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FIELD ASSESSMENT OF A SEX ATTRACTANT

FOR CONTROL OF GRASS GRUB,

Costelytra zealandica (White).

A thesis

submitted in partial fulfilment

of the requirements for the degree

of

Master of Agricultural Science

in the

University of Canterbury

by

R.B. CHAPMAN

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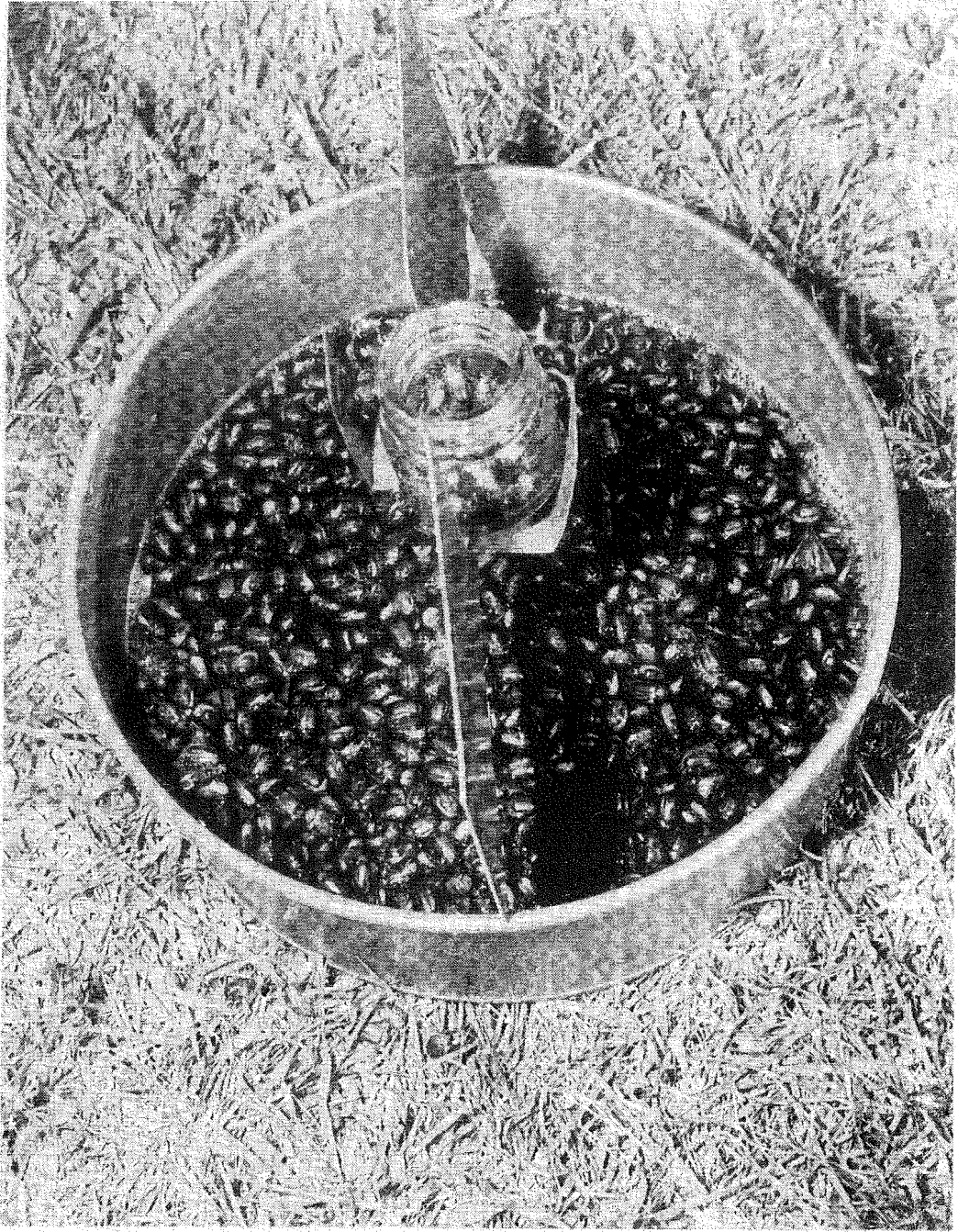
Abstract of a thesis submitted in partial fulfilment of
the requirements for the Degree of M.Agr.Sc.

