pasture is a highly variable biological material composed of

many plant species and is an outcome of the local environment, influenced by climate, soils, animal and man".

Fenemore (1966) studied pasture damage caused by grass grub and noted that the three following inter-related processes were involved. Direct losses in productivity as a result of plant stunting and death caused by the root pruning activity of larvae. Changes in botanical composition caused by weed invasion into damaged areas and possibly, a breakdown in soil structure as a result of soil ingestion by larvae.

The seasonal appearance of grass grub damage in pasture is dependent on rainfall but usually becomes visible by the end of February. After May, larval feeding declines and ceases from June onward. Pasture losses over the autumn-winter period in damaged areas may range from 5 to 85% (Rough and Haeske, 1966; McLean, 1969). With the exception of spring and early summer when the larvae are not feeding, the effects of direct pasture damage are always confounded with a grass grub induced deterioration in botanical composition. The ability of plants to tolerate the root pruning of grass grub is influenced by such factors as plant species, soil fertility and soil moisture (Radcliffe, 1971a,b).

The effect of grass grub on animal production is dependent on how pasture damage influences the principal factors governing animal production. McMeekan (1961) outlined these as: the quality, amount and seasonality of pasture production; the proportion of the pasture harvested by the grazing animal and the efficiency of utilisation of the food eaten by the animal. Of these only the latter cannot be influenced by insect damage.

II PASTURE SYSTEM

Before assessing the effects on pasture productivity and animal production that may result from grass grub damage it is essential to have an appreciation of the pasture system under study and the relationship between animal and pasture production.

Perennial ryegrass and white clover form the basic constituents of seed mixtures used for New Zealand pastures. These species may be augmented in the drier regions by cocksfoot and subterranean clover and in the wetter regions by red clover and timothy (Saxby, 1945; Levy, 1951). Many grasses, clovers and herbs became established throughout New Zealand pastures as a result of the large number of species sown during the early days of grassland farming. Recent surveys of pastures in the temperate areas of New Zealand have shown that ryegrass represents only a relatively small proportion of the plants present or herbage produced (Rumball and Grant, 1972; Palmer, 1970; Corkill, 1970). In view of this it is not surprising that Vartha's (1965) claim that New Zealand pastoral agriculture appears to be based on ryegrass and white clover has been seriously questioned (Round-Turner, 1970).

With the exception of the high rainfall areas of the South Island and Northland, seasonal pasture production patterns do not appear to differ markedly (Anon. 1972). The gross features of this pattern are brought about by the restriction of moisture over the summer and low temperatures over the winter (Rickard, 1968).

McMeekan (1945) noted that New Zealand herbage production normally exceeds animal requirements during the spring and autumn, but in winter pasture production may not be sufficient to meet these requirements. As a result, in the summer and winter months the pasture system is likely to be more sensitive to insect damage than during spring and autumn.

The feed requirements for different classes of stock change from month to month depending on their physiological condition and these have been calculated for sheep, beef cattle (Coop, 1965) and dairy cows (Hutton, 1962). Over the autumn and winter the feed requirements for beef cows and ewes are relatively low but increase in July and August. On the other hand, dairy cows have a proportionately higher feed requirement in autumn and mid winter than the other classes of stock. For this reason and because of

the poor pasture growth over the winter, dairy farmers appear to be more vulnerable to grass grub attack than other pastoral farmers.

III EFFECT OF INSECT DAMAGE ON PASTORAL PRODUCTIVITY

Insect losses in pasture production may be associated with losses in both the quality and quantity of production.

(1) Quality

There are two distinct but related aspects of pasture quality to consider, the aspect of animal nutrition or health, and the agronomic aspect.

Harris (1970) noted that weed grasses may cause mechanical damage to stock (e.g. Hordeum murinum, Atkinson and Hartley, 1972) lack palatability (e.g. sweet vernal), be poisonous to stock (e.g. fescue; "fescue rot"), have poor herbage production, or be annual species which on death leave an open sward.

Most pasture species are developed with three main objects in mind, namely, maximum production of nutritive herbage, maximum spread of seasonal production and compatibility in pasture mixtures (Corkill, 1969). A change from sown to volunteer species therefore, would be expected to interfere with these desirable pasture characters or affect stock in an undesirable way. This generalization requires examination as in many cases there is scant information on the relative productive values of different so called "weed species". For example, a range of grasses including cocksfoot, prairie, paspalum, phalaris, Yorkshire fog, kikuyu and tall fescue, some of which are regarded in certain localities as weeds, have been assessed for herbage production in many areas of New Zealand. No species has demonstrated an overall clear-cut superiority or inferiority in all districts. Rather in specific localities certain species have performed well (Anon. 1971b).

Further, little work has been carried out evaluating different pasture plants as animal foods let alone assessing the value of weeds. Evidence suggests that seasonal differences in the nutritive values of most common pasture grasses found in New Zealand is likely to be larger than differences between the more common temperate pastureland species (Joyce, pers. comm.). Ulyatt (1970) reviewed the work on animal performance on different pasture species and found that live weight gains of lambs were greater on legumes than grasses. Therefore, the loss of clover would be expected to affect not only the lamb fattening qualities of pasture but nitrogen fixation and hence herbage productivity (Sears, 1954). Conversely, a high proportion of clover in pastures is known to cause bloat in cattle (Johns, 1954) and scouring in both sheep and cattle (Hewitt, 1969). Three insect pests are known to interfere with the clover-grass balance of New Zealand pastures. Inopus rubriceps (soldier fly) (Hewitt, 1969) and Heteronychus arator (black beetle) (King, pers. comm.) induce clover dominance, whereas grass grub reduces the clover content of pastures (Kain, unpub.).

Insect damage (e.g. black beetle and grass grub) may cause a build up of litter in pasture in autumn which can precipitate outbreaks of facial eczema. The causative fungus of this disease grows in pastures on dead and decaying plant material (Thornton, 1960).

The general lack of information on the effect on productivity of weed invasion is noted by Allen and Meeklah (1972) who reported that "information on animal yield response to the removal of weeds is meagre or obscured by large parallel changes in other pasture components, especially clover, resulting from control practices."

(2) Pasture Availability

An asymptotic relationship has been reported to exist between the performance of the grazing animal and pasture availability (Willoughby, 1959; Allden, 1962; Arnold, 1963). The reason for this relationship was examined by Allden and Whittaker (1970). These authors observed that above a critical threshold

level of available pasture, intake was not affected by decreases in pasture availability. Decreases past this point caused a fall in consumption which was partially offset by an increase in grazing time. Insect damage would therefore only affect herbage consumption where the level of available pasture is below this critical threshold level or where insect losses reduce pasture availability below this level. Where there is no change in the nutritive value of pasture, losses in animal production will only occur when intake falls below the feed requirements of livestock. Consequently, if the effect of insect damage on animal production is to be accurately assessed the determination of the critical levels for pasture availability in relation to consumption for all classes of livestock throughout the year is essential.

Obviously, the sensitivity of a grazing system is influenced by factors which affect the availability of pasture, such as stocking rate, climatic variations, and animal feed requirements.

Very high levels of production per animal have been achieved with experimental stocking rates that exceed those of the local farmers (Anon, 1971c; Coop, 1967). This has been attributed to a higher percentage of pasture utilization and in part to the complicated pasture-animal interactions (Coop, 1967). Such an observation suggests that on most New Zealand farms there is a large degree of elasticity between pasture and animal production which can absorb quite large losses in pasture caused by insect attack without influencing animal productivity.

(3) Autumn-Winter Food Restrictions on Animal Production

The effect of feed restrictions on animal production over the autumn-winter period when grass grub is actively feeding varies with the class and age of stock. Young stock are generally more sensitive to changes in the amount and quality of herbage than older animals (Joblin, et al., 1972). Hutton and Parker (1973) have shown with dairy cows that gains in body weights one month before calving resulted in a 15 to 21% increase in production over the first eight weeks of lactation. Hight (1968) found that low

levels of pre-calving nutrition of run cows from late June until calving resulted in a decrease of 22% in the number of calves weaned and lower birth and weaning weights.

Lambing percentages are known to be markedly influenced both by body weight at the time of conception (March) which is called the "static effect" and increases in body weight prior to and over the mating period ("dynamic effect") (Coop, 1966).

Sub-maintenance feeding of ewes after mating have not generally induced differences in ewe death rates, ewe barrenness (Hodge, 1966; Monteath, 1971) or lamb survival (Monteath, 1971), although Coop and Clark (1969) observed under these conditions minor but significant decreases in twinning and an increased number of barren ewes. Fleece weight increases resulting from different levels of feeding over the late summer and winter have also been recorded (Wallace 1962; Coop and Hart, 1953; Clark, et al., 1965).

More extended and severe food shortage over pregnancy may reduce lamb drop (Everitt, 1964, 1966; Bennett, et al., 1964); influence birth weight, meat production and fleece characteristics of lambs (Schinckel, 1963; Taplin and Everitt, 1964; Williams and Henderson, 1971); as well as lamb growth rates (Everitt, 1967).

The assessment of the effect of insect damage in terms of animal products from the same animal, e.g. wool and meat is difficult as each product may vary in its sensitivity to fluctuations in pasture production. Joblin, et al., (1972) for instance, observed that ewe and hogget live weights are sensitive indices of short term pasture production while wool was a less sensitive index. The ability of animals deprived of food to recover through compensatory growth when food is plentiful (Joblin, 1968; Drew, et al., 1973) also complicates pest assessment studies of pasture insects.

(4) Summation

The influence of pasture composition on pasture productivity, the relationship between pasture composition and animal productivity and the relationship of pasture production to animal production must be appreciated before the pest status of grass grub can be accurately established. In summary it seems that:

- There is little New Zealand evidence to suggest that the invasion of pasture by productive volunteer grasses and herbaceous weeds is detrimental to animal production, provided that it is not at the expense of white clover, that the species are palatable, and the species do not impair the health of the animal. Insect induced changes in the botanical composition of pasture can cause disease and metabolic disorders of livestock. From available evidence it seems that differences in nutritive values of most grasses between seasons is likely to be larger than between the more common temperate pasture species.
- Herbage losses are not easily assessed in terms of losses in animal production and are influenced by the seasonal feed requirements of animals, and the available pasture. Pasture production in late autumn and winter when grass grub are actively damaging pasture is generally below animal requirements. However, the major portion of animal requirements over this period is usually met from hay grown over the spring when pasture production is in excess of animal requirements and when grass grub larvae are not actively feeding.
- The effect of herbage losses from insect damage on animal production in most commercial grazing systems is likely to be cushioned by the stocking rate effect where more stock or less pasture results in better utilization without initially a loss in production per animal. Although the majority of commercial

grazing systems may be relatively insensitive to losses in pasture production over most of the year there are well defined sensitive periods, e.g. before the mating of ewes and calving of dairy cows, when small losses may have a severe effect on animal production.

- The ability of the animal to compensate for weight losses in periods of food shortages and to tolerate wide fluctuations in food supply without large rises or falls in production, as for example the ewe, makes the problem of assessing the pest status of insect pests of pasture even more difficult.

IV INSECT DAMAGE

The method and parameters used for measuring insect damage will depend on the type of damage being studied, the factors which influence damage and the biology of the insect concerned.

While much work has been reported on the assessment of crop losses caused by insects, few attempts to assess insect damage of pasture appear in the literature. As a result, it has been necessary in this review to draw on information from pest assessment studies of crop insects.

(1) Types of Insect Damage in Pasture

Irrespective of the type of insect damage (e.g. root pruning, stem boring or defoliation) losses in pasture from insect damage can arise in three distinct but often related ways. The most obvious type of damage is where plant cover is partially or completely destroyed leaving bare ground into which volunteer weed species establish. A less obvious type is where pasture production is impaired without an associated change in the botanical composition of pasture. The third and least spectacular type of damage is an insidious change in botanical composition unaccompanied by any abrupt change in pasture production (e.g. Inopus rubriceps; Hewitt, 1969). In its more advanced stages

botanical composition may be so altered that both annual production and seasonal production patterns of pasture are affected. Changes in botanical composition may occur therefore, through the invasion of volunteer species into insect bared areas and in response to preferential feeding by the insect or differential recovery rates of damaged species.

(2) Factors Affecting Insect Damage

Damage caused by insects may be a result of the interaction of many variables which include insect numbers, population dispersion, insect behaviour, plant vigour, plant species or variety, plant maturity, cultural practices and the physical environment (Smith, 1967).

It has been pointed out by many authors that the relationship between insect numbers is not a simple linear regression (Southwood, 1966; Stern, 1966; Stern, et al., 1959; Gough, 1947; Jackson, 1965). Tammes (1961) has proposed a three stage classification between infestations and crop yield. Over the first stage injurious factors have little effect on yield because plants compensate for damage. In the second stage loss is correlated with increasing injury, whilst over the third stage yield is badly affected by increasing injury but causes proportionately less damage.

Populations which are dispersed evenly over the paddock are less likely to cause as severe damage as aggregated populations (Johnson, 1965). In addition the site of damage on the plant may affect the extent of damage (Chiang, et al., 1954). In the case of root-pruning soil insects the level of feeding in the soil (Davidson and Roberts, 1968c) has an important bearing on the severity of damage as does soil moisture, nutrient supply (Grabner, et al., 1931; Tashiro et al., 1969; Radcliffe, 1971a,b) plant species (Radcliffe, 1970) and management (Grabner, et al., 1931).

(3) Assessment of Insect Damage

Smith (1967) and Strickland and Bardiner (1967) have reviewed the methods for measuring crop losses arising from insect damage. All are adaptable for use in pasture. Those listed by Smith (1967) included such methods as: the evaluation of crop yields before and after the introduction of a pest into an area; the evaluation of damage before and after the introduction of successful control procedures; the comparison of naturally infested and naturally uninfested plants; cage studies where exclusion cages or, in the case of non flying insects, exclusion barriers are used; artificial infestations; chemical treatments; artificial removal of pests; manipulation of natural enemies and simulated damage. To these Strickland and Bardiner (1967) have added subjective estimates of yield losses arrived at from the opinions of farmers and farm advisory officers.

The approaches to measuring insect damage in pasture and alpine grasslands under field conditions are as follows:-

- Mowing herbage from plots treated with insecticide and estimating the loss caused by insect damage as the difference between the herbage production of the treated and untreated plots in terms of, green weight (Fenemore, 1966; Rough and Haeske, 1966; McLean, 1969) or dry weight (Rastrick and Upritchard, 1968; Wallace and Mahon, 1963; Allen, 1968; McLaren and Crump, 1969).
- Comparative estimates of basal cover and leaf growth (leaf length) from areas within paddocks which are infested and uninfested (Kelsey, cited Jensen, 1967).
- Visual assessments of damage based on the number or area of dead and dying plants or the size of damaged compared with undamaged plants (Fenemore, 1966; Fuelleman and Graber, 1937; Raw, 1951).
- The use of aerial photography for measuring areas suffering from insect attack (Howard, 1970).

- Estimates of consumed foliage based on controlled feeding studies under field conditions combined with population estimates (White and Watson, 1972; White, 1974; French, 1973). Methods such as these, obviously have limitations for studying root pruning-insects.
- Animal measurements such as losses in grazing days, sheep weights and wool weights estimated from insecticide treated and untreated pasture (Wallace and Mahon, 1963; Kain and Atkinson, 1972).

Fenemore (1969), from small plot insecticide trials, noted that a linear relationship exists between grass grub density and losses in herbage green weight. Kelsey (cited Jensen, 1967) defined the relationships between grass grub density and leaf length or basal ground cover. Unfortunately, with the exception of herbage dry weights and animal measurements, most of the parameters of pasture productivity used by entomologists, such as herbage green weight and leaf length or surface cover, cannot be accurately converted into animal feed units, and hence animal production.

The use of insecticides in pest assessment studies is attended by the problems that insecticides may: affect the plant directly; affect insects other than the target one, such as other pests or natural enemies; and with small plots, treated areas may leave a vacuum into which insects move and hence reduce the populations of the untreated plots (Smith, 1967). Further, the destruction of a large subterranean biomass such as that created by high populations of grass grub leads to the decay and the release of nitrogenous compounds capable of stimulating plant growth. Toms (1967) has pointed out that in studies which use insecticide we are evaluating the usefulness of chemical treatments, not the damage caused by a specific pest. Therefore, threshold levels arrived at from such studies are only useful for evaluating the value of pesticides and not other methods of control which may be used in a pest management programme.

V PEST ASSESSMENT OF PASTURE INSECTS

The problems associated with estimating economic threshold levels for crop pests are compounded for pasture pests by the fact that an economic analysis of insect losses in pasture production necessitates that losses be expressed in terms of animal production. Attention is drawn here to the pasture system and factors influencing the production of the grazing animal, discussed earlier in this review.

Only a few publications exist on the evaluation and interpretation of insect pasture damage in terms of animal production and/or monetary losses. Flay and Garrett (1942) surveyed decreases in production and farm income caused by porina (Wiseana spp.) and grass grub on Canterbury mixed cropping farms. Gordon and Kain (1972) surveyed the effect of grass grub on changes in numbers and class of stock on five badly damaged farms in the Taupo hill country. In both surveys comparisons of production were made before and after insect damage became severe, but no attempts were made in these studies to define economic threshold levels.

Jensen (1967) utilised the relationship established by Kelsey (unpub.) for the growth pattern of grass grub numbers from one generation to the next over a seven year period, and the relationship between losses in surface cover and grass grub density. He assumed the former relationship to be linear and together with the relationship between grass grub numbers and basal pasture cover carried out an economic evaluation of insecticide usage. No mention was made in this study of the time at which populations were sampled or factors which could markedly affect surface cover such as grazing management, season of measurement, pasture species, and climatic factors, e.g. rainfall. Jensen's evaluation was based on a cost-benefit analysis of strategies for the insecticidal control of grass grub at different population levels. In this work Jensen made the important assumptions that; stocking rate policies were formulated by the farmer independently of the grass grub problem, grub counts provided an accurate index of infestation, and pastures were

fully stocked. Based on the latter assumption he assumed that any decrease in pasture growth would produce a linear decrease in animal production.

Rastrick and Upritchard (1968) in a cost-benefit study of porina related the returns in pasture production from the use of insecticide to an average mid-Canterbury farm. The farm was reliant on hay and autumn saved pasture for winter feed. These authors calculated the difference between the available feed from pasture on the insecticide treated and untreated plots and the ewes' feed requirements. The cost of additional hay which was required to maintain ewes without the use of insecticide over this period was then calculated. This additional cost for hay was offset against the cost of the insecticide and the difference provided an estimate of the economic return from insecticide usage.

From small plot insecticide trials, Allen (1968) estimated the amount of herbage porina larvae consumed and expressed this in terms of potential ewe equivalents.

Wallace and Mahon (1963) studied the yield and botanical compostion of established pasture following an application of DDT to control red legged earth mite (Halotydeus destructor). From a large number of small plot insecticide trials it was estimated that the increases in herbage resulting from insecticide application to carefully selected areas, heavily infested with red legged earth mite, gave herbage response equivalent to the feed requirements of 1/3 sheep/acre. From this, the break even or economic threshold point for insecticide usage was calculated under varying costs and prices.

The approaches adopted by Allen (1968) and Rastrick and Upritchard (1968) have been criticised by McLaren and Crump (1969) on the grounds that there is no constant relationship between dry matter production and stocking rate. McLaren and Crump (1969) established that over the range of porina infestations studied on small plot insecticide trials the relationship between the

proportion of herbage production lost over a wide range of climates was adequately fitted by a linear regression ($r = 0.92$). With this relationship as a basis they proposed a model for calculating the economic threshold levels for different stocking rates. The threshold level of damage was taken as the point at which the returns from the use of insecticide covered costs. These authors like Jensen (1967) based their model on the erroneous assumption that farms are fully stocked, with the result that a decline in meat and wool production resulting from porina infestations is directly proportional to and is a linear function of the decline in herbage dry matter production. While many experimental trials may be near to being fully stocked (Coop, 1967), in most seasons it is doubtful whether many farmers even approach this situation. Therefore, a model based on this assumption will seriously over-estimate the importance of insect damage.

The difficulty of converting herbage reductions as a result of insect damage into animal production and the translation of this into economic terms, may be overcome by the development of general but accurate models of pasture systems. Such models will enable animal productivity to be predicted from pasture production and will facilitate the conversion of insect damage in pasture into losses in animal productivity for farms operating at different intensities. Freer, et al., (1970) have demonstrated the possibilities of accurately modelling pasture systems. Middleton (1973) on the other hand has developed a gross type of model for estimating the monetary value of pasture for different species of livestock and under different farming intensities. This model was developed for use in planning fertilizer programmes and with modification could be useful for calculating gross estimates of economic losses suffered from insects.

Problems of establishing accurate economic threshold levels have been discussed by Way (1973) and Adkisson (1973). These include variability between fields, the effects of unpredictable weather occurring after decisions have been made, unpredictable economic factors (Way, 1973) and grower acceptance (Adkisson, 1973;

Way, 1973). Way (1973) noted that the above variables create "a gulf between the theoretical concept of an economic injury level and its practical implementation by the farmer".

VI STUDY PLAN

In spite of the problems likely to be encountered with pasture pests it was decided to investigate the feasibility and worth of establishing accurate threshold levels for grass grub.

The general study plan adopted in these studies was as follows:

- To develop techniques for measuring losses in pasture production without the use of insecticides.
- To measure losses in pasture productivity in relation to insect density in a manner which could be assessed quantitatively in terms of animal production.
- To assess losses in pasture productivity from grass grub damage in terms of, animal production and economics.

SECTION II

METHODS

CHAPTER IV

DESCRIPTION OF STUDY SITES AND MECHANICS OF SAMPLING AND EXTRACTION

I INTRODUCTION

The purpose of this chapter is to describe the sites on which population and agronomic studies were conducted and the methods and mechanics involved in sampling and extracting grass grub from samples of soil.

II DESCRIPTION OF STUDY SITES

The research on grass grub populations and pasture damage described in this thesis may be divided into extensive and intensive studies. Extensive studies involved studies on the rate that grass grub populations build up from incipient levels, to densities at which severe pasture damage occurs and the relationship between pasture damage and population levels. The extensive studies, which are described in chapter 8 were largely confined to the Takapau Research Farm although some were carried out close by on Smith's plots.

Intensive population studies on the other hand were concerned with more precise and detailed monitoring of population changes (chapter 7) and the extent and growth of pasture damage (chapter 8). Intensive population studies were confined to two life table study plots on the Takapau Research Farm (Fig. 4-1) and another at Rukuhia near the Hamilton airport. Intensive studies of pasture damage were conducted on Smith's plots at Takapau.