FIELD ASSESSMENT OF A SEX ATTRACTANT
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Costelytra zealandica (White).

by

R.B. CHAPMAN

During the 1973 flight season of the grass grub beetle, *Costelytra zealandica* (White), an attempt was made to suppress populations on small scale field plots by mass trapping male beetles using simple water traps baited with the synthetic sex attractant, Durez 12687. Populations were monitored before, during and after the trapping period by sampling the subterranean eggs, larvae and adults. Trapping extended for three weeks during which time large numbers of beetles were captured and destroyed, however, populations in the immediate vicinity of the traps were not reduced, an outcome largely attributed to the massive immigration of male beetles on to treatment plots and low trap efficiency.



FRONTISPIECE

A typical catch of grass grub
beetles in a trap.

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CHAPTER I

INTRODUCTION

The grass grub, *Costelytra zealandica* (White), is recognised as a major pest of improved pastures in New Zealand. In the past control of grass grub has been almost exclusively directed at the larval stages, either by cultural methods or more commonly by the use of insecticides. DDT was the most widely used insecticide giving cheap and efficient control for many years, but with an increasing awareness of resistance and residue problems it was eventually banned in 1970. Since then alternative insecticides, mainly the organophosphorus compounds, have also demonstrated their problems: they are less persistent in soil, many are considerably more toxic to man and other animals, and they are more expensive to purchase and apply. Therefore it seems if efficient, economic and environmentally acceptable control of the larval stages is not assured by the present methods, then development of control strategies for other stages of the life cycle should be investigated.

Control of grass grub adults has been attempted by a number of methods, but to date, none have proven to be effective on a field scale. The discovery of a synthetic sex attractant for male beetles prompted enquiry into the feasibility of using this attractant in a manner similar to

those demonstrated in other parts of the world where sex attractants have been utilised successfully to manipulate and suppress insect pest populations [Hardee *et al.* (1970), (1971); Boyd *et al.* (1973); Roelofs *et al.* (1970)].

The main aim of this study was to determine if it was possible to remove sufficient males from a population using this attractant and traps, and so reduce the frequency of mating, resulting in a decline of the subsequent larval population.

CHAPTER II

LITERATURE REVIEW

INTRODUCTION

Sex attractants have been shown to influence the mating behaviour of many insect species. Shorey, Gaston and Jefferson (1968), Jacobson (1972) and Shorey (1973) have recently reviewed the topic. Depending on the origin of these attractants, they can be categorised into two groups; natural and synthetic products. Those of natural origin are now commonly referred to as sex pheromones, a term initially coined by Karlson and Lüscher (1959). Their nature and actions are specifically defined by Jacobson (1965) as, "chemicals which are produced by one sex of an organism to lure or sexually excite the opposite sex for the purposes of mating." A number of these sex pheromones have now been isolated and identified, but it has become apparent that some closely related compounds, not of insect origin, will also elicit a similar or identical response in the receptive sex. Knowledge of both these groups of compounds and their actions soon led to speculation that exploitation of this type of communication between the sexes may be of value in controlling some insect pests.

SEX ATTRACTANTS AND INSECT PEST CONTROL

Methods of Using Insect Sex Attractants

Literature describing sex attractants in insect pest control programmes show they may be used in a variety of ways. Shorey, Gaston and Jefferson (1968) classify them into two categories: survey and direct behavioural methods.

(i) Survey Methods - Survey methods merely involve the attractant as a population indicator; usually to ascertain the optimum time to apply a control agent.