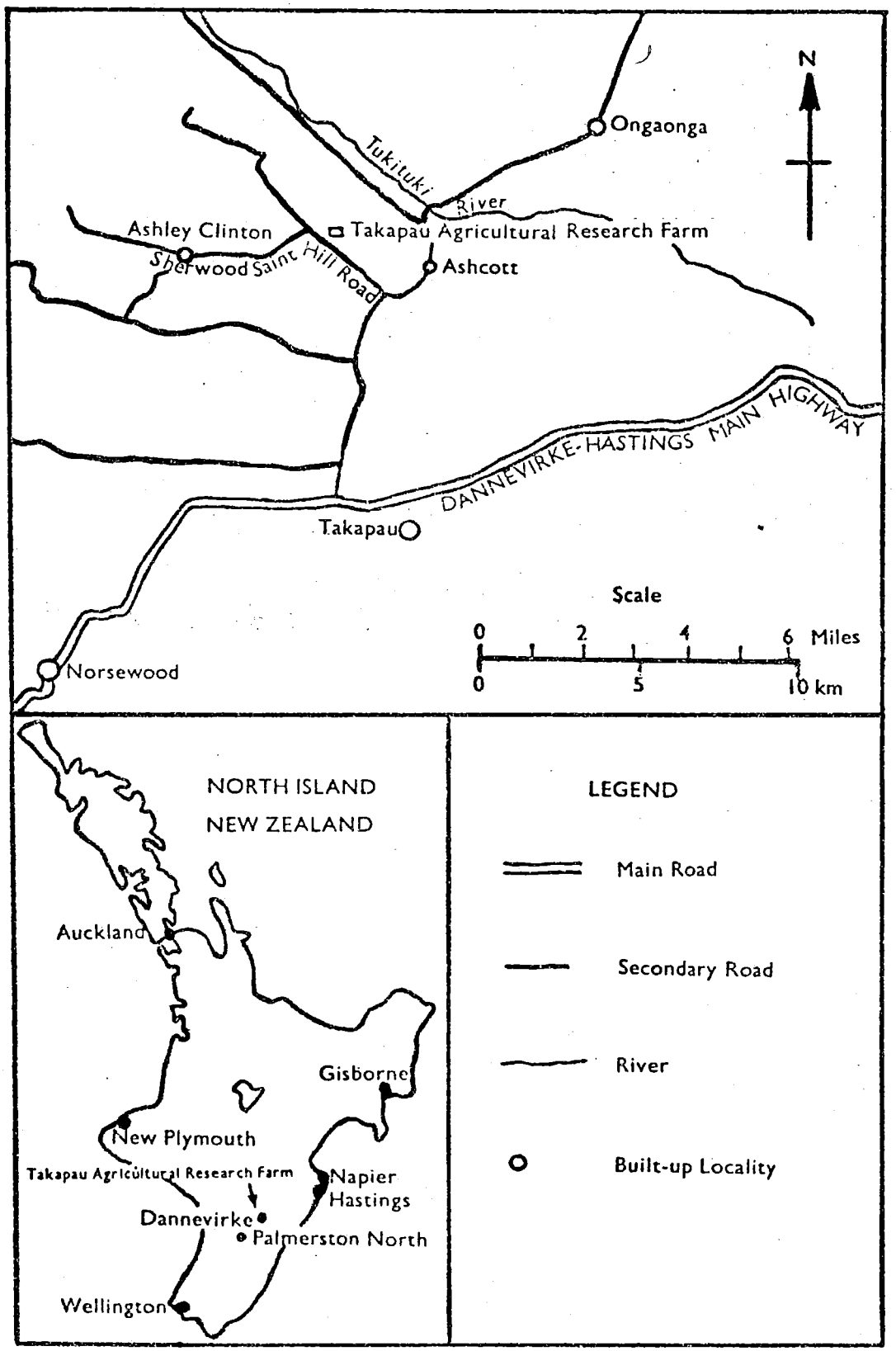


Fig. 4-1 Location of the Takapau research area.

(1) Takapau Research Farm

The Takapau Research Farm is located in Central Hawkes Bay on the Takapau Plains approximately 10 km north of Takapau and 20 km west of Waipukurau. The farm is backed by the Ruahine Mountain Range 8 km to the west (Fig. 4-1).

Climatically the area is included in Gerlach's (1972) warm zone of New Zealand. The summer temperatures in the area range from 15.6 to 29.4 °C with winter temperatures falling as low as 4.4 °C. Heavy frosts are common in winter and frequently exceed -10.0 °C. The Ruahines are usually snow clad throughout the greater part of the winter and, although winter snow falls are not uncommon on the research farm, the snow seldom lies for any length of time. The prevailing wind, from the southwest, is bitterly cold. The average annual rainfall in the area is approximately 100 cm.

Initially the soils on the research area were typed as Takapau silt loam and were included in the Takapau soil set (75) of the "General Survey of the soils of North Island New Zealand" (N.Z. Soil Bureau, 1954). A recent and more intense survey by Rijkse (1972) has described the occurrence and properties of three distinct soil types on the research farm, namely the Takapau silt loam, the Takapau mottled silt loam and the Takapau fine silt loam (Fig. 4-2). These soils are derived from a parent material consisting of a layer of loess mixed with volcanic ash and greywacke stones overlying alluvial sands and greywacke stones. The most common soil type found on the research farm is the Takapau silt loam which covers 90% of the area while Takapau mottled silt loam, a gleyed variant of Takapau silt loam caused by imperfect drainage, occupies less than 6% of the farm (Fig. 4-2). The remaining area is composed of Takapau fine sandy loam. The characteristic profiles of these soil types are shown in Appendix 4-1.

The Takapau silt loam and fine sandy loam are high phosphate fixing soils and require high inputs of superphosphate to maintain high levels of production. In comparison the Takapau

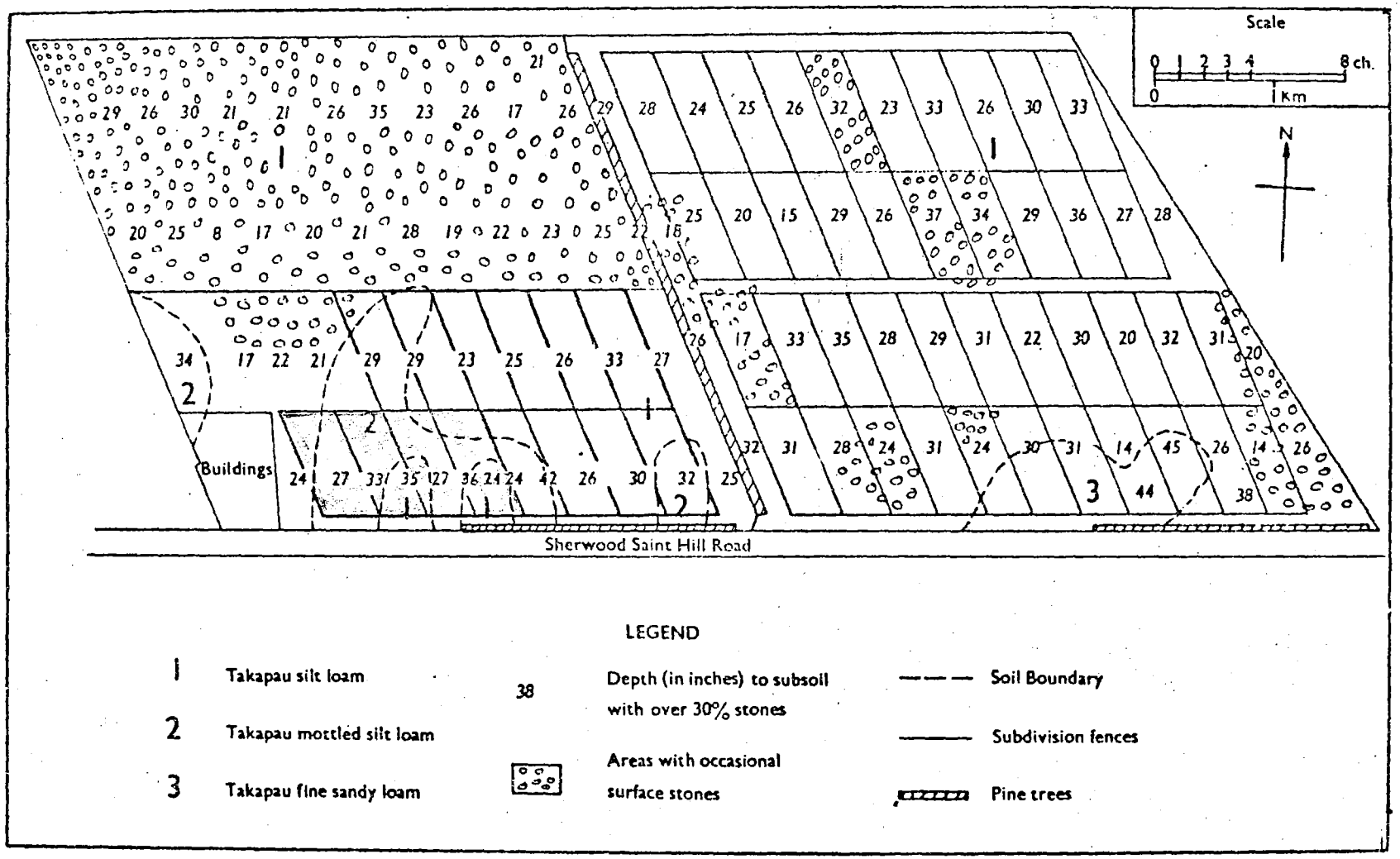


Fig. 4-2 Soil map of the Takapau research area. The shaded paddocks were involved in the life table studies. The farming systems trial area is to the east of the belt of pine trees which divides the area in half.

mottled silt loam is regarded as only a moderately high fixer of phosphate but is potentially deficient in potash. The Takapau silt loam is a free draining soil with a medium to low ability to store plant-available water in the B horizon. Under the low summer rainfall which characterises Hawkes Bay these soils are considered drought prone.

From an entomological point of view, land use on the Takapau research farm fell into two categories, namely a farming systems trial and life table plots.

(a) Farming Systems Trial (Plate 4-1). Extensive studies on the research farm were superimposed on a farming systems trial which is situated to the right of the belt of pine trees in Fig. 4-2. The aim of this trial was to evaluate the effect of different farming systems on soil fertility and structure. The farming systems were designed to provide a wide range of known edaphic and agronomic conditions so that the effect of these on plant and animal productivity and the incidence of grass grub could be assessed. As a secondary study, measurements of animal, crop and pasture productivities at different stocking rates under different farming systems were conducted.

Each of the three farming systems were run at two stocking rates giving six self-contained farmlets. The three farming systems chosen were:

- An all grass system with no provision for pasture renewal or cropping
- A limited cropping system embodying the following rotation:

Old pasture (4-5 years) - chou moellier (Dec. - July) - wheat (July - Feb.) - pasture (March)

Plate 4-1 Elevated view of the farming systems trial at Takapau

Plate 4-2 Elevated view across to the Takapau life table plots in the distance. Note the soil sample bags on the study plots in the background.

- An intensive cropping system with the following rotation:

  Old pasture (4-5 years) - Barley (Oct. - Feb.) - Ryecorn (Feb. - July)

  - Wheat (July - Feb.) - Cereal greenfeed (Feb. - Oct.) - Barley (Oct. - Feb.)

  - Pasture (March)

The trial area consisted of forty-two .40 ha paddocks which were ploughed out of pasture in the summer of 1967 and sown to perennial ryegrass - white clover pasture with 152 kg of superphosphate. One hundred and fifty-two kg per ha of 15% potassic superphosphate was applied to the area annually in late winter. Paddocks within the farming systems concerned were randomly staggered at different stages within their respective rotations. Spring cultivation severely reduced grass grub populations by crushing the delicate pupal stage (Kain and Atkinson, 1970). Hence, at any point in time paddocks which had recently been cultivated in spring provided a source of low grass grub populations for study.

Mixed age ewes were rotationally grazed on the high stocked and low stocked farmlets at 27.5 and 22.5 per ha respectively.

(b) Takapau Life Table Plots (Plate 4-2).

Intensive population studies on the Takapau research farm were confined to two plots, an unimproved and an improved plot, each consisting of two adjoining .40 ha paddocks (40 x 100 m). These paddocks are shown as a shaded area in Fig. 4-2. The three soil types found on the Takapau area were represented on the life table plots. The unimproved plot bounded the improved plot to the west. The western boundary of the plots was 90 m distant from the farm buildings. Pasture, production and botanical composition together with soil test results recorded in the second year of the studies are given for both plots in Appendix 4-2. Pasture growth rate was measured monthly from both the unimproved and improved plots by the rate of growth technique and dissected

seasonally for herbage composition (Lynch, 1960). Two 1.5 x 3 m exclusion cages were used and the pasture was harvested by a reel motor mower.

The unimproved plot received no fertiliser during these studies, possessed pasture of poor botanical composition and consequently was less productive than the improved plot. As the unimproved plot was only used for holding reserve stock for other experimental areas on the research farm the stocking rate was not consistent and the grazing pressure varied from light to moderately heavy.

The improved plot was undersown with perennial ryegrass and white clover in the autumn prior to the commencement of the studies in spring. By the following spring a good quality ryegrass-white clover dominant pasture had been established. Applications of 250 kg of 15% potassic superphosphate were made annually to the plot in late winter. The area was set stocked annually at the beginning of March with lambs. Stock numbers were adjusted for spring, summer and winter to give the respective seasonal stocking rates of 37.0, 24.7 and 49.4 per ha. These stocking rates ensured that pasture was well utilised. No supplementary feed was fed to the animals grazing this plot.

(2) Smith's Plots (Plate 4-3)

Three rectangular plots 40 x 100 m with low grass grub populations were sited side by side on a farmer's (Mr Smith) property less than 548 m south of the research farm. The soil type on the area was Takapau silt loam. The pasture on the plot had been sown out of wheat four years earlier and at the beginning of these studies had a good quality ryegrass-white clover dominant pasture containing a small amount of cocksfoot.

The plots were set stocked with ewes at a rate which changed with the season, the object being to evenly graze the pasture to a height of 1.5 to 3.5 cm. This was not always possible particularly over the spring and autumn periods when sudden flushes of growth were experienced.

Plate 4-3 Smiths plots at Takapau.

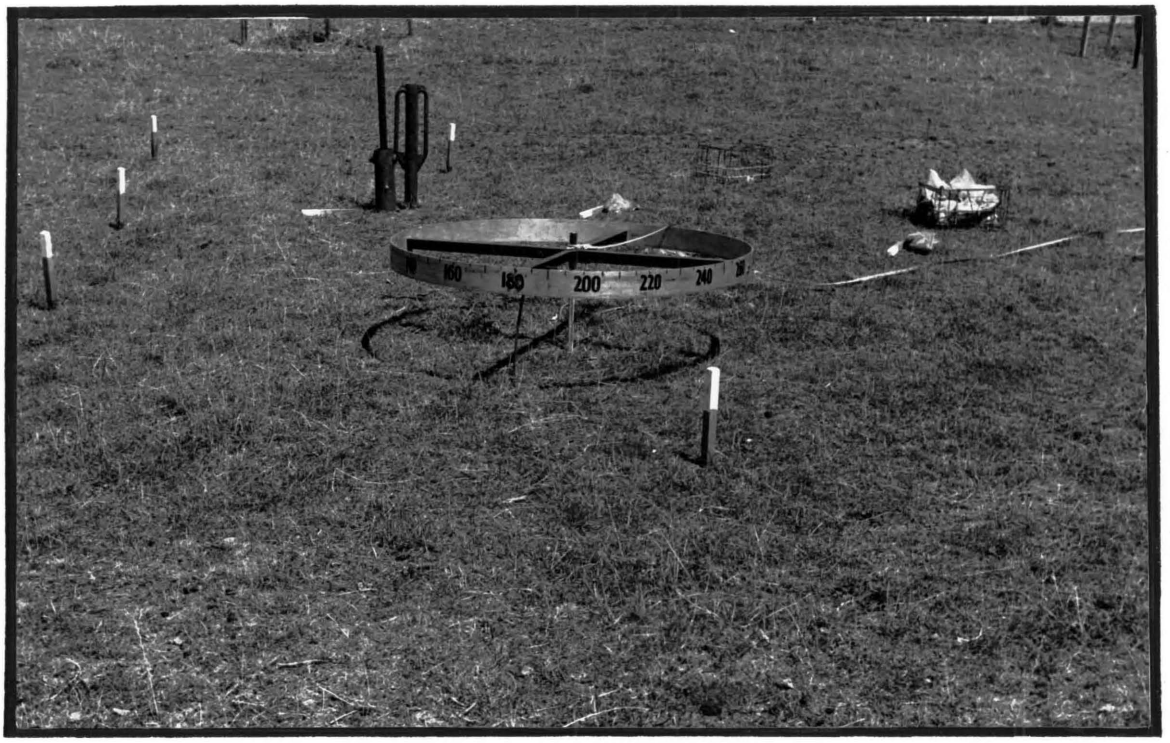


Plate 4-4 Compass wheel used for locating sample sites with the corer and crated samples in the background.

(3) Rukuhia Airport Plot

The Rukuhia Airport plot was 40 m x 100 m and was situated on the southern side of the Hamilton airport 13 km southwest of Hamilton on a flat to slightly undulating terrace. Climatically the area is characteristic of the central Waikato region with comparatively short but cold winters with frequent frosts many of which exceed -8 °C. Over the winter months the mean maximum temperature averages about 14 °C. In summer the maximum air temperature may exceed 27 °C with a mean maximum of about 22 °C.

The soil on the plot was Horotiu sandy loam which is derived from old alluvium consisting of a mixture of rhyolitic and andesitic ash deposited by the Waikato river. This soil is friable, free draining and prone to drying out quickly in summer. Naturally deficient in potassium and phosphate, large inputs of potassic superphosphate are required to obtain high levels of production. To this end the area received 153 kg of 15% potassic superphosphate each autumn.

The pasture had not been renewed for at least 25 years and was paspalum dominant during summer with ryegrass-white clover, Poa trivialis and other annual grasses constituting the major pasture components in spring and autumn. The plot was used as a holding paddock and as a result suffered periods of under and over grazing by both cattle and sheep.

III LOCATION OF SAMPLE SITES

(1) Problem

The usual method used for locating random sample points in study plots is to divide the area into rectangular subplots and locate sample positions by random rectangular co-ordinates drawn from tables of random numbers. For these studies all plots were subdivided into 20, 20 x 20 m subplots. Where it is desired to relocate sample sites the accuracy with which sample sites can be located is important. An ingenious device, developed by Farrell (1972b), for locating randomly selected points using polar co-ordinates was used in these studies. An aluminium wheel, 1.15 m

in diameter, marked off in 10^{o} intervals was suspended horizontally above the pasture, at a height of 76 cm, by its hub on a pipe driven into the centre of the subplots (Plate 4-4). Sample points were located by siting the 0^{o} angle on a given corner peg of the subplot and passing a taut measuring tape fixed to the centre of the hub over the angle given in the co-ordinates. The required distance was then measured out. Farrell (1972b) obtained polar co-ordinates by using rectangular co-ordinates drawn at random, marking the points on a plan of the subplot and converting them with a transparent polar graph overlay.

Although this method improves the accuracy with which points can be located, the drawing of polar co-ordinates prior to sampling is a major undertaking, particularly if strata or subplots are irregularly shaped. Where this occurs a high proportion of randomly drawn sample sites may have to be discarded as falling outside the required stratum.

(2) Method

In order to reduce the large input of time taken in drawing and converting rectangular co-ordinates to polar co-ordinates a computer was used. A programme was developed for an IBM 1130 computer for generating random rectangular co-ordinates and converting them to polar co-ordinates. Further, the programme was extended to handle two irregularly shaped strata within each subplot. To accomplish this each subplot was mapped out into 1.66 x 1.66 m plotlets and classified into stratum one if it showed grass grub damage and stratum two if undamaged. In the case of the Rukuhia Airport plots where damage was not clearly visible, because of the tolerance of paspalum to grass grub damage, occurrence maps were drawn up from systematic sampling based on a 1.66 x 1.66 m grid. The sample unit used in this work was a 14 x 14 cm spade spit. Mapping whether based on pasture damage or grass grub occurrence allowed the division of each subplot into 12 columns ($\underline{X}_{n}$) and 12 rows ($\underline{Y}_{n}$) giving 144 plotlets. These were stored by the computer in a two dimensional array

$X_n Y_n$ (n = 1 -12). The strata into which these plotlets were classified were stored in another two dimensional array $X_n Y_n$ (n = 1 -12). The number of samples required from each stratum within each subplot was set. Random rectangular co-ordinates were generated and drawn for each subplot. These were then tested to see if the plotlet in which they fell was in the required stratum. Where this was not the case the co-ordinates were rejected and the process was repeated. Co-ordinates that were accepted were translated into polar co-ordinates and listed. For the Takapau plots suitable adjustments had to be made in drawing rectangular co-ordinates and converting these into polar co-ordinates. This was necessitated by the shape of the study plots which were 30° parallelograms.

With the assistance of this programme the time taken to draw random rectangular co-ordinates, transform them to polar co-ordinates and list them was minimized. In cases where it was desired to divide the plots into strata and where the time factor for manual drawing, converting and listing co-ordinates made the exercise prohibitively slow the use of this programme made it practical.

It was claimed by Farrell (1972b) that, with the compass wheel and the use of polar co-ordinates, it was possible to locate 300 sample sites per hour. The times taken to prepare for and to locate sampling positions in the present study are given in Table 4-1.

The time given for shifting and setting up the measuring device includes the time to shift the wheel from the centre of one subplot to the next, align the 0° mark of the compass wheel with the corner peg of the subplot and fix it in position. Another fixed cost was the time taken to pick up the markers from the previous subplot. This gave an average total fixed time per subplot of one minute. The type of markers found most suitable for marking sample positions were 20 cm x 4 cm x .32 cm thick yellow plastic pegs which were sharpened at one end. These were easily identified in the pasture and as many as 25

Table 4-1 The time in minutes for one man to prepare, locate and mark 20 sampling sites per subplot.*

Operation	Time	± S.E.
Numbering sample bags	2.0	± 0.4
Moving siting wheel from subplot to subplot and setting it up	0.6	± 0.1
Picking marking pegs up after sampling	0.6	± 0.2
Average time for unordered polar co-ordinates	5.6	± 0.5
Average time for locating ordered polar co-ordinates	2.8	± 0.3
Total time using unordered polar co-ordinates	11.6	
Total time using ordered polar co-ordinates	6.0	

* subplot size = 20 x 20 m

could be held in one hand, while sample sites were located and pegged. In a two man team the activities of locating and marking sample sites were carried out simultaneously.

Large savings in the time to locate sample sites were made by having the computer sort and record the co-ordinates in numerical order, based on the size of the polar component. With sorted co-ordinates sample sites could be located and marked 50% faster than with unsorted sample sites, the increase in speed being equivalent to an additional helper.

Location of sampling sites for the more extensive population studies, in which the relocation of sample points was not considered necessary, was obtained by pacing out the rectangular co-ordinates which had been randomly drawn and listed by the computer.

IV SAMPLING

(1) Choice of Sample Unit

The choice of the sample unit size for studies was determined by a cost variance analysis.

Three sample unit sizes were tested for their suitability, a 5 cm and a 10 cm diameter corer and a 14 x 14 cm spade spit. All samples were taken to a depth of 25 cm. Since corers had to be manually driven into the soil, a 10 cm diameter corer was considered the upper limit that it was physically possible to drive into the soil for an extended sampling period, especially over the summer months. On the other hand a 14 x 14 cm spade spit was considered to be the minimum size square sample that could conveniently and accurately be taken with a spade. With smaller samples problems arose with the accuracy with which square spade spits could be taken.

Sample points were located randomly with rectangular co-ordinates. Samples were drawn from 20 x 20 m plots sited on low, medium and high populations of third instar larvae in May. In these, as in all extensive population studies, samples were hand sorted and examined on trays in the field.

The estimates of variance ($\underline{s}^2$) were computed for each sample unit. According to Finney (1946) a relative measure of precision for each sample unit is given by the ratios of their $\underline{s}^2$ per common area. Variances for each sample unit were calculated and expressed per m^2. The relative area required to be sampled for equal precision was assessed by dividing the smallest sample unit $\underline{s}^2$ per m^2 into the $\underline{s}^2$ per m^2 estimates for the other sample units. The relative number of samples required for equal precision was then estimated on a m^2 basis. The total time required to sample with equal precision with the different sample units was calculated by multiplying this with the total time taken to locate, take and sort each sample. The results of this cost variance analysis are given in Table 4-2.

Variance increased with the size of the sample unit as did the $\underline{s}^2$ per m^2. For all population densities the 10 cm diameter corer on a time for equal precision basis was much more efficient than the other sample units.

It is recognized that extrapolation of these findings, outside the conditions under which the evaluation was conducted is dangerous as changes in the extraction method, or stage of the insect, could markedly change the efficiency of the different sample units.

(2) Sampler

An essential prerequisite for population studies of grass grub and other soil insects is an efficient soil sampling tool. The attributes of a sampler suitable for such studies are given below.

- The sampler is efficient in terms of time required for taking samples and in the cases of intensive population studies placing them in containers for transport to the extraction laboratory.
- The sampler has the capacity to sample a wide range of soil types under a variety of seasonal conditions.

Table 4-2 Statistics of different size sampling units for sampling third instar larvae

Population	Unit c m.	Mean /unit	Mean $/m^2$	No. units	** s^2/ unit	s^2 $/m^2$	Area for equal precision	No. for equal precision	Time Locate	Time Sample	Time Sort	Time Total	Total time for equal precision
Low	+ 5.0	0.080	39.39	80	0.094	46.64	1.00	493.00	0.33	0.55	0.71	1.59	783.87
	+10.0	0.255	31.43	80	0.457	56.31	1.21	149.13	0.33	0.92	1.41	2.66	396.68
	*14.0^2	0.809	41.44	80	1.833	93.92	2.01	103.00	0.33	2.00	4.80	7.13	734.39
Medium	5.0	0.270	133.11	80	0.303	149.38	1.00	493.00	0.33	0.55	0.71	1.59	783.87
	10.0	0.769	94.83	80	1.269	156.40	1.04	128.18	0.33	0.92	1.41	2.66	340.95
	14.0^2	1.699	87.08	80	4.088	209.47	1.40	71.74	0.33	2.00	4.80	7.13	511.50
High	5.0	0.319	157.56	80	0.401	197.94	1.00	493.00	0.33	0.55	0.71	1.59	783.87
	10.0	1.560	192.24	80	2.996	369.29	1.86	229.24	0.33	0.92	1.41	2.66	609.77
	14.0^2	3.189	163.40	80	12.369	633.79	3.20	163.97	0.33	2.00	4.80	7.13	1169.11

+ 5 and 10 c m. diameter core samples were 5.08 and 10.16 respectively

* 14 x 14 c m. spade spit samples were 13.97

** s^2 = variance

A longitudinally split barrel corer 10 cm in diameter was developed for studying grass grub populations. One half of the barrel was fixed, the other articulated on a hinge. The corer was driven manually into the ground with a hammer. A corer was chosen as a sampling tool rather than a spade as it was less subject to personal bias in the size of the sample taken. This instrument enabled a wide range of compact agricultural soils to be sampled rapidly to a depth of 25 cm. Compression of samples was kept to a minimum by the method by which samples were released. Soil samples held in the corer could be exposed for sectioning, on soil profile characteristics or other biological phenomena, by withdrawing the hinge pin and removing the hinged side of the corer. Because of the absence of a power unit the corer can be easily manoeuvred on small plots which is a decided advantage when sampling intensively.

The technical aspects of the corer's construction are given in Appendices 4-3 and 4-4.

(3) Sampling

The corer was driven into the soil by sliding the hammer up and down the guide tube (Plate 4-5). On impact the force was conveyed from the hammer directly down the walls of the corer. Both halves of the corer were held firmly together during driving by the pressure of the soil on the tapered outer surface of the cutting head. At the required depth of 25 cm the corer was pushed from side to side in order to sever the base of the sample. On withdrawal the fixed side of the corer was kept downward to support the sample. The sample was released by rotating the corer through 180°. In so doing the weight of the sample was transferred from the fixed to the hinged side of the barrel. Under the weight of the sample the hinged side fell open and the released sample slid out as illustrated in Plate 4-6. No problem was encountered in sampling soil with a friable subsoil providing the corer was withdrawn on a 45° angle to the surface of the ground.

Plate 4-5 Sampling the Takapau life table plots.

Plate 4-6 Release of sample unit into a polythene bag in preparation for transport from the field.

The speed with which samples could be taken was dependent on a number of factors, particularly, soil type, soil moisture, soil compaction and plot size. Presented in Table 4-3 is a summary of the mean number of samples that were taken randomly over .40 ha plots and placed in containers during an eight-hour man-day. The mean number of samples are given for a range of compacted soil types under relatively dry (January-March) and wet (May-June) conditions.

Approximately 25% of the time involved in sampling was taken up in moving between sites.

In dry summers if sampling became too difficult for one operator, a two-man hammer was used which had a 15 cm by 45 cm rectangular handle running lengthwise down either side of the hammer barrel. The use of this hammer increased the sampling speed under these conditions by approximately 40-60 samples per eight-hour man-day.

V PACKING, FILLING SAMPLE HOLES AND STORAGE OF SAMPLES

(1) Packing and Filling Sample Holes

The polythene bags in which the soil cores were deposited from the corer were packed horizontally into steel crates for transport.

Prior to packing, the hole from which the core was drawn was filled and rammed with local soil similar to that found on the plot. Soil and cores were carted to and from the field by tractor and trailer (Plate 4-7). The total time taken per sample to carry out these operations was 50 seconds. A further 20 seconds per sample was required to load the samples on to a truck for freighting back to the laboratory and unloading and placing them in cool storage on arrival.

Table 4-3 Mean number of cores (per man day) sampled at random from .40 ha plots and placed in containers.

Location	Soil Type	Time of Year	No. obs.	Mean number of samples taken per man per day (8 hrs.)
Mid Hawkes Bay	Takapau silt loam (75)	Jan - Feb	8	175 ± 11.2
		May - June	6	232 ± 13.6
Taupo and	Taupo sandy silt (18)	Jan - Feb	7	246 ± 24.1
Waikato	Horotiu silt loam (83)	May - June	6	274 ± 16.3

(2) Storage of Samples

Egg samples were stored at an arbitrarily chosen temperature of 5 °C while samples of other stages were held at approximately 8 °C. In the case of the egg samples 5 °C prevented egg development while 8°C markedly retarded development of all larval, pupal and teneral beetle stages. Recent work by Wightman (1972) has shown that 5 °C approximates to the lower threshold for egg development.

Usually samples were processed within two weeks of being placed in the cool store but on occasions were kept for up to a month without any apparent ill effects on the insect.

VI EXTRACTION

(1) Review. A method for the extraction of grass grub from soil samples suitable for life table studies should ideally have the following qualities:

- An acceptable level of recovery for the different developmental stages of interest.
- The ability to recover insect cadavers which are used for assessing the causes of mortality.
- A fast processing rate.
- Have no adverse affect on the insect's viability.

Although mechanical methods of extraction, in comparison with dynamic methods which rely on the behaviour of the animal in response to some stimuli, have the disadvantage of requiring a high input in time and energy, they offered more scope for meeting these requirements.

The extraction of the later stages of scarabaeid larvae from soil samples has been achieved by manually breaking up the soil and hand sorting (Raw, 1951; Burrage and Gyrisco, 1954a;

Plate 4-7 Transporting crates of samples from the study plots.

Guppy and Harcourt, 1970). This method has proved unsuitable for the smaller developmental stages which are less easy to detect (Burrage and Gyrisco, 1954a). These stages have been successfully extracted by dry sieving after the soil sample has been manually broken up (Burrage and Gyrisco, 1954a; Carne, 1948; Guppy and Harcourt, 1970). Although dry sieving has the advantage that it is easily mechanised (Carne, 1948; Lange, et al., 1954), the method usually requires a high input of hand sorting and the more delicate stages of development are damaged by abrasion. This method, however, has been used with notable success for the extraction of the third larval stage of grass grub. (Fenemore, pers. comm.).

Hand sorting of soil for grass grub was found to be inaccurate and time consuming particularly in the early developmental stages. Experience has suggested that, depending on soil texture and colour, errors in sorting for eggs and first instar larvae range from 15 to 26%.

Ladell (1936) designed a process for extracting soil inhabiting arthropods which combined the principles of wet sieving, flotation, mechanical agitation and aeration. In this process soil samples were broken down by the joint action of mechanical agitation and aeration. Buoyant constituents of the sample were then poured off and retained in a sieve. The method was subsequently used in modified forms by many workers (Baweja, 1939; Glasgow, 1939; Strickland, 1945; Read, 1958; Wilcox and Oliver, 1971). Salt and Hollick (1944), working on wireworms, devised a process which incorporated some of Ladell's principles. This process consisted of differential wet sieving, flotation to remove the heavier debris from the buoyant material and separation of fauna from organic matter at an oil-water interface. The specific gravity of the aqueous magnesium sulphate used for flotation was 1.1. This method in a modified or standard form has been used for many insects (Salt, et al., 1948; MacFadyen, 1953; Raw, 1955; Sheals, 1957). Although adaptable, the method

has a slow process rate of 1.5 samples (10 cm diameter x 40 cm) per man hour. Cockbill, et al., (1945) modified the Salt and Hollick method for wireworm surveys by breaking down samples on the sieves with a strong jet of water. Thirteen samples (10 cm diameter x 15 cm) were processed in one man hour by this method. To achieve this, samples were bulked for processing into lots of ten. The efficiency of recovery by this method was 95% compared with 99% for the Salt and Hollick process.

The Salt and Hollick technique can only be significantly speeded up by the elimination of the oil-water interface separation stage. This has been shown to be practical for macro-arthropods which are visually distinguishable from organic matter by the naked eye and can therefore be removed by hand from a brine bath in which the sieve is immersed (Read, 1958; Feeney, 1967). An additional advantage of deleting this stage is that insects are recovered alive.

(2) Description of the Process and Extraction Unit.

The layout of the unit and items of equipment are shown diagrammatically in Fig. 4-3 both in a floor plan and in a sectional elevation.

Essentially the extraction process consists of four basic operations; the break-down of soil samples by hand, the separation of fauna and organic matter by flotation, differential sieving and washing, and inspection, counting and recording.

(a) Break-down of Soil Samples. Soil samples (10 cm diameter x 25 cm) were carefully crumbled by hand into trays on the sorting table. The slotted angle iron table had a wire gauze top which permitted dust and soil particles to fall to the floor. Any large roots or other debris were cleared of soil and discarded. When extracting eggs, turf which was known not to be frequented by this stage was removed, inspected for beetles, and discarded. The trays on to which the samples were crumbled were rectangular (36 cm x 56 cm) with a V-like

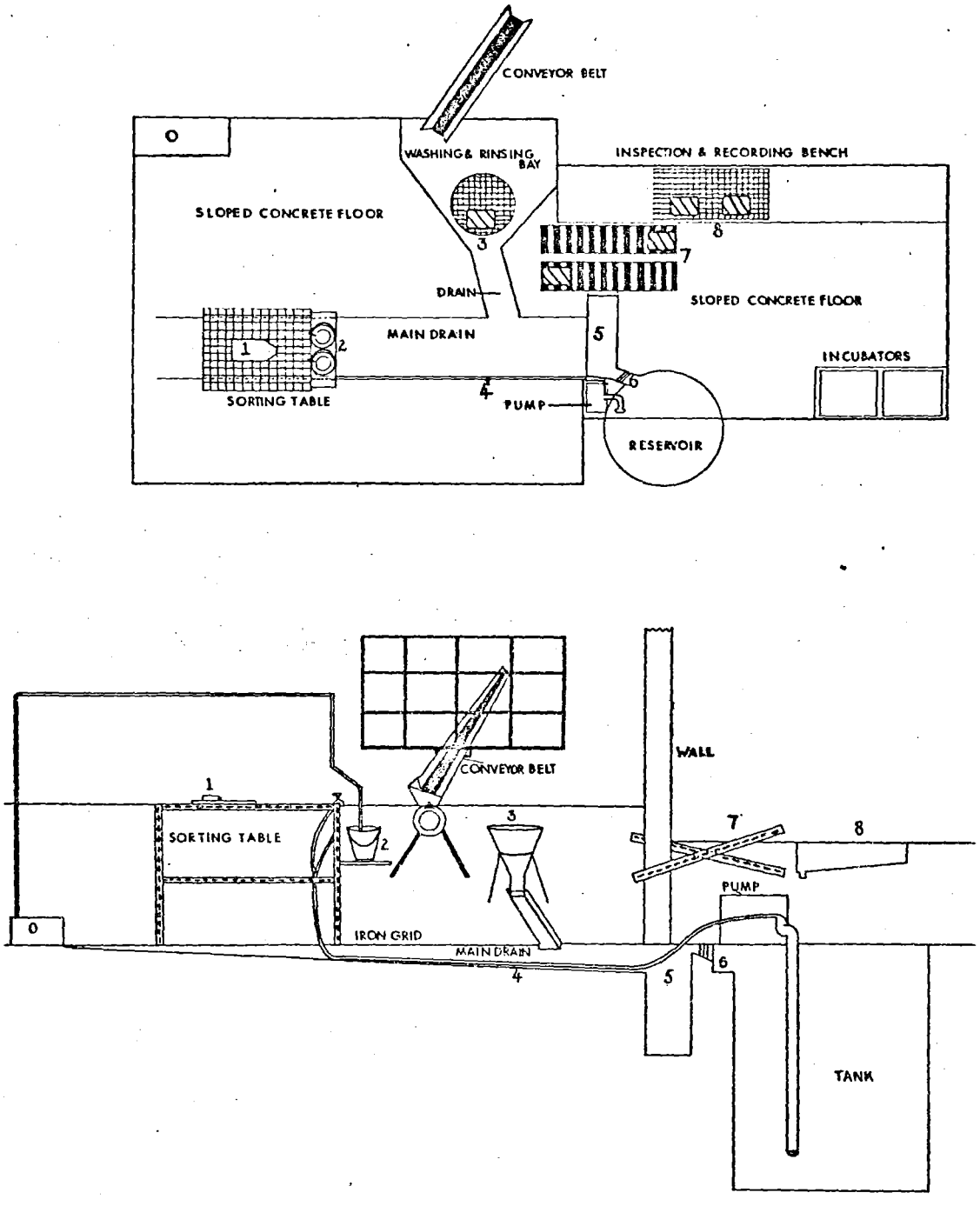


Fig. 4-3 Extraction unit showing a floor plan (above) and a sectional elevation(below): 0, compressor; 1, soil trays; 2, flotation bucket; 3, drainage funnel for differential sieving; 4, pipe for recirculating Mg SO_4 from the tank to the sorting table; 5, sediment trap; 6, filter; 7, roller races for conveying the sieves to and from the recording bench; 8, inspection bench.

constriction at one end. This constriction terminated in a 15 cm wide mouth, through which the crumbled soil was poured.

Efforts to mechanise the break-down operation foundered because the early developmental stages of the insect were easily squashed. In this condition they were adhered to by soil particles and sank during flotation.

(b) Flotation. Attached to the sorting table was a bucket stand serviced by two, high pressure, lever action, gate valve taps through which a magnesium sulphate solution was pumped from the reservoir. The solution was reticulated in 3.8 cm alkathene pipe.

Crumbled samples were poured slowly into 15 litre plastic buckets of magnesium sulphate solution over a steady stream of air bubbles. Air was introduced from a compressor unit through a perforated nozzle into the bottom of the bucket. A gradual transfer of samples from the tray into the solution ensured that there was sufficient agitation and dispersion of soil particles to prevent the buoyant material which includes the fauna from being trapped by an overburden of soil and carried to the bottom of the bucket.

Constant checks were made during the running of the unit to see that the specific gravity of the magnesium sulphate solution did not fall below 1.09. Although all stages of grass grub float in the laboratory in magnesium sulphate solutions with a specific gravity of 1.07, in practice a higher percentage recovery has been obtained with magnesium sulphate solutions with specific gravities of between 1.09 and 1.10.

(c) Differential Sieving. All buoyant material was decanted from the buckets into three sieves placed in series over the drainage funnel in the washing bay. The mesh size of the sieves varied according to the stage of the insect being extracted. For the egg and first instar stages, sieve sizes of 4, 6 and 20 meshes per 2.5 cm were adequate. For all other

stages the 20 mesh sieve was replaced in the series by a 12 mesh sieve.

After the magnesium sulphate solution and buoyant material had been decanted into the sieves the residual soil was retained in the bucket. This was then flooded with magnesium sulphate, reticulated into the washing bay, by carefully playing the hose on the upper side of the bucket which was held in the pouring position. With the bucket held in this position, the solution flowed over the sloping surface soil, dislodged any buoyant material and swept it into the sieves. The soil remaining in the bucket was emptied on to a conveyor belt, transported outside the unit and deposited on to a trailer. The conveyor was designed so that the belt ran in the floor of a trough. A rubber flap situated at the feeding end prevented the sludge running back on to the floor.

Sieves were then quickly washed with aqueous magnesium sulphate. This removed as much of the fine debris as possible and sorted the material on a size basis on to the appropriate sieve. The magnesium sulphate solution was used for this purpose rather than fresh water to prevent dilution of the solution in the reservoir. Foliage, long root fibres and sticks were usually retained in the coarser top sieve while smaller roots, soil particles and other debris together with a range of macro-arthropods were caught on the mid and lower sieves.

(d) Inspection. After rinsing, the sieves together with the numbered polythene sample bag were transported to the inspection bench by means of a roller race. Here, sheltered from splash they were examined over a grated inspection sink with the aid of an illuminating magnifying glass. Runoff from the wet sieves was caught by the sink and drained on to the floor. All fauna of interest were rinsed in fresh water, placed in containers counted, recorded and stored in incubators for further study. If too much debris was retained in the sieves it was necessary to place the sieves in baths of magnesium sulphate solution and pick the floating fauna from the surface. In some instances it was

found beneficial to colour the solution red with 25 grams of Rhodamine B per 500 cc. This gave a better background against which to identify the whitish-grey grass grubs.

Sieves were returned to the washing bay by a second roller race, cleared of debris, fitted together and replaced on the drainage funnel in preparation for the next sample.

The unit was characterised by its recirculating system. Magnesium sulphate was dissolved in water to the required specific gravity in a 682 litre reservoir. The tank was fitted with a thermostatically controlled heater which maintained the temperature of the solution at approximately 26 ^{o}C. This was not only conducive to operator comfort, but prevented magnesium sulphate crystallizing out of solution with drops in temperature and not being rapidly redissolved as temperatures increased. Aqueous magnesium sulphate was pumped from the reservoir to the bucket stand or the washing bay by a centrifugal pump with a delivery rate of 5456 litres per hour. Any magnesium sulphate solution that was spilt on to the sloped floor or was decanted through the sieves, was drained in grating covered drains into the sediment trap. Here the coarser soil settled out and the overflow was led into the reservoir through a series of three sieves which retained any debris capable of blocking the pump.

A gate valve which directed the output from the pump to the taps at the sorting table into a bypass hose enabled the unit to be sluiced down and the debris on the floor to be swept into the sediment tank for removal from the unit.

(3) Performance

(a) Speed of Extraction. The extraction unit was laid out so that it could be worked by two to four operators. With two people the operations were divided so that one person was responsible for the break-down, flotation, decanting and differential sieving, leaving the other person to take care of the inspection, counting and recording. With three people, the

breaking up of soil samples became the responsibility of the additional operator. With four, two people were employed inspecting, counting and recording. Operators could move to help with the operation which at any time was limiting the processing rate. This fluidity of labour enabled the unit to be worked with approximately equal efficiency (number of samples per man day) by two to four people. The mean time together with its SE to complete each operation is given in Table 4-4.

Eggs and first instar larvae took longer to extract than the more advanced stages as they were smaller and more difficult to identify in the sieves. The removal of the turf mat from the egg samples facilitated a quicker breakdown rate than for samples of other stages. The time involved in flotation and decanting operations were similar for all stages. Differences in the breakdown time between the larval stages reflects the hardness of the soil over the summer months.

Samples which contained large amounts of dry litter, seeds or poorly weathered pumice which were buoyant and were retained on the sieves made identification more difficult and reduced the processing rate, particularly for the early stages of development.

The extraction rate of samples was governed by the speed of the slowest operation. For a three man team, the actual time taken per sample was faster than the slowest operator which for all but the extraction of the third instar larvae and teneral beetle stage was the counting and recording. This was achieved by the movement of labour between the fastest and slowest operator and represents a saving for eggs of over a third of a minute per sample. With an additional operator to help with the inspection and counting of eggs and the first instar larval stage, less time was expended on this operation and the gains from the movement of labour with four operators were therefore reduced. This was also the case with the extraction of the more advanced stages, with three operators where the duty time of each operator were similar.

In comparison with Salt and Hollick's (1944) and Cockbill's

Table 4-4 Time (minutes), taken by three operators to process one sample and each operator to complete each operation in the extraction of *C. zealandica* from soil samples.