(ii) Direct Behavioural Methods - These methods involve either releasing the attractant in moderate concentrations from an insect trap so as to outcompete the naturally produced sex pheromone (the mass trapping technique), or by releasing the attractant in high concentrations so that pheromonal communication between the sexes is prevented or greatly reduced (the confusion technique).

The two techniques, although singular in aim, are obviously quite distinct in the manner employed to minimize mating. While the mass trapping technique aims to remove as many as possible of one sex from the population before mating occurs, the confusion technique endeavours to prevent one sex from detecting the minute amounts of pheromone produced by the other without actually destroying any individuals in the population. The ultimate result in both is a decline in mating success.

Both techniques have been argued from theoretical standpoints. Knipling and McGuire (1966) contend that mass trapping can successfully reduce an insect population as long as there is a sufficiently high ratio of attractant to wild females in the population. Their models predict that boll weevil and codling moth can be controlled by mass trapping, but the combination of this and other control strategies, e.g. release of sterile individuals, has only a marginal effect on the degree of control expected.

With respect to the confusion technique, Wright (1965) suggests the lowest concentration of an odorous chemical needed to elicit a response in an insect is about 10 molecules per mm^3 . He also predicts the amount required to saturate the chemical receptors and block responses to mating behaviour would be about 10^5 molecules per mm^3 higher than the behaviour threshold. He therefore concluded, that with a chemical having a molecular weight of 200, the air would need to be permeated with 10^{12} molecules of pheromone per litre to have an effect.

The value of these theoretical predictions, while they demonstrate the basis of each technique, appear to be limited as, in each case, they involve relatively static situations with little regard for the effects of external modifying factors that occur in nature.

Field Use of Sex Attractants for Pest Control

Both the confusion and mass trapping techniques have been used successfully to reduce insect populations.

(i) Confusion Technique - Gaston, Shorey and Saario (1967) record the first successful attempt at disrupting pheromone communication between the sexes with the cabbage looper moth, *Trichoplusia ni* (Hubner). Synthetic sex attractant released from regular points over a small plot prevented males from locating virgin females caged in a centrally located trap. By comparison, males had no difficulty in locating females caged in traps on the control plot. Shorey *et al.* (1972) also disrupted sex pheromone communication between male and female cabbage looper moths and obtained up to 80% reduction in trapped males compared to catches on control plots.

Although both these instances demonstrate that sex pheromone communication can largely be inhibited in this species, there is no evaluation in either case of the effects this inhibition had on the mating success of the entire population for that generation, i.e. there was no post-treatment population assessment.

In experiments where post-treatment assessments have been carried out, reduction in numbers of the following generation have been achieved by disrupting pheromone communication between males and females of the parent

population. Shorey, Kaae and Gaston (1974) released hexalure in cotton fields to disrupt pheromone communication between adult male and female pink bollworms, *Pectinophora gossypiella* (Saunders), and gained reductions in the resultant larval population in the order of 83-93%. Likewise, Beroza *et al.* (1974) gained a marked reduction in the number of egg masses of gypsy moth, *Porthetria dispar* L., in areas treated with a microencapsulated formulation of disparlure, thereby demonstrating that the degree of confusion achieved was adequate to maintain populations of this pest at a low level.

These examples of the confusion technique show that pheromone communication between the sexes of some Lepidoptera species can be disrupted to such an extent to result in useful reductions of numbers in the following generation. From inspection of the methods used in these experiments, the success of this technique appears to be largely determined by the incidence, volatility, longevity and concentration of the attractant sources in the field.

(ii) Mass Trapping Technique - Mass trapping with sex attractants for insect pest control has been convincingly demonstrated with the boll weevil, *Anthonomus grandis* Boheman, and the red banded leaf roller, *Argyrotaenia velutinana* Walker.