Operators	Operation	Eggs	1st instar	2nd instar	3rd instar and teneral beetle
1	Breakdown of soil samples	1.30 ± 0.026	1.58 ± 0.094	1.58 ± 0.09	1.44 ± 0.09
2	Flotation in magnesium sulphate	0.44 ± 0.017	0.44 ± 0.017	0.44 ± 0.02	0.44 ± 0.02
2	Decanting and differential sieving	1.22 ± 0.054	1.22 ± 0.054	1.22 ± 0.05	1.22 ± 0.05
3	Inspection, counting and recording	2.44 ± 0.087	2.17 ± 0.048	1.39 ± 0.04	1.27 ± 0.03
	Gain*	- 0.35	- 0.14	- 0.012	- 0.20
	Actual total number of minutes per sample	2.09 ± 0.05	2.03 ± 0.15	1.65 ± 0.16	1.46 ± 0.06

* Time saved with three operators by the movement of operators to the operation which was limiting = total time - time for slowest operator.

et al., (1945) methods, this method had a faster processing rate. This was made possible by the early separation of the buoyant portion of the sample containing the fauna and the disposal of the larger and heavier soil fraction, and the development of labour-saving devices. These include the use of a recirculating system to minimise the time and effort involved in replenishing supplies of magnesium sulphate solution. Apart from a daily addition of magnesium sulphate to replace that which was removed from solution and disposed of in the waste soil, a tank of magnesium sulphate could service over 2,000 samples. At this time the system required to be cleaned and the solution renewed. The other labour-saving devices used were roller races for transporting sieves to and from the inspection bay and a conveyor belt for the movement of waste soil out of the unit into a disposal trailer.

(b) Percentage Recovery. The percentage recovery for each stage is recorded in Table 4-5. Two methods were used for assessing the percentage recovery of the larval stages, namely the seeding of samples with a given number of tagged larvae and the use of uninfested samples seeded with untagged stages. The tagging of large numbers of larvae was accomplished by holding them in a medium of soil and cow dung liberally treated with Rhodamine B. With the passage of this medium through the gut, the larvae took on, over a few days, a crimson tinge. Tagged larvae were then placed in samples with the aid of a cork borer or mixed with the crumbled sample. Egg and beetle recovery rates were checked by seeding samples taken from an area of similar soil which was known to be uninhabited by grass grub.

No noticeable differences in the recovery rate were recorded between soil types. The similar recovery rates for eggs and first instar larvae were lower than that for the more mature stages.

(c) Recovery of Cadavers. Cadavers of larvae were shown to have a recovery rate equal to that of live larvae provided remnants of the body were still attached to the head

Table 4-5 Recovery of different stages of C. zealandica from soil.

Stage	No. tests	No. of insects used	% recovered
Egg	10	753	95.85 ± 1.47
1st instar larvae	15	834	95.48 ± 1.22
2nd and 3rd instar larvae	15	697	98.45 ± 0.47
Teneral beetles	14	475	97.78 ± 0.95

Table 4-6 Effect of extraction on the viability of the different developmental stages of C. zealandica.

Stage	Control			Processed		
	No. tests	Total No.	% survival	No. tests	Total No.	% survival
Eggs	20	164	95 ± 3.23	10	50	94 ± 4.78
1st instar larvae	10	50	98 ± 2.15	10	50	96 ± 3.14
2nd instar larvae	10	50	96 ± 1.80	10	50	98 ± 2.12
3rd instar larvae	10	67	97 ± 1.24	10	54	96 ± 1.15
Teneral beetles	13	120	96 ± 1.32	10	100	98 ± 1.68

Table 4-7 Cost in time (man minutes) required to locate, sample and process Takapau soil samples, for C. zealandica.

Operation	Stage			
	Egg	1st instar larvae	2nd instar larvae	3rd instar larvae, pupae and teneral beetles.
Locating sample sites	0.30	0.30	0.30	0.30
Sampling	2.74 (1.95)*	2.74 (1.95)	2.74 (1.95)	2.06 (1.75)
Packing, replacing soil and storage	1.16	1.16	1.16	1.16
Extraction	6.28	6.10	4.94	4.40
Total time	10.48 (9.69)	10.30 (9.51)	9.14 (8.35)	7.92 (7.61)
No. units/8 hour man day	45.80 (49.55)	46.66 (50.47)	52.28 (57.48)	60.60 (63.07)

* () Rukuhia samples

capsule. Larval exuviae were successfully extracted and a large range of macro-arthropods, both phytophagous and predaceous, were also recovered.

(d) Effect on Viability. Tests carried out to determine the effect of the extraction process on the viability of eggs and first, second and third instar larvae as well as teneral beetles are recorded in Table 4-6. Survival of larval and teneral beetle stages were assessed two days after treatment while the viability of the egg stage was assessed on hatching. For comparative purposes, a control treatment consisting of individuals which had been hand sorted was also included.

Differences between mortalities of the control and processed treatments for each stage and differences within treatments were not significant.

(4) Conclusion

This method and unit allowed the extraction of all stages of grass grub without adversely affecting their viability. The extraction rate of the method was sufficiently fast to enable the large number of samples required for precise estimates of population density to be processed with available resources (see chapter 5).

VII TOTAL COST OF SAMPLING AND EXTRACTION

The total costs of sampling and extraction are summarised in Table 4-7. The egg and first instar stages were more costly in terms of time to sample than the later stages.

Overall, the sampling and extraction techniques allowed large numbers of samples to be taken and processed in a manner which fulfilled the prescribed conditions laid down at the outset of these studies. Many factors concerned with the insect and the study sites permitted these rapid sampling and extraction techniques to be developed. The relatively shallow

depths that grass grub inhabit in the soil profile meant that it was unnecessary to sample below a depth of 25 cm into the more compact subsoil. Over the autumn and early winter months most larvae were found within 7.5 cm of the soil's surface. The fact that eggs are seldom laid in the top 2.5 cm of soil has permitted the elimination of the top portion of the samples prior to processing. This minimises the amount of buoyant material required to be processed and makes inspection and identification easier.

The relatively large size of all stages of grass grub and the reduced amount of unhumified organic matter in most of the agricultural soils of New Zealand has enabled all stages to be identified in a magnesium sulphate bath without recourse to the time consuming oil-water interface separation process.

VIII TIMING OF SAMPLING

If worthwhile and reliable population estimates are to provide adequate estimates of the population density at different age intervals, and the operation of mortality factors within each age interval, then the timing of sampling is critical (Morris, 1955). The problems and considerations involved in the correct timing of sampling have been reviewed in chapter 2.

Observations by other authors have suggested that in improved grasslands parasites of grass grub were nonexistent and generally invertebrate and vertebrate predators were few and of little or no importance in controlling grass grub populations (Kelsey, 1951; Given, 1967, 1968). For this reason, the choice of sampling intervals was based on ecological factors associated with changes in the insect's physical environment (season), as well as physiological considerations.

Little attention was paid to sampling at relatively stable periods in the life cycle. The rapid sampling and extraction methods used, combined with cool storage to retard the insect's

development prior to extraction overcame the problems encountered by Morris (1955). Sampling problems concerned with highly mobile insects were of no consequence in studying grass grub as rapid movement was only associated with adult flight and estimates of beetles could be made at the teneral stage from soil sampling before flight commenced.

The sampling stages chosen initially were eggs, first and second instar larvae, third instar larvae in May and August, pupae and teneral beetles.

It is important that sampling be carried out when peak numbers occurred in each stage so that an accurate estimate of the numbers reaching each developmental stage is gained. In order to achieve this it was necessary to use criteria that ensured that the sampling of each stage was initiated at the most optimal time. Because the Takapau studies were conducted 365 km distant from where the author was based, in Hamilton, criteria were chosen which involved as few detailed on the site observations as possible.

Preliminary studies (Kain, unpub.) of flight and oviposition behaviour at Taupo, Rukuhia and later studies at Takapau showed that the oviposition period of the adult female in the paddock of origin is very short and lasts about 2-3 weeks. Peak numbers of eggs are present in the soil immediately after the major flights over pasture have finished. At this stage there may be as many as 20% of the female beetle population present in the soil. Usually these females have mated and have invariably laid at least one cluster of eggs. Females extracted from the soil after the major flights and placed in pots of soil in the insectary and fed on white clover foliage laid few eggs and usually died within a week. It is known that few, if any, beetles are present in the paddock of origin long enough after mating to oviposit more than twice (Kain, unpub.). In view of this and since more than 70% of the eggs laid in the first two clusters are laid in the first cluster, the proportion of the total eggs unlaid two to three weeks after peak flight would appear to be very small. Based on this information egg sampling was initiated soon after the major flights had ceased. Sampling of first instar larvae

was conducted when over 75% of a sample of viable eggs which had been extracted during egg sampling but not subjected to cool storage had hatched following incubation at 16 °C. The choice of 16 °C as an incubation temperature was made as it approximated field temperatures at depths in the soil between 7.5 and 10 cm where eggs are usually laid. Subject to a rudimentary pilot sample, second instar larvae were sampled at the beginning of February, third instar at the beginning of May and again in August, and pupae, also subject to a pilot sample, four weeks from the beginning of peak flight. Beetle sampling was conducted when the majority of beetles were still in the teneral stage and was initiated when small but consistent flight catches were recorded in rotary flight traps, sited on the study plots.

Over the period that these studies were conducted at Takapau, the flight periodicity of grass grub on the life table plots was very consistent. The commencement date of the annual flight season and the occurrence of the major flights occurred within a 10 day period.

The worth of the criteria used for initiating age specific sampling can be gauged from Table 4-8. In these studies it was felt that a 90% occurrence of the stage required to be sampled was desirable. As is evident from Table 4-8, the percentage occurrence of the required stage in certain generations for second instar larval, pupal and teneral beetle samplings was lower than that considered desirable. The development of individuals which were retarded by diseases are not included in these estimates.

In the case of the second instar sampling the majority of larvae which were not second instar larvae had advanced to the third stadium and could therefore be assessed as having passed through the stage sampled. The lower percentage occurrences in the pupal and teneral beetle samplings arose as a result of the high number of individuals in the pre-pupal and pupal stages

Table 4-8 Percentage of C. zealandica populations in the developmental stage on which age specific sampling was based.

Generation / Stage	1968-69		1969-70		1970-71		1971-72		1972-73	
	Im*	Un*	In	Un	Im	Un	Im	Un	Im	Un
Egg	91.7	99.6	99.8	96.2	94.4	97.6	89.8	91.3	94.3	87.5
1st instar larvae	-	-	84.2	79.8	-	-	-	-	-	-
2nd instar larvae	-	-	92.1	87.6	85.2	94.6	86.9	90.7	87.7	95.8
3rd instar larvae (May)	96.6	99.3	93.0	96.7	100	100	95.5	95.3		
3rd instar larvae (August)	100	100	94.4	98.4	100	100	100	100		
Pupae	91.0	92.7	72.4	76.3	78.9	86.4	74.7	83.3		
Teneral beetle	96.3	97.8	87.4	82.9	75.4	78.6	84.0	82.0		

*Im = Improved Takapau life table plots

*Un = Unimproved Takapau life table plots

respectively. Where this occurred, these earlier stages were incubated on damp filter paper in ice cube trays in sealed containers at 14 ^{o}C to determine the percentage of these individuals which were capable of developing into the developmental stage being sampled. If these were included in Table 4-8, the percentage occurrence for these stages would exceed 90%. The low occurrence of first instar larvae was not considered important as the sampling of this stage was discontinued in 1970 as it was not practical to take and extract both an egg and first instar sample within one month, with available resources.

The reason for sampling third instar larvae in both May and August was to allow the measurement of mortality occurring over the winter. The choice of this sampling interval allowed generation mortality to be partitioned not only between most age intervals but also between seasons.

CHAPTER V

STATISTICAL ASPECTS OF SAMPLING AND THE DEVELOPMENT OF A SAMPLING PLAN

I. INTRODUCTION

The object of these sampling studies was to design a sampling programme for all developmental stages of grass grub which would enable the mean population densities to be estimated to within a given level of precision, for a minimal cost. The relative level of precision sought in these studies was $\pm$ 10% SE, an arbitrary level which was first proposed by Morris (1955) for life table studies and has since been widely adhered to (Harcourt, 1969) (see chapter 2).

II. SPATIAL DISTRIBUTION

(1) Stratification

The advantages of stratified sampling compared with simple random sampling, such as increased representativeness of samples and error minimising properties have been discussed earlier, in chapter 2.

It is known that maximal gains in precision are obtained when stratification is based on criteria which are well correlated with insect density. Objective stratification of plots for insect sampling usually involves the use of information based on previous samplings, some knowledge of the species' behaviour or the use of criteria which indicate the presence of the insect.

Since pasture damage offered the only convenient criterion for the stratification of grass grub study plots, such factors as, the relationship between grass grub occurrence and damage, the proportion of grass grub populations found at given distances away from areas of visible damage, the extension of damage within a

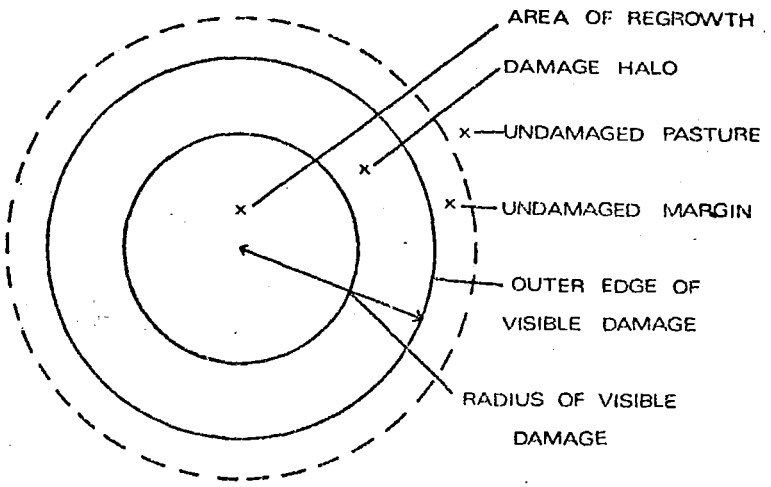


Fig. 5-1 Boundaries and areas in individual patches of damaged pasture, caused by C. zealandica larvae.

generation and from generation to generation, and the rate of appearance of new areas of damage from one generation to the next were investigated.

(a) Location of Grass Grub Relative to Damage.

The typical aggregated nature of low grass grub populations in Hawkes Bay is well illustrated by the pattern of damage shown in Plates 6-1 and 6-2. Grub populations in the early colonising stage consist of discrete circular colonies or aggregates, the majority of which may be located by the visible pasture damage that they cause. Close examination will reveal that the larger patches of pasture damage are encircled by a halo of severe damage while the pasture in the central area has re-established itself. To avoid confusion in terminology, a diagram of a patch of pasture damage and the terms used to describe various aspects of it are given in Fig. 5-1. All the area within the outer edge of visible damage was termed the inner zone and the area of interest outside this the outer zone.

Six circular areas of damage of similar size, approximately 1.84 m in diameter, were selected for study at Takapau in late March of 1969. Two 15 x 20 cm deep trenches were dug and divided into 15 x 15 cm sections from each patch of damage from the outer edge of visible damage .60 m toward the centre and .75 cm out into visibly undamaged pasture. Each section of soil was hand sorted and the number of grass grub recorded.

This procedure was repeated in late September during the pupal-teneral beetle stage. The results are given in Table 5-1. The complete absence of grass grub .60 m past the outer edge of visible damage in late March and the near complete absence of grubs in this zone in September are clearly shown.

Further studies on the movement of larvae relative to the outer edge of visible damage were conducted by subdividing six small spherical damaged patches not exceeding 5 m in diameter into the following strata; the inner area consisting of the area of regrowth, the damage halo, and three .60 m wide concentric margins extending into undamaged pasture. Within the size range

Table 5-1 Density ($/m^2$) of *C. zealandica* in relation to visible damage.

Distance from the outer edge of visible damage (m).	3rd instar larvae (March)	Pupae - teneral beetles (September)
Inner zone		
0.45 - 0.60	172.2 ± 65.3	64.6 ± 21.8
0.30 - 0.45	180.8 ± 63.0	37.7 ± 12.7
0.15 0.30	180.8 ± 34.4	53.8 ± 18.2
0.00 - 0.15	267.0 ± 25.2	53.8 ± 18.2
Outer zone		
0.00 - 0.15	241.1 ± 46.4	32.3 ± 10.9
0.15 - 0.30	241.1 ± 55.5	21.5 ± 7.3
0.30 - 0.45	155.0 ± 55.5	26.9 ± 9.1
0.45 - 0.60	43.1 ± *	13.2 ± 1.8
0.60 - 0.75	0.0 -	3.3 -

* Too few samples in which grass grub occurred to allow a meanful S.E. estimate.

Table 5-2 Density ($/m^2$) of *C. zealandica* larvae in different strata based on pasture damage.

Strata	Time			
	March	April	May	August
Inner zone	154.0 ± 33.3	200.0 ± 36.7	154.0 ± 51.8	146.3 ± 55.4
Damage halo	499.1 ± 63.4	462.1 ± 21.8	375.8 ± 34.3	264.9 ± 58.1
Distance from OEVD*				
0.0 - 0.60 m	24.6 ± 17.9	141.7 ± 45.3	117.1 ± 61.1	129.4 ± 46.1
0.60 - 1.20 m	0.0	0.0	0.0	6.1 ± 6.1
1.20 - 1.80 m	0.0	0.0	0.0	0.0

* OEVD = Outer edge of visible damage

of damaged patches chosen the boundaries in between these strata were easily defined. Samples were taken monthly or every second month from March to August at random. Four, 10 cm diameter soil cores were taken from each patch of damage within each stratum. Samples were broken up by hand and the grass grubs counted. The density of larvae found in each stratum are summarised in Table 5-2. These results confirmed those obtained from transect samplings, that the numbers of grass grub decrease suddenly .60 m from the outer edge of visible damage, and few larvae are found within the .60 m - 1.20 m stratum. This trend did not change substantially, throughout the larval season, although initially, the density in the undamaged margin closest to the outer edge of visible damage increased. The relative density of grass-grub found in this stratum however, was highest in August.

(b) Proportion of Grass Grub Population found in or Near Damage. Following studies of larval dispersion in the vicinity of damaged patches, a number of paddocks in the Takapau farming systems trial, with a wide range of grass grub populations, were sampled in March and May. These paddocks were kept evenly grazed over the late summer early autumn period to ensure that damage was as clearly defined as possible. Paddocks were divided into ten 20 x 20 m subplots. Eight to ten, 10 cm cores were sampled at random from each subplot and the number of grass grub in each recorded. Samples were marked as those originating from within visible damage, within .60 m of the outer margin of visible damage, or from areas where pasture damage was not apparent. Estimates of the percentage of the grass grub populations in each stratum are summarised in Table 5-3.

On average, in excess of 85% of the larvae in March and May were located in areas of visible damage and within a .60 m wide undamaged margin. More usually this figure was closer to 90% and frequently exceeded 95%. The percentage of samples taken from each stratum is also given in Table 5-3 and provides an estimate of the proportion of the paddock which fell into these strata.

Table 5-3 Mean percentage ± S.E. of C. zealandica larval populations found in or near visible damage

Stage	Range in pop density (/m²)	No. Pdks+	% population D*	% population DM**	% population D+DM	% plots in strata D	% plots in strata DM	% plots in strata D+DM
2nd & 3rd instar (March)	0-120	5	54.2 ± 2.02	34.7 ± 9.91	88.9 ± 2.41	6.7 ± 0.64	20.2 ± 4.76	27.0 ± 4.83
	120-240	14	72.0 ± 4.13	32.9 ± 6.65	94.1 ± 1.73	20.4 ± 2.29	22.0 ± 2.71	42.7 ± 4.47
	240	24	48.2 ± 3.96	25.1 ± 3.63	96.4 ± 0.81	37.6 ± 3.11	31.4 ± 2.23	69.7 ± 2.89
3rd Instar (May)	0-120	8	48.5 ±12.91	12.5 ± 8.54	85.0 ± 8.25	3.7 ± 0.93	0.78± 0.62	4.4 ± 0.85
	120-240	15	57.3 ± 4.34	30.2 ± 3.80	87.5 ± 2.54	30.4 ± 3.81	33.3 ± 3.34	61.8 ± 6.07
	240	7	60.5 ± 4.19	33.4 ± 3.72	92.5 ± 1.92	33.2 ± 2.10	34.8 ± 2.87	63.2 ± 5.23

D* = Visibly damaged areas

DM** = Damaged margin (.60m wide area surrounding visible damage)

Pdks+ = Paddocks

Table 5-4 Extension of the outer edge of visible pasture damage caused by C. zealandica over the larval season (1972) and its relationship with the initial radius of the visible patch of damage.

Time	No. obs.	Mean distance (cm)	± S.E.	r*
Feb - April	30	13.9	± 2.82	0.117
April - June	30	4.1	± 2.83	0.042
Overall increase (Feb - June)	30	8.1	± 2.56	0.034

*r = correlation coefficient

It can be seen from this, that the proportion of grass grub populations found in areas of visible damage and the surrounding margin far exceeded the proportion of the paddock that these strata occupied.

(c) Extension of Grass Grub Damage. The extension of visible damage within the larval season was measured in 1972 from mid-February to June at approximately two monthly intervals. As with all studies involving the measurement of damage, the outer edge of visible damage was referenced to a peg usually located in the centre of each damaged patch. Measurements were made along marked transects running in cardinal directions from the centre of the damaged area to the outer edge of visible damage. The mean extensions of visible damage within a generation are shown in Table 5-4. These results show that after April the outer edge of visible damage tends to recede. The extension of the outer edge of visible damage within a generation did not exceed 22 cm and was not influenced by the initial radius of the damaged area it encircled.

Extension of the outer edge of visible damage between successive generations are given in Table 5-5. Measurements were not made in the same month in each generation. However, as within generation movement of damage is relatively restricted, the following observations seem valid. In all seasons the movement of the outer edge of visible damage from one year to the next seldom exceeded 1.20 m and in most instances did not exceed 1.0 m, while the mean increases between each pair of years studied ranged from .26 to .82 m. The extension of damage from one generation to the next overall generations was significantly related to the initial radius of the patch of damage (Table 5-5).