The first attempt at control of boll weevils by Hardee *et al.* (1970) aimed at reducing the numbers of overwintering

weevils which emerge in June, July and August in West Texas. Traps placed around the boundaries of cotton fields baited with live males (male produced pheromone) caught sufficient numbers of overwintering females to suppress the populations from April until early August, a period which would normally require insecticide treatments. Hardee *et al.* (1971) followed these experiments with a more extensive trapping programme involving 625 ha and by the end of August no weevils or oviposition punctures were found in over half the fields treated. The largest single attempt at mass trapping boll weevils involved 30,375 ha in West Texas where Boyd *et al.* (1973) used 26,521 traps baited with live males from April to July. Boll weevils were not detected until early July, thus indicating that trapping had successfully suppressed the population in the early and middle parts of the growing season.

Successful sex pheromone trapping of the red banded leaf roller has also been demonstrated on a number of occasions. Although Roelofs *et al.* (1970) trapped approximately 90% of the males estimated to be present in a moderately high population, control was not achieved, as an analysis of the fruit samples showed 32% to be damaged. However, with a relatively low leaf roller population, two traps per tree caught sufficient males to result in negligible fruit damage. Trammell, Roelofs and Glass (1974) conducted a more extensive red banded leaf roller trapping programme in two orchards over three years. Using traps baited with a mixture of dodecylacetate and RibLuRe, over

the three years fruit injury levels were maintained at an average of less than 1% compared with over 9% in control areas. Taschenberg, Cardé and Roelofs (1974) also substantially reduced red banded leaf roller damage in two vineyards. Mass trapping on these occasions compared favourably with normal insecticide treatments and the percentage of fruit damage was well below that of the control plots.

The striking features of these mass trapping programmes are that they are conducted over relatively large areas or relatively contained agro-ecosystems, e.g. orchards; they involve large numbers of traps which invariably require regular maintenance and appear to be more successful at lower pest population densities. Under these conditions, mass trapping can, like the confusion technique, result in useful reductions of insect pest populations. Mass trapping may also have some advantage over the confusion technique in that the quantities of attractant used and wasted when the insects are not active may be less and the removal of one sex could reduce feeding damage if the adults are active feeders, e.g. boll weevils.

ATTRACTANT STUDIES WITH GRASS GRUB BEETLES

Investigations into chemical attractants for New Zealand adult grass grubs, *Costelytra zealandica* (White), were initiated following observations that they were strongly attracted to the common elder bush, *Sambucus nigra* L. (Osborne and Hoyt, 1968). They found elder flowers to be more attractive than elder leaves, and, in particular, the first few millilitres of a steam distillate were highly attractive to female beetles.

Osborne and Hoyt (1969) also screened a number of chemical compounds that have been reported to attract the adults of other Scarabaeid species. Those compounds tested were eugenol, geraniol, citronellol, valeric acid, caproic acid, sorbic acid, n-propyl sorbate, anethol, isoeugenol, limonene and 2-methoxy ethanol. However, none of these compounds proved to be attractive to the beetles.

From a parallel study to determine what part, if any, site or contact played in the attraction of males to females, Osborne and Hoyt (1969) found that a commercially available adhesive (Pliobond, manufactured by the Goodyear Tyre and Rubber Co., Akron, Ohio, U.S.A.) was highly attractive to the males of this species. Newly emerged virgin females that had been extracted with diethyl ether and ethanol, and dead and thoroughly dried beetles of both sexes attached to the elder with this adhesive were rapidly surrounded by males, and attempts at mating were observed. Traps

subsequently baited with small amounts of dried adhesive also attracted and caught only male beetles.

Some constituents of this adhesive and other related compounds supplied by the manufacturer were tested in 1969 (Osborne and Hoyt, 1970). One particular component, a thermosetting phenol-formaldehyde resin, Durez 12687, proved highly attractive to male beetles. Although this compound is not present in Pliobond, a related compound which was less attractive is thought to be present in this adhesive and could account for its activity. From chemical studies and what is known of the resin, Osborne and Hoyt (1970) associated its activity with the phenol content, although several other phenolic compounds are also present.

Osborne and Hoyt (1970) showed that traps baited with Durez 12687 consistently caught more beetles than those baited with Pliobond, while attempts to compare the relative attractant activities of phenol crystals and the resin were not clear, although on several occasions the resin attracted more beetles than did pure phenol. A concentration effect was suggested to be the cause of fewer beetles being caught in the phenol baited traps as many beetles were seen to congregate some distance from the traps. Osborne and Boyd (1975) have since suggested the presence of an odour synergist in this resin may be responsible for the higher catches obtained with Durez 12687 than with phenol or aqueous phenol solutions. Henzell (1970) also tested a number of phenolic compounds as attractants for male grass

beetles. They were phenol, 1-naphthol, 2-phenyl phenol, 2-cresol, 4-cresol, guaicol, vanillin, thymol, carvacrol, catechol, resorcinol, quinol, pyrogallol, 1,2-dihydroxy-3 allyl benzene and 1,2-dihydroxy-4 allyl benzene. Of these compounds only crude phenol attracted male beetles. Aqueous phenol solutions also proved to be attractive with concentrations ranging from 1-480 ppm catching approximately equal numbers.

Fenemore, Read and Esson (1971) studied the relative attractant activities of two samples of Durez 12687, varying quantities of Durez 12687 per trap, acetone solutions of Durez 12687 deposited on glass beads, granules containing 0.1% Durez 12687 and various concentrations of aqueous phenol. One sample of Durez 12687 apparently attracted more beetles than did the other sample and this is almost certainly to be due to the different moisture contents (Osborne, pers. comm.). A comparison of the quantities of Durez 12687 per trap showed that 2 mg per trap attracted significantly fewer beetles than did 10, 50 and 250 mg per trap on some nights, but this trend was not repeated on other nights. Ten milligrams of Durez 12687 proved to be more attractive than glass beads coated with Durez 12687 and was also more attractive than the 0.1% formulated granule. The granules were less attractive than the glass beads. A comparison of 50 mg Durez 12687, 40 and 400 ppm aqueous phenol per trap showed that on most nights the resin attracted more beetles than did the aqueous phenol solutions. This is consistent with the earlier observations

of Osborne and Hoyt (1968) and more recently of Boyd (1975) where Durez 12687 baited traps caught approximately 18 to 20 times the numbers of beetles caught in traps baited with aqueous phenol or phenol crystals. Moist Durez 12687 is considerably more attractive than the dry resin powder. Boyd (1975) demonstrated that traps baited with 0.01, 0.1, 0.3 and 0.8 ml water per 100 mg Durez 12687 caught an average of 95, 163, 285 and 209 beetles per trap, per night respectively.

Penemore, Read and Esson (1971) in field tests with repeatedly run traps baited with Durez 12687 on plots in Canterbury with traps at 10 m and 25 m spacings, and plots in Nelson at 10 m spacings caught a total of 45,601, 18,763 and 3,539 beetles respectively. Actual numbers expressed as a percentage of the estimated number of available males were 3.43% (25 m spacings) and 19.8% (10 m spacings) from the two Canterbury plots. From these trials it was concluded that insufficient males had been removed from the population to suppress mating and to cause a decline in the subsequent generation. This was substantiated by sampling the larval populations at a later date.

A further study by Read (1975) during the 1972-73 flight season where the traps were arranged at 1 m intervals in large field cages showed a substantial decline in larval numbers of the following generation in treatment cages; 0.86 larvae per spade square sample compared with 13.78 per spade square sample in the control cage.

Observations on the behaviour of the male beetles to Durez 12687 are also described by Fenemore, Read and Esson (1971). Glass headed pins coated with minute amounts of Durez 12687 placed in field observation cages attracted only a few male beetles, and obviously did not adequately mimic the female form as on emergence of a female the pins were rapidly abandoned. Durez 12687 broadcast at a rate equivalent to 2 kg/ha on the floors of these cages also failed to prevent the males from locating females, however, at a rate equivalent to 20 kg/ha mating was largely suppressed although the effect wore off after three days.