(d) Appearance of New Colonies. The relationship between the rate of appearance of new colonies as evidenced by new patches of damage (eruptions) and the percentage of the area damaged in each paddock is given in Fig. 8-10. With low populations, a larger percentage of the total area suffering visible damage arose from individual areas of damage which had

Table 5-5 Extension of the outer edge of visible pasture damage, between successive generations of C. zealandica, and the relationship between this and the initial radius of the damaged patch.

	no.of patches	Mean radius increase (m)	range	r^+	Slope ± 95%	Intercept ± 95%
1968 - 1969	19	0.826 ± 0.042	0.49 - 1.26	0.224	0.093 ± 0.208	0.675 ± 0.091
1969 - 1970	6	0.637 ± 0.063	0.47 - 0.92	0.499	0.456 ± 1.704	0.107 ± 0.265
1970 - 1971	22	0.265 ± 0.022	0.08 - 0.43	0.146	-0.126 ± 0.400	0.312 ± 0.047
1971 - 1972	17	0.416 ± 0.018	0.29 - 0.54	0.046	-0.023 ± 0.273	0.429 ± 0.040
Overall	64	0.506 ± 0.035	0.08 - 1.26	0.814 **	0.369 ± 0.067	0.184 ± 0.039

Levels of Significance * = $P<.05$
** = $P<.01$

r^+ = correlation coefficient

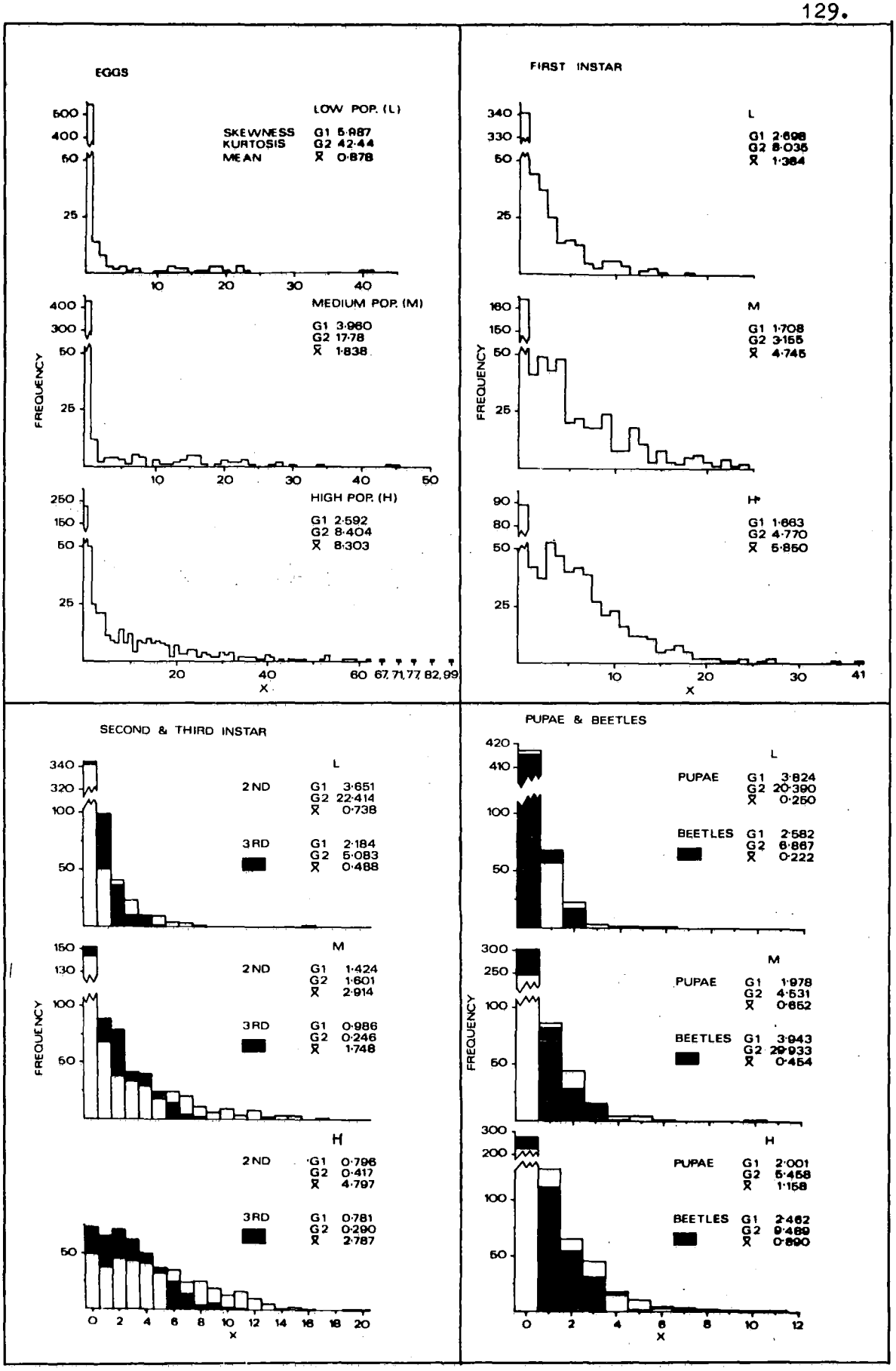


Fig. 5-2 Frequency distributions of different developmental stages of C. zealandica from low (L), medium (M), and high (H), populations.

not been visible during the previous generation. This proportion decreased as the area of damage increased.

It is thought that new colonies were initiated as a result of a single deposition of eggs and that over a minimum of three generations larval density increased to the point where pasture damage became apparent. The smallest visible areas of damage detected were approximately .33 m in diameter (see chapter 8).

(e) Practicability of Stratifying on Damage.

As a result of the sedentary reproductive behaviour of the female grass grub beetle described in chapter 1 and the limited dispersal of larvae shown in these studies, it appeared feasible at an early stage during the study to divide study plots into two strata, damaged and undamaged.

Visible damage was mapped in the manner described in chapter 3. Included in the damaged stratum was a .60 m undamaged margin which encircled the outer edge of visible damage. In the following season prior to egg sampling a 1.20 m margin was added to the damage stratum to allow for the extension of damage from one generation to the next.

At Rukuhia, where pasture damage was poorly defined due to the tolerance of paspalum, and usually, a higher summer rainfall which masked damage, an intensive systematic sampling carried out on a grid basis allowed plots to be stratified on grass grub occurrence (see chapter 3). Again an allowance of 1.20 m for the extension of these strata boundaries between generations was made. Further adjustments were made to the strata at Takapau in March when pasture damage became visible and at both Takapau and Rukuhia when sampling located new areas of occurrence.

(2) Frequency Distribution

The frequency distributions representative of all developmental stages of grass grub from low, medium and high populations in the damaged and undamaged strata of the Takapau study plots are shown in Fig. 5-2, together with means ($\bar{x}$) and statistics of skewness (G_1) and kurtosis (G_2). The respective standard deviations of G_1 and G_2 are given for all samplings in Appendices 5-1 and 5-2 and the relationship of the mean ($\bar{x}$) with G_1 and G_2 are shown in Figs. 5-3 and 5-4. For normal distributions G_1 and G_2 do not depart significantly from zero.

All frequency distributions exhibited marked positive skewness and kurtosis. The extreme degree of positive skewness was most pronounced at the egg stage and tended to decrease up until the May third instar sampling. Thereafter skewness increased slightly up to the beetle stage. Skewness was less marked at high than low populations. Kurtosis followed a similar trend to that shown by skewness.

In all stages and for all populations the zero class of the frequency distributions was usually much larger than any of the other class intervals, and constituted more than 90% of all observations on low egg populations.

The frequency distributions for all stages and population levels were tested against the negative binomial model. The maximum likelihood estimate of the exponent k was used and either the u or t statistic, whichever provided the most efficient test for the k and mean concerned were used for testing the adequacy of fit of the model.

The results of these analyses are given in Appendices 5-3 and 5-4 and for four generations of grass grub in the damaged stratum of the improved life table plot at Takapau in Table 5-6. Also given are the results of the chi-square test for goodness of fit. Where u or t are less than their respective standard errors, SEu and SEt, the negative binomial may be taken as a satisfactory model. A large negative value of u or t indicates that the distribution is more skewed than that described by the negative

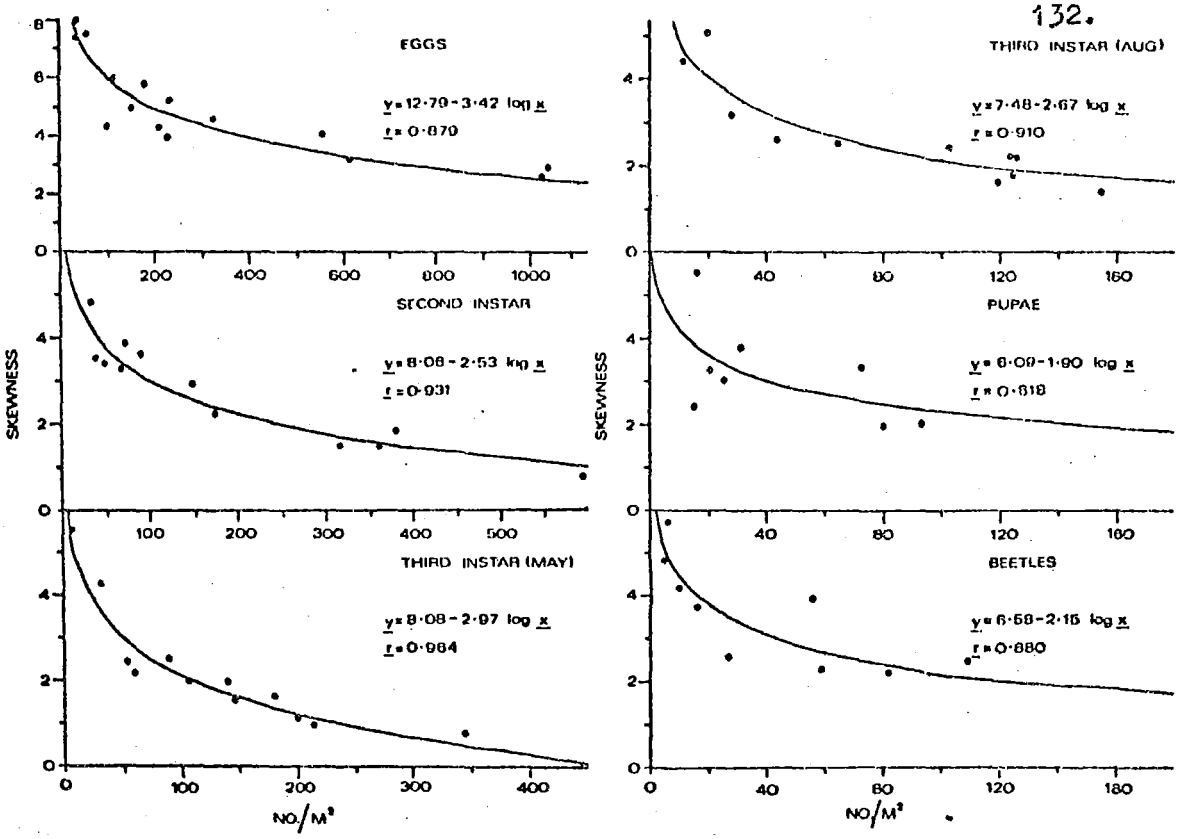


Fig. 5-3 Relationship between skewness and mean population density for different developmental stages of C. zealandica.

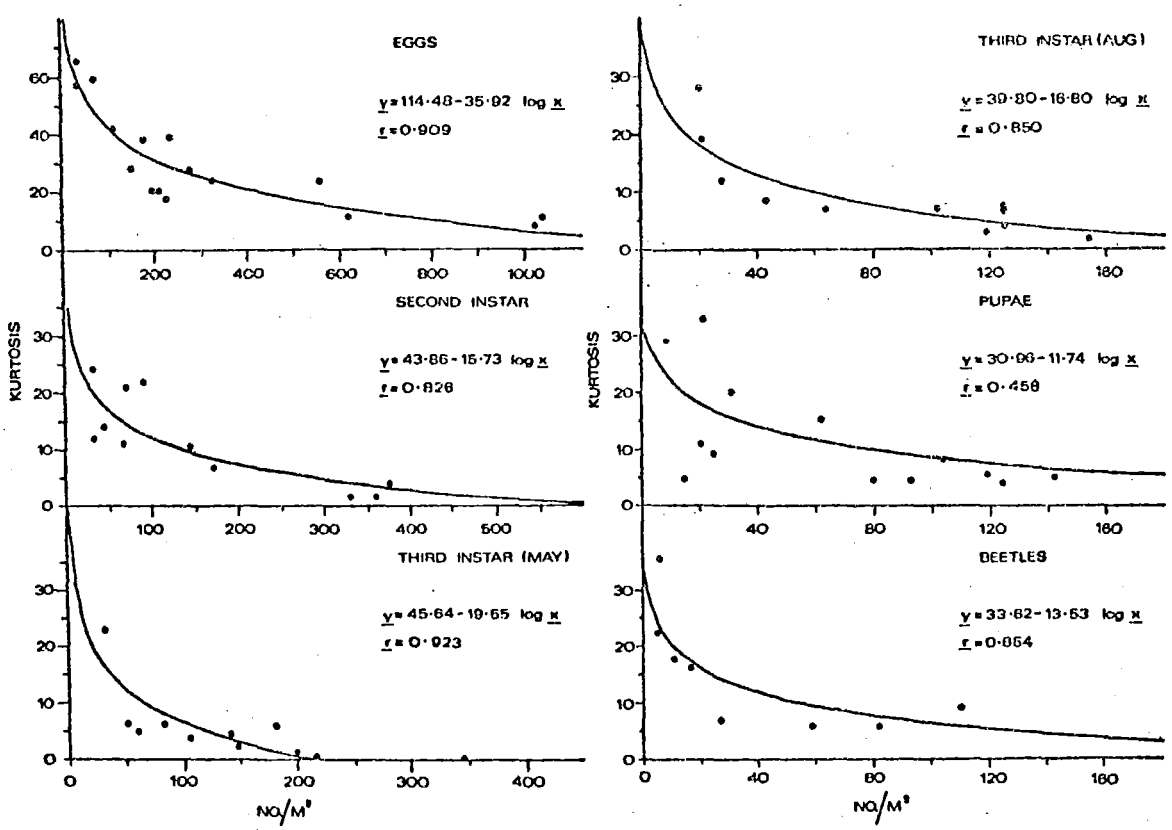


Fig. 5-4 Relationship between kurtosis and mean population density for different developmental stages of C. zealandica.

Table 5-6 Tests for the adequacy of fit of the negative binomial (NB) for counts of *C. zealandica* recorded from the improved life table plot at Takapau

(* signified appropriate test of NB for $\bar{x}/k$)

Stage	SINGLE SAMPLE UNITS - DAMAGED STRATA									
	$\bar{x}$	s^2	k	x^2	df	T	SET	U	SEU	$\bar{x}/k$
+1968-69 Eggs	1.59	33.70	0.0310	67.24	7	- 550.40	4928.88	- 49.07*	27.75	51.22
3rd (May)	0.63	1.96	0.2571	9.67	7	- 2.62	3.70	- 0.49*	0.30	2.64
3rd (Aug)	0.83	2.24	0.3720	13.65	8	- 1.78	3.22	- 0.44*	0.29	2.23
Pupae	0.59	1.71	0.2414	7.30	7	- 0.96	2.86	- 0.29*	0.24	2.42
1969-70 Eggs	8.41	233.78	0.2052	63.98	29	-2329.93	6987.35	-120.84*	49.12	40.90
2nd	2.91	12.41	0.6700	21.49	13	- 31.46	27.49	- 3.17*	1.56	4.34
3rd (May)	1.74	3.33	1.4796	21.12	9	- 3.43*	1.89	- 0.48*	0.28	1.17
3rd (Aug)	1.01	1.95	1.1022	10.63	7	0.45	0.85	0.01*	0.14	0.92
Pupae	0.84	1.57	0.9526	3.53	7	0.17	0.68	- 0.02*	0.11	0.88
Beetles	0.66	1.19	0.7979	1.96	6	- 0.27	0.51	- 0.02*	0.09	0.83
1970-71 Eggs	4.95	143.72	0.0722	105.52	13	-2801.59	16903.77	-201.05*	71.60	68.50
2nd	2.68	11.90	0.5490	31.11	13	- 34.44	33.29	- 3.96*	1.68	4.90
3rd (May)	1.61	2.90	1.6251	13.05	8	- 2.02*	1.47	- 0.32*	0.24	0.99
3rd (Aug)	1.01	1.95	1.2149	2.35	7	0.43	0.79	0.10*	0.14	0.83
Pupae	0.75	1.39	0.8109	2.14	6	- 0.49	0.70	- 0.05*	0.11	0.92
Beetles	0.48	0.73	0.8704	1.18	5	- 0.09	0.24	- 0.007*	0.04	0.55
1971-72 Eggs	1.86	36.24	0.0523	42.92	13	- 230.76	2021.07	- 32.04*	16.13	35.64
2nd	1.16	5.20	0.2788	11.69	11	- 7.26	11.92	- 0.78*	0.67	4.16
3rd (May)	0.42	0.72	0.4660	4.13	5	- 0.30	0.35	- 0.07*	0.06	0.90
3rd (Aug)	0.23	0.34	0.4572	1.10	4	- 0.04	0.10	- 0.004*	0.02	0.50
Pupae	0.16	0.23	0.3044	3.26	4	- 0.06	0.09	- 0.018*	0.01	0.53
Beetles	0.13	0.18	0.2892	1.27	4	- 0.03	0.06	- 0.007*	0.01	0.45
1972-73 Eggs	0.87	15.85	0.0291	15.43	7	- 180.35	904.48	- 11.46*	8.47	29.90

$\bar{x}$ = mean

s^2 = variance

k = dispersion parameter of NB

x^2 = chisquare test

df = degrees of freedom in x^2

T = test of NB

SET = SE of T

U = test of NB

SEU = SE of U

+1968-69 study plot was not stratified on damage

binomial. For these data the u test proved to be the most efficient as the values of k and means were generally low.

The negative binomial only provided a satisfactory fit for the damaged stratum over the later developmental stages including the August third instar larvae through to the teneral beetle stage, (Table 5-6). In the undamaged stratum all stages were generally adequately described by the negative binomial model.(Appendix 5-3).

It is of interest to note that, had the t statistic been used to test the adequacy of the negative binomial model over the range of k and means encountered, the negative binomial would have been accepted on many occasions as a satisfactory model. On the other hand, the chi-square test generally indicated that apart from the eggs and first instar larvae the frequency distribution of the other developmental stages were satisfactorily described by the negative binomial series.

If samples were independently pooled, a process which reduces the proportion in the zero class interval, the adequacy of the negative binomial model was not improved (Appendix 5-5).

For stages where the negative binomial gave an adequate fit of individual frequency distributions, based on counts from single sample units, tests were carried out to ascertain whether the k value was common to the frequency distributions of a particular stage irrespective of the mean population density. The use of a common k (kc) for transforming insect counts and planning sampling programmes has been previously reviewed. The graphic method adopted in these studies was that described by Bliss and Owen (1958). First y^1 and x^1 were calculated from the following formulae: $y^1 = s^2 - \bar{x}$, where s^2 and $\bar{x}$ are the variance and mean, respectively and $x^1 = \bar{x}^2 - s^2/n$, where n is the total number of samples. kc is given by the slope of y^1 on x^1. Where there is no relationship between $1/kc = y^1/x^1$ and $\bar{x}$ for individual samplings, then the estimate of a kc is valid. The graphic tests for each stage are given in Fig. 5-5. From these it can be seen that an estimate of kc is applicable only for pupae and teneral beetles.

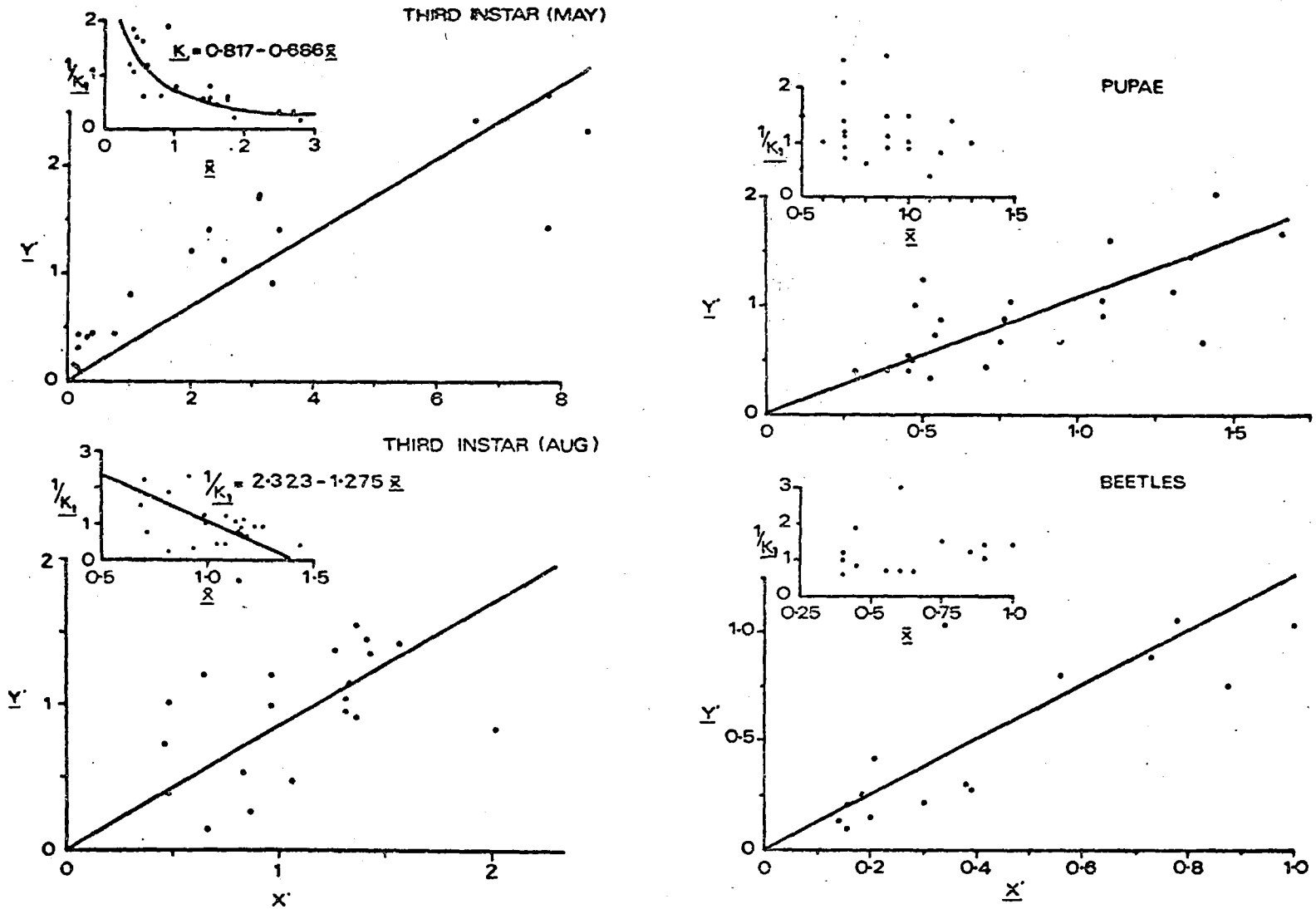


Fig. 5-5 Graphic computations of a common dispersal parameter ($\underline{k}$) for the negative binomial frequency distribution model calculated for different developmental stages of C. zealandica.

III TRANSFORMATION

Skewness and kurtosis invalidate the basic assumptions of the analysis of variance (ANOVA) and the t test. A transformation capable of eliminating the problems arising with highly skewed frequency distributions in using parametric statistics, such as the dependence of variance on the mean and the nonadditivity of variance, was therefore sought.

The relationship of the variance with the mean for all developmental stages of grass grub is shown in Fig. 5-6 together with the expected relationship for the Poisson series for which the variance equals the mean. The relationship of the variance (s^2) to the mean ($\bar{x}$) for all stages of grass grub was best described by Taylor's (1961) power law in which $s^2 = ax^{-b}$, where a is the intercept of the log s^2 on log $\bar{x}$ regression and b is the slope. In the pupal and teneral beetle stages for which the k values were common (kc) to each stage the variance is given by $s^2 = \bar{x} + \bar{x}^2/kc$. This relationship between the variance and the mean is also fitted to the observed data in Fig. 5-6.

The transformations tested for their ability to stabilise the variance for all stages in the development of grass grub included: the square root; log $(x + 1)$; a transformation estimated from Taylor's power law by which x the raw variable was transformed to x^p, where $p = 1 - \frac{1}{2}b$ in which b is the slope of log s^2 on log $\bar{x}$, and

$$\sin h^{-1}\sqrt{\frac{b - 1}{a + 1}}$$

where x is the raw variable and b is the slope and a the intercept of the linear relationship between mean crowding (m^*) (see chapter 2) and the mean ($\bar{x}$). The relationships of (m) on $\bar{x}$ and log s^2 on log $\bar{x}$ are given in Table 5-7.

The ability of the transformations to break the dependence of the mean on the variance was assessed as it is known that this is the most important basic assumption, on which the ANOVA is based, to satisfy.

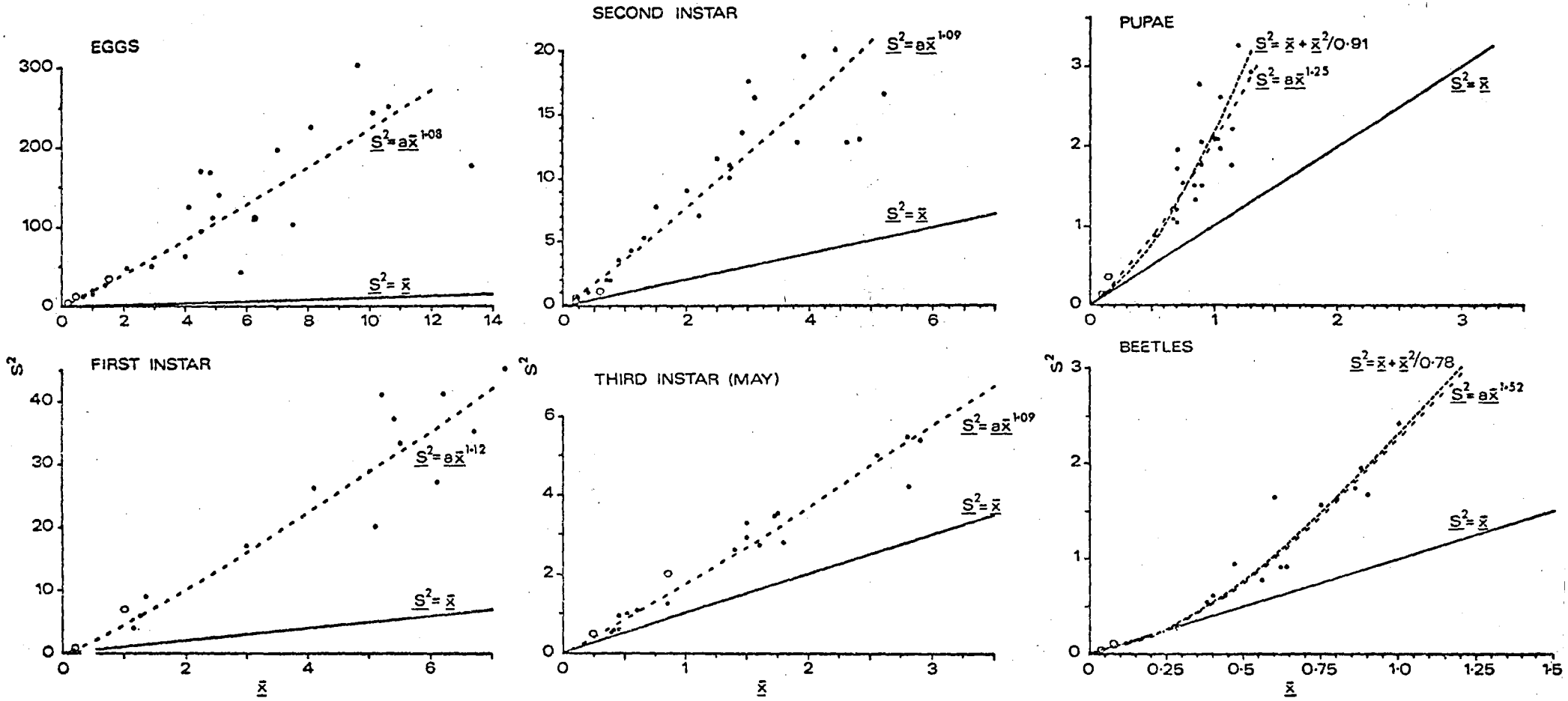


Fig. 5-6 Relationship between the variance (S^2) and the mean density ($\bar{x}$) of different developmental stages in C. zealandica

Table 5-7 Relationships of log variance (s^2) on log mean ($\bar{x}$) and mean crowding (m^*) on mean ($\bar{x}$) for all developmental stages of *C. zealandica*

Stage	No. of samples pooled	$\log s^2$ on $\log \bar{x}$				m^* on $\bar{x}$			
		Slope	± 95%	Intercept	± 95%	Slope	± 95%	Intercept	± 95%
Eggs	1	1.083	0.195	1.268	0.067	1.216	1.084	20.171	3.722
	2	1.201	0.134	1.164	0.045	1.471	0.554	17.447	3.288
	3	1.201	0.150	1.122	0.050	1.246	0.412	18.279	3.649
1st instar	1	1.124	0.127	0.068	0.048	1.212	0.320	3.732	0.716
	2	1.163	0.138	0.606	0.052	1.136	0.186	3.514	0.834
	3	1.217	0.120	0.575	0.045	1.132	0.126	3.712	0.849
2nd instar	1	1.089	0.140	0.551	0.043	1.030	0.310	2.841	0.453
	2	1.121	0.165	0.495	0.051	1.048	0.209	2.655	0.610
	3	1.103	0.167	0.483	0.052	1.022	0.130	2.681	0.568
3rd instar (May)	1	1.091	0.076	0.235	0.023	1.102	0.123	0.611	0.104
	2	1.049	0.092	0.224	0.028	1.030	0.077	0.678	0.130
	3	1.050	0.146	0.193	0.044	1.027	0.075	0.590	0.191
3rd instar (Aug)	1	0.876	0.369	0.284	0.032	0.811	1.097	1.167	0.213
	2	0.828	0.397	0.338	0.034	0.753	0.630	1.499	0.243
	3	0.682	0.422	0.457	0.036	0.760	0.433	1.820	0.249
Pupae	1	1.252	0.269	0.316	0.028	1.548	0.996	0.554	0.210
	2	1.245	0.303	0.257	0.031	1.303	0.583	0.591	0.242
	3	1.137	0.323	0.269	0.033	1.115	0.378	0.871	0.229
Beetles	1	1.521	0.215	0.351	0.030	2.405	1.067	-0.116	0.215
	2	1.575	0.248	0.207	0.034	1.760	0.663	-0.096	0.266
	3	1.521	0.251	0.104	0.034	1.492	0.349	-0.146	0.210

* For definitions see text

Data were transformed by the above transformations and the means and variances of the transformed data were regressed against one another to determine whether the dependency of the variance on the mean was broken. Owing to the large number of zeros in the data from all stages, no transformation eliminated the dependence of the variance on the mean for counts from single sample units (10 cm diameter cores) (Table 5-8).

Andersen (1965) reported that where the mean and the $\underline{k}$ of the negative binomial are very small (less than three and approaching zero respectively) the dependence of the mean on variance is unlikely to be broken by any common transformation. He noted that this could be overcome by the independent pooling of sample units. Units were independently pooled into twos and threes. The data were then transformed as before. Transformations involving Taylor's power law did not change substantially for pooled sample units; however, those based on the mean crowding parameter did (Table 5-7). Of the transformations used, those based on Taylor's power law were generally the most effective over all stages, in breaking the relationship between the mean and variance. With the exception of eggs, for which three units were required to be pooled, this relationship was usually broken by the independent pooling of two units. The effect of Taylor's power law transformations on normality of individual frequency distributions from high, medium and low populations for all developmental stages of the insect for one, two and three sample units pooled, was examined. Even with two and three sample units pooled, skewness and kurtosis were less marked than with one, but were not eliminated (Appendix 5-6).

IV CENTRAL LIMIT THEOREM

With the highly skewed frequency distributions which characterise grass grub populations, particularly in the earlier developmental stages, the question arises as to what the sample size (number of sample units) must be before it can be assumed that the central limit theorem is applicable. It is known that

Table 5-8 Correlation coefficients (r) between variance and means of counts of *C. zealandica* using different transformations.

Stage	No. of samples pooled	df	x	$x^{0.5}$	$\log(x+1)$	$*x^{p}$	$+\sin h^{-1}$
Eggs	1	19	0.811 **	0.865 **	0.892 **	0.868 **	0.879 **
	2	19	0.884 **	0.785 **	0.597 **	0.674 **	0.828 **
	3	19	0.872 **	0.594 **	0.138	0.353	0.511 *
1st instar	1	12	0.932 **	0.832 **	0.775 **	0.776 **	0.908 **
	2	12	0.924 **	0.520 *	0.113	0.077	0.764 **
2nd instar	1	19	0.865 **	0.617 **	0.592 **	0.552 **	0.859 **
	2	19	0.788 **	0.217	0.054	0.007	0.684 **
3rd instar (May)	2	19	0.956 **	0.321	0.263	0.135	0.921 **
Pupae	1	19	0.804 **	0.695 **	0.731 **	0.680 **	0.772 **
	2	19	0.750 **	0.177	0.270	0.229	0.574 **
Beetles	1	12	0.935 **	0.962 **	0.966 **	0.980 **	0.968 **
	2	12	0.924 **	0.855 **	0.904 **	0.074	0.915 **

$*x^{p}$ = Taylors power law transformation where $p = 1 - \frac{1}{2}b$ where is the slope of log S on log $\bar{x}$ (see table 4-6)

$+\sin h^{-1} = \sin h^{-1}\sqrt{\frac{b-1}{a+1}}\ x$ Where b and a are the slope and intercept of mean crowding on mean respectively (see table 4-6)

Levels of significance * = $P < .05$
** = $P < .01$

this will increase with skewness. The sample size at which it is safe to assume that the central limit theorem is applicable was estimated for all stages in grass grub development by the formula given by Cochran (1963), $SN = 25C_1^2$ (see chapter 2). The relationship of SN to population mean is presented in Fig. 5-7 and the SN and the number of samples required, to give an estimate of the mean population density with an SE equal to or less than ±10% for the Takapau populations are given in Table 5-9. Generally, the latter required approximately two to three times as many samples as the former.

V THE QUESTION OF TRANSFORMATION

The problems associated with transformation of life table data have been discussed at length in the review of literature. Despite the fact that the grass grub frequency distributions are characterised by marked skewness and kurtosis, a decision was made not to transform for the reasons that follow:

- Although it was possible to adequately transform counts, at least pairs of sample units required independent pooling in order to stabilise variance and in spite of this different transformations were required for different stages.
- Studies on soil enchytraeids which exhibit similar degrees of skewness and kurtosis to that encountered in sampling the damaged stratum have shown that although transformations did improve a one way ANOVA, the improvement was marginal for recommending transformation (Abrahamsen and Strand, 1970).
- Sample sizes large enough to assume that the central limit theorem was applicable were not prohibitively high and with the sampling and extraction techniques developed in these studies, were within the capabilities of the available resources.

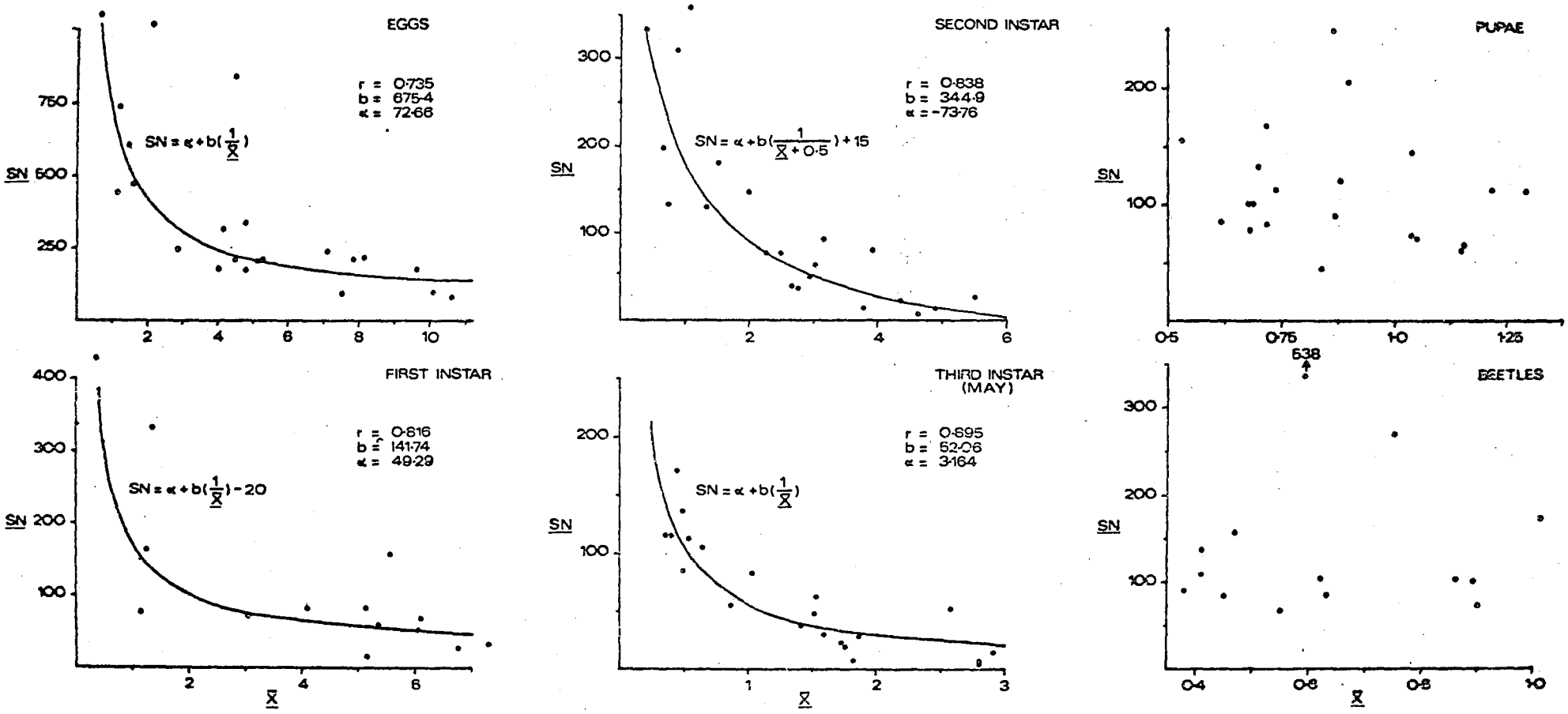


Fig. 5-7 Relationship between the mean population ($\bar{X}$) and the sample size (SN) required before it can be assumed that the central limit theorem is applicable.

Table 5-9 Number of sample units (SN) required in order that the central limit theorem is applicable and the sample size required to provide an estimate of the mean population of C. zealandica with a precision of ±10% SE

Generation 1968-69				1969-70			1970-71			1971-72			1972-73		
Stage	+$\bar{X}$	SN	10%S.E.	$\bar{X}$	SN	10%S.E.	$\bar{X}$	SN	10%S.E.	$\bar{X}$	SN	10%S.E.	$\bar{X}$	SN	10%S.E.
IMPROVED LIFETABLE															
Egg	1.59	473	1336	8.42	213	328	4.96	251	587	1.86	691	1042	0.87	896	2055
1st	not sampled			4.74	72	148	not sampled			not sampled					
2nd	not sampled			2.91	50	146	2.70	52	165	1.16	210	387			
3rd (May)	0.68	155	428	1.75	24	109	1.61	31	111	0.42	146	413			
3rd (Aug)	0.13	147	326	1.01	123	190	1.01	123	191	0.23	251	646			
Pupae	0.58	274	502	0.84	128	220	0.75	102	247	0.16	270	868			
Beetle	*Sampled with a spade			0.66	119	270	0.48	130	320	0.13	360	1052			
UNIMPROVED LIFETABLE															
Egg	1.84	392	1015	8.30	168	277	4.52	417	635	1.22	623	1441	1.69	463	1207
1st	not sampled			5.85	69	89	not sampled			not sampled					
2nd	not sampled			4.80	15	61	3.06	89	173	0.74	333	434			
3rd (May)	1.15	103	244	2.79	15	66	1.20	59	155	0.49	119	326			
3rd (Aug)	1.01	83	205	1.26	47	155	0.97	67	170	0.35	164	400			
Pupae	0.97	114	227	1.16	100	177	0.65	97	251	0.25	365	698			
Beetle	Sampled with a spade			0.89	151	239	0.45	388	396	0.22	167	563			

+ $\bar{X}$ = population mean

* Sampled with a spade and therefore not applicable

- Finally, the equation for estimating the variance from stratified sampling does not assume homogeneity of the within stratum variance (Cochran, 1963).

VI DEVELOPMENT OF A SAMPLING PLAN

The early development of a sampling plan for long term population studies allows the feasibility of carrying out these studies with the available resources to be assessed.

The development of a sampling plan should consider such factors as:

- The worth of stratifying the universe, in order to gain a more representative sample and increase the efficiency of variance estimates and thereby decrease sampling costs.
- The allocations of samples with a view to optimising sample allocation between strata, to obtain the required precision for the lowest cost.
  In these studies the cost of erecting and sampling from different strata was in terms of the overall sampling costs considered negligible.
- The number of samples required for each developmental stage and hence the cost and time likely to be involved in such studies.

(1) Precision obtained

The first step in the above studies was to examine the level of precision obtained by the method of stratification and sample allocation used and the number of samples taken.

With the exception of the 1968-69 generation when study plots were only divided into subplots, all study plots both at Takapau and Rukuhia were subdivided into strata based on pasture damage and grass grub occurrence, respectively. Allocation of samples between strata and total number of samples to be taken were decided more or less arbitrarily, but with regard to

Table 5-10 The relative level of precision obtained, the mean and the number of samples taken from the Takapau life table plots

IMPROVED STUDY PLOT															
Generation Stratification	1968-69 subplots			+dam 1969-70 x subplots			dam 1970-71 x subplots			dam 1971-72 x subplots			dam 1972-73 x subplots		
Stage	no./m^2	no. samples	% S.E.	no./m^2	no. samples	% S.E.	no./m^2	no. samples	% S.E.	no./m^2	no. samples	% S.E.	no./m^2	no. samples	% S.E.
Eggs	195.0	500	16.2	537.9	800	8.1	510.7	783	9.7	194.1	781	14.0	93.9	800	16.7
1st instar	not sampled			321.4	795	5.2	not sampled			not sampled			not sampled		
2nd instar	not sampled			202.7	600	6.2	269.0	500	6.2	114.8	597	8.6			
3rd instar (May)	83.5	400	9.7	146.1	700	4.7	165.1	595	5.4	41.6	600	8.1			
3rd instar (Aug)	77.7	406	9.3	104.4	600	6.2	107.9	400	6.7	25.2	600	10.0			
Pupae	72.1	400	12.7	86.7	600	7.4	76.6	400	7.9	21.2	600	11.7			
Beetles	52.7	100*	50.0	50.2	600	7.8	19.5	400	9.6	7.5	600	12.0			

* Sample unit a 15 cm spade spit.

+dam = pasture damage

Table 5-10 cont. The relative level of precision obtained, the mean and the number of samples taken from the Takapau life table plots

UNIMPROVED STUDY PLOT															
Generation Stratification	1968-69 subplots			1969-70 See footnote			1970-71 subplots			1971-72 subplots			1972-73 subplots		
Stage	no./m^2	no. samples	% S.E.	no./m^2	no. samples	% S.E.	no./m^2	no. samples	% S.E.	no./m^2	no. samples	% S.E.	no./m^2	no. samples	% S.E.
Eggs	221.8	500	14.3	608.5	800	7.5	559.0	598	10.4	155.4	608	15.8	208.3	600	24.0
1st instar	not sampled			472.9	800	4.3	not sampled			not sampled			not sampled		
2nd instar	not sampled			354.9	600	4.2	382.4	500	5.5	91.2	500	8.1			
3rd instar (May)	141.6	400	7.3	238.6	710	3.5	190.6	498	4.3	60.5	500	8.0			
3rd instar (Aug)	124.4	400	6.8	158.5	500	5.4	119.3	400	6.3	42.7	500	8.6			
Pupae	119.9	400	7.1	135.7	500	6.0	79.8	400	7.9	30.8	500	11.6			
Beetles	73.9	100*	37.0	93.3	500	6.9	25.1	427	8.8	19.1	500	10.2			

Footnote: Damage x subplots up to 3rd instar (May) thereafter solely by subplots.

the degree of aggregation of the developmental stage being sampled and the time required to sample and extract grass grub from soil samples. It was appreciated early in this study that the younger stages of grass grub were more aggregated and would therefore need more intensive sampling to attain the required level of precision.

The number of samples allocated to each stratum were allocated proportionately to the damaged and undamaged strata in each subplot to ensure that sampling within strata was as representative as possible.

The overall proportions of the study plots and subplots in each stratum are tabulated in Appendix 5-7 and the stratum sample sizes in Appendix 5-8. With the exception of Smith's plot, generally 1 in 2 to 1 in 5 samples were drawn from the undamaged stratum. The usual number of sample units taken ranged from 500 to 800 for egg sampling down to 360 for the later developmental stages (Appendix 5-8). Overall, the density of grass grub in the damaged stratum was 3 to 10 times as great as that in the undamaged stratum (see page 152). As can be seen from Appendix 5-7, the size of the damaged stratum on the improved Takapau plot was enlarged by a factor greater than two after May 1970 to include areas of pasture damage which had become obvious during the autumn and early winter. At the same time damaged strata were abandoned on the unimproved plot for reasons that will become apparent later in this chapter.

It was recognised early in these studies that the number of sample units taken in many samplings would probably exceed that required for the level of precision sought. This over-sampling continued since it allowed the study plots to be divided while still providing a reasonably precise estimate of the mean population density within each subdivision. This enabled density dependence in mortalities and comparisons of the manner with which density dependent mortalities operated to be studied (see chapter 7).