Henzell and Kain (1972) also tested the confusion technique but in this case on a field scale as a possible control method. In these experiments aqueous phenol solutions contained in 3.2 mm diameter PVC tubes each 64 m long and spread over the pasture surface at 1 m intervals released phenol at an average rate of 5 g/h/ha. Release of phenol at this rate did not prevent sexual communication between the sexes. Males were however completely disorientated on small plots when the release rate of phenol was considerably higher and in this instance liquified phenol was placed in open trays spaced 9 m apart.

The latter part of the preceding review shows that attempts to control grass grub on a field scale by both the mass trapping and confusion techniques have failed. Possible reasons for their failures may be as follows.

(i) Confusion Technique - In the experiment involving the release of phenol from PVC tubes, it appears that sufficient phenol may not have been released to disrupt pheromone communication. This was substantiated in the same study when phenol was released in much greater quantities from open trays, although this apparent disruption or confusion may have in fact been a repellent reaction to high concentrations. The release of phenol alone may not be sufficient to result in complete inhibition of pheromone communication between the sexes. Multi-component attractants are known for a number of insect species and it may be possible that the odour synergist demonstrated by Osborne and Boyd (1975) is essential in this situation. The suppression of mating with Durez 12687 in small cages where the phenol release rate would be much lower also supports this assumption. However, until the effects of the individual components of this resin are known, discussion of this point can only be speculative.

(ii) Mass Trapping Technique - The trapping trials by Fenimore, Read and Esson (1971) showed that too few numbers of males were withdrawn from the population to have an effect on the overall mating success, i.e. the incidence of traps and attractant and/or the efficiency of the traps were insufficient in relation to the density of the test population. The success of a higher density of the same traps was demonstrated by Read (1975), however the trial was not replicated.

GRASS GRUB ADULT BEHAVIOUR AND BIOLOGY

Because sex attractants often have a profound effect on the sequence of events leading up to mating, a review of aspects of normal mating behaviour provides a base line for the interpretation of their effects.

The life cycle and biology of *C. zealandica* have been reviewed by Dumbleton (1942), Miller (1945), Kelsey (1951) and Pottinger (1968). There is usually one generation per year with three subterranean larval instars, the adults being the only stage to emerge above the surface. The sex ratios of adult males to females does not usually differ from 1:1 (Kain, 1968).

Flights

Tenereal beetles are soft and whitish in colour and remain in the soil until the integument hardens (Miller, 1921). Adults can be found in the soil from September to February (Kelsey, 1951), and normally start emerging during October, although there can be considerable variation. Miller (1921) records that beetles may begin emerging as early as September and may be found flying as late as May, but these are exceptions.

In Canterbury the flight season normally begins mid-October, with peak numbers between 15-24 November and may continue into February (Kelsey, 1951). The flights commence at Manatuke and Rukuhia in mid-October and one week later at Wairekei, Tangoio and Winchmore and two weeks

later again at Invermay (Helson, 1967). He also states that seasonal variations may account for delays in the flights starting by 7-10 days. Kain (1968) records flights starting in October around Hamilton with the development there being one month ahead of Taupo. Development may also vary between different aspects and soil types by up to two weeks. Flights at Redwoods Valley, Nelson, commence in late October and continue until early December with most flights occurring between 1-20 November (Farrell and Wightman, 1972).

Delays in the commencement of beetle flights throughout the country therefore appear to vary by up to one month, a trend concomitant with increasing altitude and latitude.