The relative level of precision obtained in sampling was calculated in the normal way. The formulae for estimating the variance of the mean from stratified sampling ($V_{st\bar{x}}$) are given

Table 5-11 The relative level of precision obtained, the mean and the number of samples taken from Rukuhia and Smith's plots

RUKUHIA					SMITHS				
Stratification			See footnote				damage x subplots		
Generation	Stage	no./m^2	no. samples	% S.E.	Generation	Stage	no./m^2	no. samples	% S.E.
1968-69	3rd instar (Aug)	72.7	400	7.4	1970-71	Egg	123.1	454	11.6
	Pupae	46.8	200	12.1		2nd instar	46.8	434	9.4
1969-70	Egg	288.4	800	7.3					
	2nd instar	25.9	600	9.5					
	3rd instar (May)	6.2	600	19.8					
	3rd instar (Aug)	7.4	500	20.5					
	Pupae	3.1	400	27.7					
1970-71	Egg	24.7	430	29.4					
	Pupae	2.1	360	40.3					

Footnote: From the egg to 3rd instar (May) stage of the 1969-70 generation, plots were stratified by occurrence x subplot. For the remaining samples plots were stratified by subplots.

in Appendix 5-9. Where these are calculated, the % SE is estimated as follows:

$$\% \text{ SE} = \left(\underline{\text{Vst}\bar{x}/\bar{x}}\right) \cdot 100$$

With the exception of the egg stage, the relative level of precision obtained was within or close to ±10% SE (Table 5-10). Only in years when egg populations were high did egg sampling provide the degree of precision sought. However, with the exception of the 1970-71 generation egg sampling at Rukuhia, even with low egg populations the relative level of precision obtained did not exceed ±20% SE (Table 5-11).

(2) Components of Variance

Analysis of variance was used to identify the magnitude of the within stratum components of variance. Each stratum was analysed separately since differences between the strata variances were extreme, and the within stratum variances were likely to be more homogeneous.

The sums of squares for each stratum were partitioned between and within subplots in a one way ANOVA (Table 5-12). The variance components are given in Appendix 5-10 and a summary of the percentage of stratum variance contributed by each component is presented in Table 5-12. The most consistent feature of these analyses is the large contributions made to total stratum variance by the within compared with the between subplot component. This trend is contrary to that observed by Guppy and Harcourt (1973) for other pasture scarabaeids belonging to the genus Phyllophaga where the between subplot variance was much larger than the within subplot variance (Guppy and Harcourt, 1973).

Significant differences between subplots were recorded in the damaged stratum from the Takapau study plots over the egg, second instar and May third instar sampling of the 1969-70 generation (Appendix 5-10). With the exception of the egg sample from the unimproved study plot, the same was true of the undamaged stratum. From May onwards differences within subplots within each stratum

Table 5-12 Mean and range of the percentage variance (s^2) of each stratum contributed by the variance components.

s^2 components						s^2 components					
		Between SP*		Within SP				Between SP*		Within SP	
Stage	Strata	$\bar{x}$	range	$\bar{x}$	range	Stage	Strata	$\bar{x}$	range	$\bar{x}$	range
Eggs	D+	1.7	0.2 - 3.9	98.3	96.1 - 99.8	3rd instar	D	4.2	0.0 - 11.0	95.8	89.0 - 100.0
	U+	2.10	0.0 - 5.2	97.9	94.7 - 100.0	(August)	U	12.9	1.3 - 13.4	87.1	76.0 - 99.0
1st instar	D	4.4	0.0 - 6.9	95.5	93.1 - 100.0	Pupae	D	2.5	1.1 - 5.0	95.5	95.0 - 99.0
	U	7.8	0.0 - 15.7	92.2	84.3 - 100.0		U	9.1	5.8 - 12.5	90.8	87.5 - 94.2
2nd instar	D	7.6	1.6 - 9.1	92.3	84.4 - 98.4	Beetles	D	2.6	1.7 - 3.8	97.4	96.1 - 98.2
	U	5.7	1.8 - 10.1	94.2	89.2 - 98.2	(teneral)	U	3.7	1.3 - 6.2	95.7	94.0 - 98.0
3rd instar (May)	D	4.4	0.9 - 8.5	95.6	91.5 - 99.1						
	U	9.1	0.6 - 19.1	90.6	80.9 - 99.4						

$\bar{x}$ = mean variance

* SP = subplot

\+ U = undamaged stratum

\+ D = damaged stratum

Partitioning of the within stratum sums of squares

Source of variation	df	E (ms)
Between subplots	p-1	$\sigma e^2 + \frac{\sum ni^2}{\sum ni} \sigma p^2$
Within subplots	n-p	σe^2

p = number of subplots
n = total number of samples
E(ms) = expected mean square

were generally not significant. On the unimproved study plot this trend was predictable since by May damage had become widespread and ill-defined. In view of this, stratification of the unimproved study plot based on pasture damage was abandoned and the complete plot was classified as falling into the damaged stratum. Samples were then proportionately allocated between subplots. With this sampling design consistent and significant subplot differences were recorded.

In spite of the rather ill-defined nature of the damage the maintenance of damage strata on the improved study plot at Takapau continued as the undamaged area was low lying and was therefore wet in winter. This division of the study plot enabled the low area of this plot to be studied separately.

At the Rukuhia study plot, differences between subplots within the damage strata were not consistently significant up until the time when the population collapsed.

The above findings suggest that overall the subdivision of the damage strata into subplots is unlikely to produce large gains in precision over stratification based solely on pasture damage or, in the case of Rukuhia, grass grub occurrence.

(3) Efficiency of Stratification

The subdivision of the study plots in the manner described earlier in this chapter and in chapter 4 allowed the variance minimising efficiencies of different patterns of stratification to be compared. The efficiency of the following sampling designs; simple random, stratification by pasture damage (in the case of Rukuhia on grass grub occurrence), stratification by subplots, and stratification by damage within subplots were assessed.

To avoid confusion in terminology, in the ensuing discussion, subplots are termed geostrata, strata based on pasture damage or grass grub occurrence, damage strata, and the division of damage strata on a subplot basis damage-geostrata.

The relative efficiencies (RE) of estimating the variance of the mean using different sampling designs and assuming

proportional allocation of samples between strata was assessed. This was done by relating the variance estimates for simple random with those from different forms of stratification in the below equation:

$$\underline{RE} = (\underline{Vran\bar{x}} - Vst\bar{x}/Vran\bar{x}) \cdot 100$$

The formulae for estimating the simple random variance of the mean ($\underline{Vran\bar{x}}$) and the stratified random variance of the mean ($\underline{Vst\bar{x}}$) is given in Appendix 5-9 and a worked example presented in Appendix 5-11. The strata, weights, means and variances are summarised in Appendices, 5-11 and 5-12 and the relative efficiencies of different methods of stratification in Appendix 5-13.

It is known that stratification usually results in a smaller variance compared with simple random sampling unless the number of samples taken from each stratum are far from optimal when stratified sampling can give a higher variance.

(a) Takapau Study Plots. Over the 1969-70 generation in the improved Takapau life table plots stratification by geostrata, damage strata and damage-geostrata produced relative efficiencies up to May 1970 of 33% but were usually close to 20%. After May gains from stratification were neither consistent nor in most instances large. In spite of this, stratification of the improved life table plot, irrespective of the method used, overall produced minor increases in relative efficiency.

With the exception of egg sampling in 1969 and damage-geostratification for the first instar sampling in 1970, the gains in relative efficiency, on the unimproved plot, from the different methods of stratifying were of the same order as those obtained on the improved plot. After May, the plot was classified as falling completely into the damaged stratum and small relative efficiencies were recorded from geostratification.

Results from stratifying by damage were most spectacular on Smith's plot where relative efficiencies of 66 and 75% were

Table 5-13 Number of samples required to provide an estimate of C. zealandica with a precision of ± 10% S.E. of the mean.

Generation	Stage	* $\bar{X}$	OPTIMAL +RAN	OPTIMAL DAM	OPTIMAL DAM-GEO	PROPORTIONAL DAM	PROPORTIONAL GEO	PROPORTIONAL DAM-GEO
IMPROVED TAKAPAU STUDY PLOT								
1969-70	Eggs	4.26	737	461	329	529	599	556
	1st instar	2.60	325	237	150	240	242	218
	2nd instar	1.64	297	226	162	238	258	233
	3rd instar (May)	1.19	230	173	131	227	179	183
	3rd instar (Aug)	0.83	247	218	194	267	184	243
	Pupae	0.70	294	259	210	321	335	326
	Beetles	0.52	361	317	248	404	388	366
1970-71	Eggs	3.98	744	648	542	768	796	798
	2nd instar	2.19	220	171	156	194	194	192
	3rd instar (May)	1.31	157	131	117	164	164	178
	3rd instar (Aug)	0.87	244	197	159	198	198	182
	Pupae	0.62	310	262	214	270	260	246
	Beetles	0.38	409	345	302	357	378	348
1971-72	Eggs	1.57	1203	1077	807	1311	1237	1533
	2nd instar	0.93	490	394	340	399	530	433
	3rd instar (May)	0.34	502	442	381	453	439	402
	3rd instar (Aug)	0.20	751	726	560	727	717	594
	Pupae	0.17	857	850	679	816	835	600
	Beetles	0.12	1196	1153	847	1161	1383	870
1972-73	Eggs	0.76	2365	2245	1294	2462	2450	2232
UNIMPROVED TAKAPAU STUDY PLOT								
1969-70	Eggs	4.99	561	429	320	493	503	468
	1st instar	3.83	176	151	105	121	133	147
	2nd instar	2.88	141	101	80	106	121	107
	3rd instar (May)	1.73	139	111	86	138	125	106
RUKUHIA STUDY PLOT								
1969-70	Eggs	2.34	630	427	369	430	473	430
	2nd instar	0.21	696	649	544	665	627	538
	3rd instar (May)	0.05	2568	2413	1188	2424	2472	2352
SMITH'S STUDY PLOT								
1970-71	Eggs	0.99	2953	240	97	998	1176	658
	2nd instar	0.38	1139	178	127	379	798	285

+RAN = Simple random

DAM = Stratified solely on damage

GEO = Stratified solely by sub plots

DAM-GEO = Stratified by both damage and sub plots

* $\bar{X}$ = mean population per sample unit.

recorded for both egg and second instar sampling. Interestingly, just geostratification gave relative efficiencies for the egg and second instar larval samplings of 60 and 34%, respectively.

Since the purpose of intensive sampling studies on Smith's plots was solely to investigate the worth and practicability of stratifying study plots with low grass grub populations, these studies were not continued after the second instar sampling in 1971.

(b) Rukuhia Study Plot. At Rukuhia for the egg, second instar and May third instar sampling stratification by grass grub occurrence gave the respective relative efficiencies of 32, 4 and 6%. These were not markedly superior to those obtained by geostratification. In comparison damage-geostratification gave relative efficiencies of 32, 23 and 8% for eggs, second instar and May third instar sampling, respectively (Appendix 5-13).

(4) Number and Allocation of Samples

Up to this point calculations of the relative efficiencies in variance estimates have assumed proportional allocation between strata. The potential for, and feasibility of, increasing the efficiency of variance estimates and hence minimising the number of samples required for a given level of precision by optimising the allocation of sample units between strata was assessed. The sample size required to obtain a relative level of precision of $\pm$10% SE assuming optimal and proportional allocation was estimated (Table 5-13). The calculations involved are summarised in Appendix 5-11.

Optimal compared with proportional allocation of sample units for a particular method of stratification reduced the sample size required to obtain the level of precision sought. In many instances these differences between the two methods were extremely large. Unfortunately, the number of samples required to be taken from each stratum with optimal allocation varied widely in a manner which seemed unrelated to the size of

Table 5-14 Number of samples required to estimate the mean population density ±10% S.E. of eggs and second instar larvae of C. zealandica, at different stages in population development, assuming proportional allocation.

Stages in population development relative to pasture damage	Stage	++ $\bar{x}$	Stratification				
			Random	Damage	Geostrata	Damage — geostrata	Undamage deleted
+ * Low well defined damage	Egg	1.05	2948	996	1176	658	192
(Smiths plots)	Second Instar	0.38	1139	369	798	285	160
**High well defined damage (Takapau 1969-70)	Egg	4.24	737	529	599	556	*NA
	Second Instar	1.64	297	238	258	233	*NA
High ill defined damage (Takapau 1970-71)	Egg	3.98	744	768	796	798	*NA
	Second instar	1.32	220	194	194	192	*NA
Population collapse damage ill defined (Takapau 1972-73)	Egg	0.76	2365	2462	2450	2232	*NA

* Not applicable

**High = large proportion of plot visibly damaged

+* Low = small proportion of plot visibly damaged

++ $\bar{x}$ = population mean

the strata or predictable from the previous sampling. In view of this it became clear that any practical sampling plan at least in the initial stages of a study must be based on proportional allocation of samples.

(5) Conclusion

From the data presented in these studies it is suggested that the most efficient method of stratification will be considerably influenced by the level of the population being studied and the accuracy with which damage and hence areas inhabited by grass grub can be identified. When considering intensive sampling, there appear to be four important stages in the development of grass grub populations. The number of sample units required at each stage of population development to obtain a relative level of precision $\pm 10\%$ SE for the egg and second instar stage with different sampling designs and proportional allocation of sample units are presented in Table 5-14.

The first stage is the colonising period when patches of damage are clearly defined and circular in shape, as was the case on Smith's plots. Over this period large gains from stratifying solely on damage have been demonstrated. These gains were increased by dividing the plot into damage-geostrata. However, the greatest gains in the efficiency of variance estimates were obtained by deleting the undamaged stratum which harboured less than 2% of the population. Further improvements were obtained when the damaged stratum was divided into geostrata (Appendix 5-14). When sampling populations at this stage a limit may have to be placed on the number of samples to be taken, otherwise a large proportion of the area harbouring grass grub may be disturbed and this could significantly influence the population's performance.

Later in the development of a grass grub population visible damage becomes more extensive and less well defined as exemplified by the improved life table plot at Takapau during the

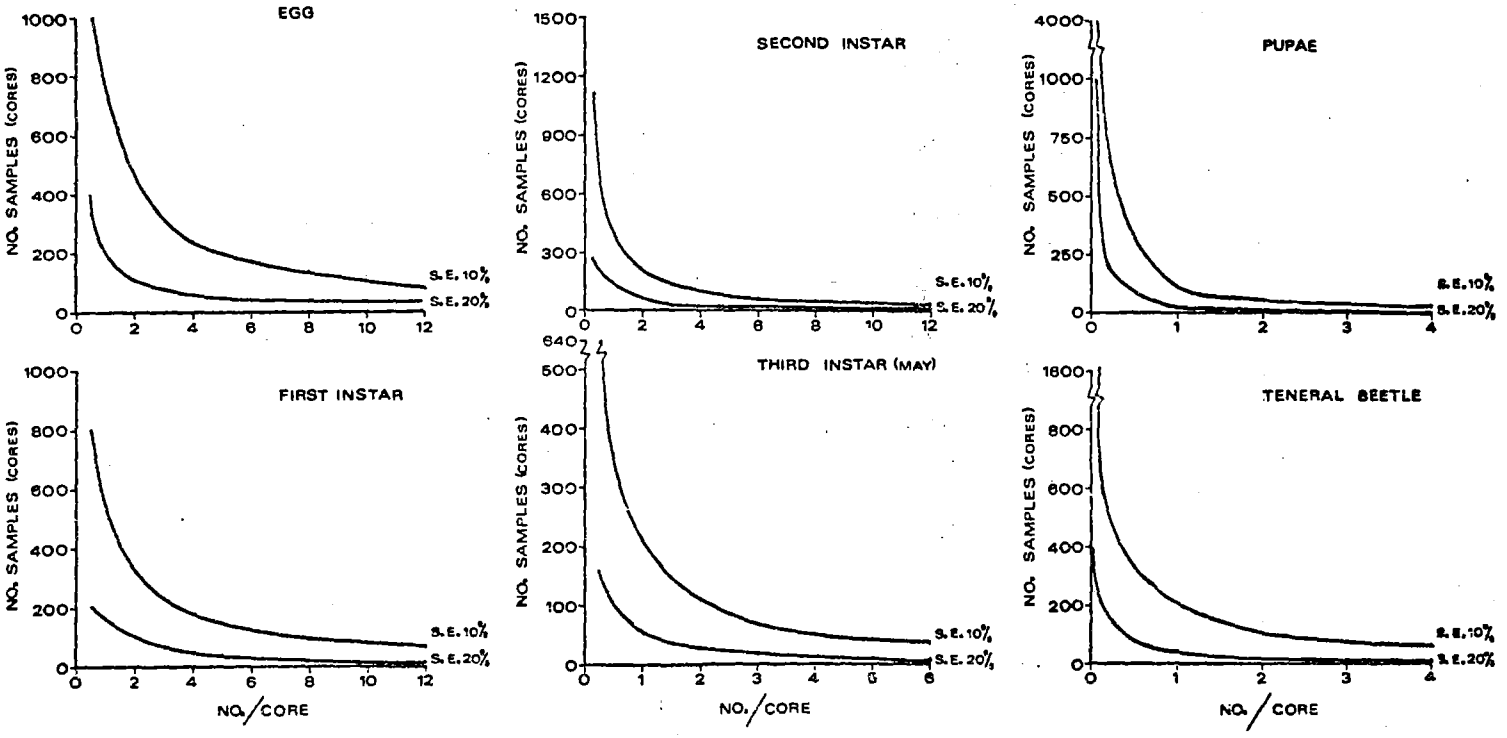


Fig. 5-8 Relationship between population mean per sample unit and the number of sample units required for given levels of relative precision

1969-70 generation. At this stage, the undamaged stratum cannot be deleted on the grounds that it contains an insignificant proportion of the population. Over this period, damage-geostratification was consistently more efficient than other methods of stratification. It is considered that the end of this stage was reached at Takapau during the egg and first and second instar sampling of the 1969-70 generation.

Over the period of peak populations, for example the Takapau life table plots during the 1970-71 and 1971-72 generations, no consistent improvements in efficiency were produced by any one sampling design. At this stage in population development pasture damage is ill-defined and, in the interests of sample representativity, geostratification is considered worthwhile. The construction of geostrata was found to be less time consuming than the other methods and provided minor increases in efficiency over simple random sampling.

The fourth stage followed immediately after a population collapse when population density was very low and an extremely large number of samples were required to obtain a level of precision $\pm$10% SE. As with the preceding stage, gains in efficiency from any method were minor and variable but in the interest of obtaining a representative sample the use of geostrata is considered worthwhile. An indication of how many samples are required to obtain an estimate of the mean with relative levels of precision of $\pm$10% and 20% SE at this stage were calculated at different population levels from the log variance - log mean relationship given in Table 5-6. These are presented in graphic form in Fig. 5-8 and show the inverse relationship between the number of samples required for a given level of precision and population density per sample unit.

As populations begin to build up again, circular areas of damage become obvious and the method of stratification outlined above can begin again.

For environmental conditions where damage is not easily identified, stratification based on the presence or absence of larvae, as was described for the Rukuhia plot, is worthy of further investigation.

VIII ALTERNATIVE PROCEDURES FOR SAMPLING

The potential for obtaining the largest gains in sampling efficiency rests with the optimal allocation of sample units between strata. In view of this a preliminary investigation was made to increase the efficiency of sampling by tapping this potential.

If the weight of the different strata are known and the mean insect density in each stratum can be predicted approximately, then the variances for the stratum may be calculated from the linear relationship established between log variance and log mean. From this relationship the statistics of stratified sampling and hence the number of samples required for a 10% relative level of precision can be computed. The survivorship curve of grass grub (see chapter 7) is characteristically linear when plotted on a semi-log scale and could give a worthwhile guide to population density in the different strata. The accuracy of this estimate will improve as more becomes known about the population dynamics of grass grub, but in the initial stages of population studies this approach is unlikely to provide worthwhile results.

At high population levels of grass grub when pasture damage is ill-defined or immediately after a catastrophic population collapse, gains from stratified sampling are usually minimal and variable. In these instances the adoption of a sequential sampling plan is likely to produce worthwhile gains in efficiency over the more conventional sampling methods for which sample size is predetermined. In many cases sample size in the initial stages of population studies, is arrived at arbitrarily. The efficiency of

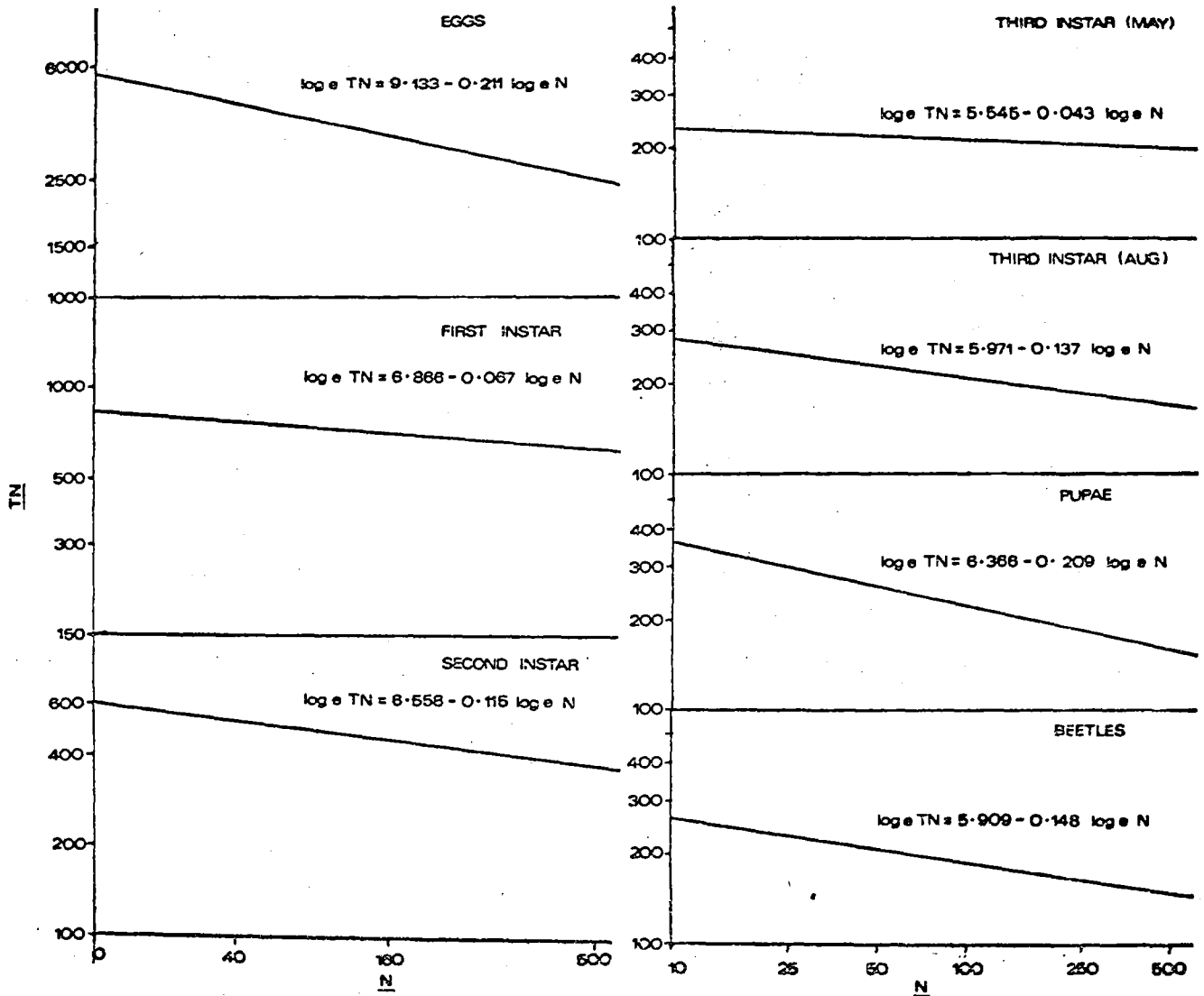


Fig. 5-9 Sequential sampling plans for all developmental stages of C. zealandica giving the stop lines which define the point at which the required level of precision (±10% SE) has been reached.

sequential sampling methods stem from the fact that sampling is terminated when a decision (Wald, 1945) or a fixed level of precision is reached (Kuno, 1969; Green, 1970). As the sampling of most developmental stages of grass grub involves laboratory extraction, sequential sampling methods do not reduce the number of samples needed to be taken but do reduce the number required to be processed. Processing samples involves a large proportion of the total sampling time and for this reason a sequential sampling plan was developed. The formulae for estimating stop lines are given in chapter 2.

The plan given in Appendix 5-15 was calculated from the relationship between the mean and variance in the manner described by Green (1970). A graphic presentation of the plan with a ±10% SE level of precision for all developmental stages of grass grub is given in Fig. 5-9.

VII TOTAL COST OF SAMPLING

Studies on sampling have been conducted over a period which saw both a rise and fall in the density of grass grub populations. In view of this a reasonably objective estimate can be made of the range in total cost likely to be involved in conducting population studies with population estimates of ±10% SE.

These studies have shown that the total time required to sample is influenced by the size of sample, different developmental stages, population density, heterogeneity within strata and the effectiveness of stratification.

The ranges in sample size and total time involved in taking and processing samples are summarised in Table 5-15 and are based on Takapau data from the improved life table plot and Smith's plots (Table 5-13). These cover most stages in the population dynamics of grass grub, at least for the eggs and second instar stages, and include: low populations with well defined pasture damage (Smith's plots, 1970-71); high populations with

TABLE 5-15 Ranges in total cost required for estimating population density of the different stages of C. zealandica with a relative level of precision ± 10% SE.

STAGE	No./sample RANGE	No. of units RANGE	Time / unit (Man Min.)	Total cost (8 hr. man days) RANGE
Eggs	0.76-4.26	556-2450	10.48	12.14-53.49
First instar	2.60-2.60	218-218	10.30	4.68- 4.68
Second instar	0.38-2.19	194-530	9.14	3.69-10.09
Third instar (May)	0.34-1.31	164-439	7.92	2.71- 7.24
Third instar (Aug)	0.20-0.87	184-717	7.92	3.04-11.83
Pupae	0.17-0.70	260-835	7.92	4.29-13.87
Beetles	0.12-0.52	378-1383	7.92	6.24-22.82

less well-defined pasture damage (Takapau life table plot, eggs 1969 to May 1970) when the worth of stratification based on pasture damage was marginal; high population with ill-defined pasture damage (Takapau life table plot, eggs 1970 to beetles 1971) and following a population collapse, low populations with ill-defined pasture damage (Takapau, life table plot, eggs 1971 to eggs 1972). All calculations in Table 5-15 are based on the following assumptions:

- Samples are proportionately allocated between damage-geostrata over the period that damage is clearly defined (e.g. Takapau life table plot, egg 1969 to May third instar 1970).
- At high population levels, when damage covers a larger area and is less clearly defined and through periods of population collapse, samples are proportionately allocated between geostrata.
- Over the period after collapse, when populations begin to build up again and circular areas of damage become evident, samples are allocated proportionately between damage-geostrata.

With the exception of low egg and possibly low beetle populations it appears feasible to sample grass grub populations with the requisite level of precision with relatively small resources. It is of interest to note that the stage in population development which, with given resources (20 man days), defied the level of precision sought, was just after the Takapau populations had collapsed (1971-72 generation). Yet at Smith's where pasture damage was well-defined but the mean population density was not much higher than the 1971 - 72 generation for the Takapau life table plots, damage-stratification placed the level of precision required ($\pm$ 10% SE) within the scope of available resources.

In practice where it is not possible to sample with the required level of precision, as many samples as possible should be taken. From Fig. 5-8 it can be seen that such an approach should permit a relative level of precision of $\pm$ 20% SE to be attained.

CHAPTER VI

DEVELOPMENT OF TECHNIQUES AND THE METHODS USED IN STUDYING GRASS GRUB DAMAGE IN PASTURE

I INTRODUCTION

The purpose of this chapter is to describe the methods and the development of techniques used for studying the growth of grass grub damage in pasture and the relationship between losses in pasture production and grass grub density. Such studies require estimates of population densities, the measurement of the area damaged, the measurement of losses in herbage production in damaged areas, and the measurement of changes in botanical composition.

The description of the Takapau farming systems trial and Smith's plots where the studies described in this chapter were conducted are given in chapter 4.

II SAMPLING GRASS GRUB

Estimates of grass grub densities were carried out in the following manner. A minimum of four but usually eight cores were taken at random from each of the ten (20 x 20 m) subplots in the 140 ha paddocks or plots in March, when most grass grubs were in the late second to early third instar larval stages, and again in May when near mature third instar larvae were present. In March larvae were extracted in the laboratory by the wet sieving method described in chapter 4 but in May larvae were counted in the field from hand sorted samples.

III MEASUREMENT OF AREA DAMAGED

In order to measure and study the area and growth in area of damaged pasture the nature and distribution of individual damaged patches were studied by ground observations and photographic

Plate 6-1 Patches of pasture damage caused by C. zealandica on Smith's plot: note the circular shape of the areas and the re-establishment of pasture in the centres.

methods.

(1) Nature of Damage

In the early summer months grass grub aggregates are usually so dense that where they occur the pasture root system is completely undercut by February and affected pasture begins to yellow and die. From studies described in chapter 4 it appears that within areas of pasture damage, larvae migrate outward in search of live root tissue. Pupation, emergence, mating and oviposition occur close to the point at which larval feeding terminates. Because of this characteristic pattern of adult and larval behaviour the extension of the outer edge of visible damage occurs both within and between generations (see chapter 5). Initially the shape of individual patches of pasture damage is circular (Plates 6-1, 6-2). Over the course of time, as a result of growth and coalescence, the shapes of patches of damage become more irregular.

(2) Ground Measurement

Ground measurements of individual areas of pasture damage were referenced to marker pegs located in the centre of each damaged patch. These were driven into the ground in late April in the centre of patches which had not been visible in the previous season. The appearance of new areas of damage (eruptions) in each subplot were counted. Eruptions which occurred on the subplot margins were included in the subplot in which the largest proportion of them occurred. The measurements of extensions in the outer edge of visible damage within and between generations has been described in chapter 5.

Individual patches of damage which were circular were estimated by measuring a mean radius for the patch and calculating the area. Less regular patches were estimated by measuring the largest rectangle it was possible to fit into the area of damage. Portions not included were assessed by overlaying a 2 x 2 m piece of (15 x 15 cm mesh) reinforcing steel and summing the squares and fractions of squares that covered damage.

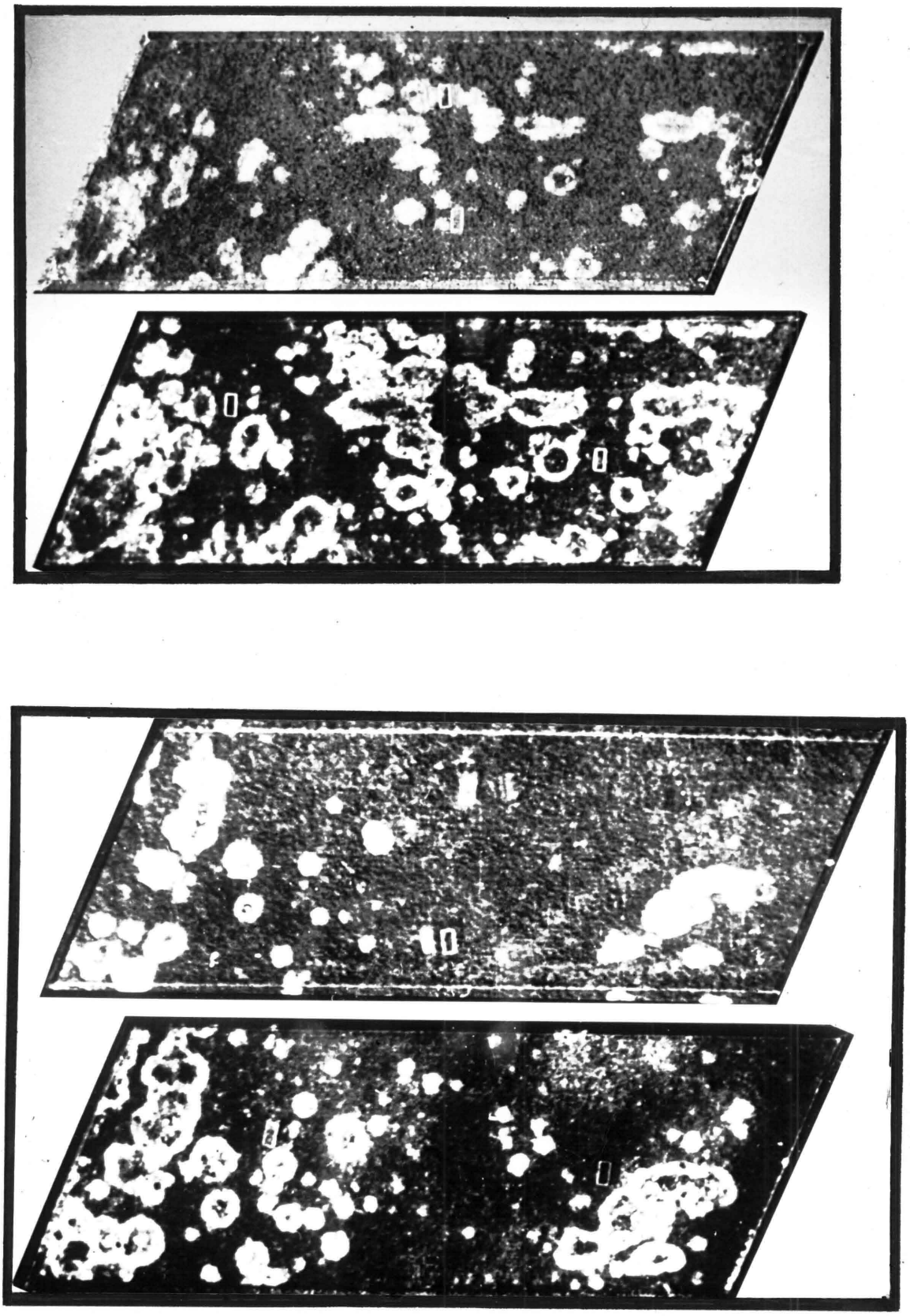


Plate 6-2 Aerial photographs of two paddocks (0.40 ha) taken in May of successive years showing the growth of pasture damage caused by C. zealandica.

(3) Photographic Measurement

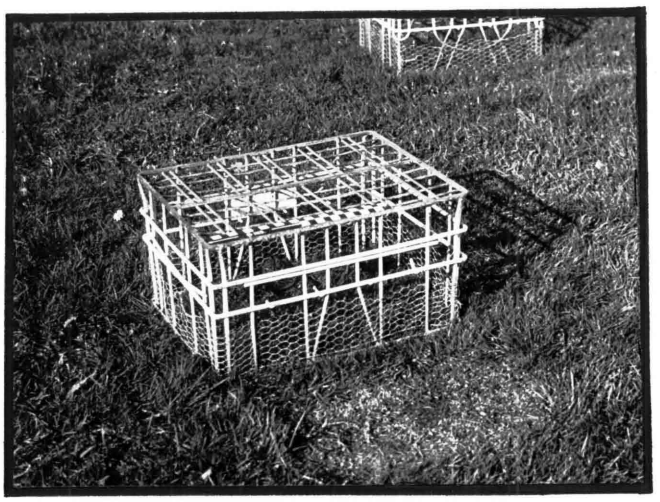
A detailed evaluation of the use of aerial colour photography for detecting, measuring and recording grass grub damage is given in Appendix 6-1. These studies showed the following.

- Colour infra red film was more versatile than colour film for detecting and measuring grass grub damage.
- The projection of near vertical positive transparencies taken at a height of 200 m, on to high quality bond paper and the tracing, cutting out and weighing of areas showing damage, allowed areas visibly damaged by grass grub to be accurately measured.
- The use of aerial photography allowed extensively damaged areas, the measurement of which was impossible from the ground, to be measured.
- In less extensively damaged areas where ground measurement was possible, measurement by aerial photography was up to ten times faster than ground measurement.

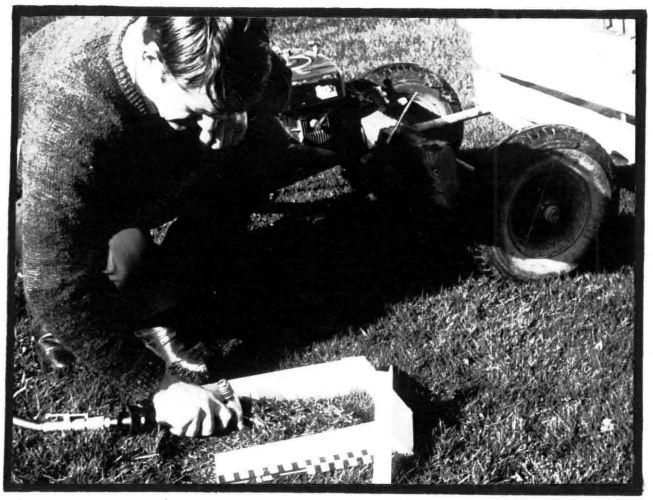
IV MEASUREMENT OF LOSSES IN PASTURE PRODUCTION

(1) Pasture Quantity

A difference method rather than a direct harvest technique was used to measure pasture production. These methods allow estimates of losses in net pasture production arising from decaying herbage, the amount of herbage consumed by the animal together with other parameters of grazed pasture to be made (Campbell, 1966a,b). Campbell (1966c) noted that while statistical variability was reduced with direct harvesting methods these techniques only reduce variability at the expense of realism.



A



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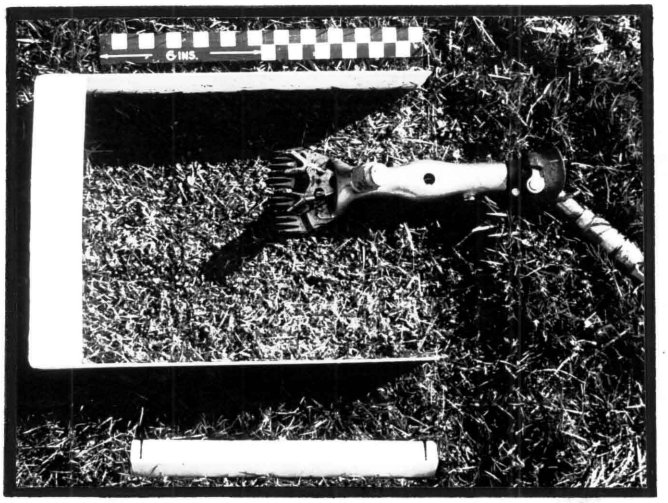

C

Plate 6-3 Equipment used for sampling pasture :

A : stock exclusion cages;

B : harvesting pasture;

C : quadrat and cutter.

For example, with the pre-trim direct harvesting methods negative pasture production resulting from herbage senescence in autumn or insect losses cannot be measured.

The difference technique employed in these studies has been described by Nevens (1945). In this method, yields from caged areas are subtracted from yields of open pasture at the time of cage placement to give net pasture production. Cages (C) were placed at random within a stratum and pasture was harvested from an adjacent open area (A) matched for botanical composition and amount of herbage present. Another matched open area (B) was marked with a .60 x .60 m, 1.2 cm diameter iron rod frame set into the pasture and was harvested when the caged area was clipped. From these data the following can be estimated:

Available herbage	= C
Net pasture production over the time interval of interest	= C - A
Herbage consumed	= C - B

Twelve wire cages (45 x 35 x 30 cm high) were placed in each stratum and pinned down with steel pins to prevent the cages being knocked or shifted by stock (Plate A, 6-3). The areas harvested in cages and open areas (.45 x 30 m) were cut to ground level by a portable sheep shearing plant (Plate B, 6-3). In most cases herbage required sorting to exclude sheep dung and dirt. Where herbage was contaminated by mud, samples were washed prior to drying. All herbage harvested in these studies was dried at 80°C for three days.

(2) Pasture Composition

Changes in herbage compostion were monitored seasonally by herbage dissection. Fresh foliage was subsampled and a 10 g subsample was dissected out into its component species. These were dried and expressed as a percentage by weight of the dried subsample.

In autumn 1972 point analysis was used to record the changes in ground cover along transects running through the centre of damaged patches and extending 1.5 m out either side of the damaged areas into undamaged pasture. The needles of the point analyser were spaced 10 cm apart and the number of points recorded varied with the size of the damaged area, but always exceeded 25. Only the plant species that the needles first hit were recorded.

(3) Stratification and Assessment of Damage

Preliminary observations revealed (chapter 5) that in individual areas damaged by grass grub there were three well defined zones (Fig. 5-1): a central area which had been damaged by previous generations and in which plants had established or re-established; a halo of more severe damage which was caused solely by the feeding activity of the current generation; and an outer 60 cm undamaged margin, in which grub density declined rapidly with distance from the outer edge of visible damage. Studies had shown that the extension of the outer edge of visible damage between generations seldom exceeded 1.20 m and within generations 20 cm.

The hypothesis was proposed that the density of grass grub found in the discrete colonies or aggregates which make up grass grub populations are similar, irrespective of population levels. If this hypothesis was correct it would obviate the need to study damage in paddocks with different population levels. It could therefore be assumed that population levels increase, not by an increase in density within areas of occurrence, but by the occupation of a larger proportion of the habitat. In order to test this, the density of larvae in March and May found in areas of visible damage and in the surrounding .60 m wide undamaged margin were regressed against the mean population density for each paddock or plot (Table 6-1). Significant linear relationships were found to exist between these variables indicating that as grass grub population levels increased larval density in or close to damaged areas also increased.

Table 6-1 Relationship between larval density (/m^2) of C. zealandica in different strata.

Strata			Visible damage					Undamaged margin				
Time	Generation	No	*r	Slope	± 95%	Intercept	± 95%	r	Slope	± 95%	Intercept	± 95%
March	1968-69	23	0.551	0.761	0.525	758.4	94.86	0.530	0.551	0.402	149.5	72.54
	1969-70	20	0.319	0.407	0.598	540.3	60.18	0.456	0.429	0.415	62.4	41.94
	All generations	43	0.342	0.583	0.514	658.6	75.90	0.439	0.487	0.319	115.9	46.73
May	1968-69	14	0.130	0.143	0.681	500.8	54.50	0.588	0.629	0.548	158.0	43.88
	1969-70	8	0.713	0.789	0.774	122.1	29.02	0.651	0.975	1.136	6.47	42.57
	All generations	22	0.360	0.732	0.885	277.3	63.32	0.639	0.839	0.472	80.6	33.72

Strata		Visible damage and undamaged margin					
Time	Generation	No	r	Slope	± 95%	Intercept	± 95%
March	1968-69	23	0.656	0.703	0.369	389.7	66.61
	1969-70	20	0.329	0.355	0.506	308.5	51.16
	All generations	43	0.568	0.568	0.346	341.1	51.10
May	1968-69	14	0.456	0.345	0.426	362.0	34.11
	1969-70	8	0.897	0.840	0.413	76.21	15.49
	All generations	22	0.500	0.787	0.633	194.6	45.03

* r = correlation coefficient

At an early stage in these studies the following became obvious.

- Grass grubs were so aggregated in the early part of their life cycle that in most areas of larval occurrence root pruning was so severe that visible pasture damage resulted.
- The severity of pasture damage in similarly sized patches in high or low populated plots or paddocks did not differ.

In fested paddocks were divided into three strata: areas damaged by previous generations, new areas of damage located on the margins of last season's damage and undamaged areas. Within these strata there was no marked difference in the extent of damage between large and small patches of damage. For this reason, in these studies, herbage sampling over each grass grub generation was confined to only two .40 ha paddocks or plots.

Study plots were stratified at the end of January before the new areas of damage became visible. Since most new damage occurred approximately within a 1 m margin of last year's damage, it was possible to position cages in this stratum before new damage became visible.

This method of sampling herbage was not completely representative as the .60 m wide margin surrounding the individual areas of pasture damage was included in the undamaged stratum. Measurements of autumn pasture production from and larval density under 20 x 20 cm quadrats extending from the outer edge of visible damage 40 cm into the centre of visible damage and 60 cm out into visibly undamaged pasture are given in Table 6-2. A significant depression in herbage production occurred 20 cm out from the outer edge of visible damage.

Estimates of the losses in pasture damage which were not accounted for by the described method were assessed for differently sized patches of damage ranging from 1 to 5 m in diameter.

Table 6-2 Density of C. zealandica larvae and pasture production (D.M. kg/ha) assessed from individual patches of damage in late autumn at distances out from the outer edge of visible damage.

Zone	Distance from O.E.V.D.*	No. of transects	No. /m^2	±95%	D.M. kg/ha	± 95%
Visible damage	20–40 c.m.	8	154.1	50.76	191	27.72
	0–20 c.m.	8	292.7	111.55	503	212.00
	O.E.V.D.					
Visibly undamaged area	0–20 c.m.	8	107.8	71.55	1350	450.88
	20–40 c.m.	8	0.0		1803	564.49
	40–60 c.m.	8	0.0		1739	552.63

* outer edge of visible damage

Pasture production from the previously damaged stratum, the newly damaged stratum and the peripheral 20 cm wide margin were weighted on the average proportion that each stratum contributed to that particular sized patch of damage. These weights were based on observations from six similar sized patches of damage. Calculations showed that the average loss in pasture production not accounted for in these studies, expressed as a percentage of the total loss in pasture production in 1 m, 2 m, 3 m, and 4 m diameter patches was 13%, 7%, 4% and 2.5%, respectively. Therefore, as there is such a large range in the size of damaged patches in grass grub damaged pasture it was concluded that the losses on a paddock basis, unaccounted for in these studies were well below 10%.