Most beetles emerge within the first hour of darkness, although Kain (1968) found that 25% of a population near Hamilton emerge between 1 a.m. and 6 a.m. In Canterbury, Kelsey (1968b) records flights commencing on most nights between 8.15 and 8.45 a.m. and ceasing at around 9.20 p.m. Duration of the flights varies from 7-48 minutes, with an average of 28.5 minutes (Kelsey, 1968b). Fenemore and Perrott (1970) investigated the duration of the periods of primary emergence and found that 95% of all beetles emerged within 19-23 days, depending on location. Males tend to develop and emerge earlier in the year than females (Kelsey, 1951), yet Fenemore and Perrott (1970) found no evidence of this from soil samples and concluded that there was probably a behavioural difference between the two sexes in this respect.

Only the beetles that emerge in the first hour of each night fly (Kain, 1968). Males emerge before females each night and, as a result, most females are mated as soon as they emerge (Kelsey, 1951). Net collections above the pasture surface in the first five minutes of a flight usually contain less than 2% females, while collections through the pasture sward comprise about 25% females (Kelsey, 1951). If females are not mated as soon as they emerge they invariably take to wing. Kain (1968) found that males tend to fly closer to the ground in search of mates, moving over the pasture in a random manner, while females tend to fly in a more directional manner to feeding sites. Farrell and Wightman (1972) also observed the two types of flight. Kain (in East, 1972) determined that beetles may fly up to 275 m from pasture to feed trees and back.

Flights usually only occur if wind speeds are below 4.5 m/sec, there is either little or no rain, and grass temperatures are above 9.4°C (Kelsey, 1951). However, Kelsey (1951) records that beetles will emerge under more adverse conditions and a few may even fly. At wind speeds below 0.9 m/sec beetle flight is random, but above that speed they generally fly into the wind (Kelsey, 1968b).

Mating and Oviposition

Beetles are sexually mature on emergence and mating usually takes place on the pasture surface, or in the soil (Kain, 1968; Fenemore and Perrott, 1970), or on feed trees (Kelsey, 1951). Males are attracted to females by a sex

pheromone (Kelsey, 1966), which is produced by symbiotic bacteria in the collateral glands (Hoyt, Osborne and Mulcock, 1971). Henzell *et al.* (1969) reportedly have isolated and identified phenol as the natural pheromone.

Once females break through on to the surface, they usually remain stationary and if left undisturbed are mated within a few minutes. Males however, tend to fly immediately after emerging (Fenemore and Perrott, 1970). Females that are not mated fly to feeding sites where mating will invariably take place (Kelsey, 1951). Kelsey (1967) states that females do not fly on the nights they are mated.

Eggs may be laid 2-16 days after mating (Kelsey, 1951; Miller, 1945; Kain, 1968). Kain (1968) found up to five ovipositions may occur, but two are usual, with 72% of the eggs laid in the first and 94% laid by the second oviposition. Radcliffe and Payne (1969) found a range of 4-32 eggs per cluster for the first oviposition and 2-16 eggs per cluster for the second. Feeding is necessary for the subsequent ovipositions and many female beetles fly to feeding sites after they have laid their first batch of eggs (Kain, 1968). A single mating is sufficient to fertilise all eggs a female produces. Eggs are laid in the soil 8-18 cm below the surface depending on soil moisture (Kelsey, 1951).

Kelsey (1957, 1968a) examined the preference for oviposition sites and found covered ground was preferred to bare ground, with apparently no discrimination for different types of ground cover. Radcliffe and Payne (1969) also

found no preference in pot experiments ^s as did Radcliffe and Kain (1971) with emergent beetles reared *in situ*.

The longevity of beetles in the field has not been accurately determined. Fenimore (1966) suggests 2-3 weeks.

CHAPTER III

MATERIALS AND METHODS

INTRODUCTION

Mass trapping experiments with grass grub beetles were carried out over two flight seasons during 1972 and 1973. In 1972, non-replicated field experiments of a preliminary nature were carried out to test the effect of various trap densities and layouts on trapping performance and to establish the optimum numbers of samples required to achieve reliable estimates of larval densities. The results of trapping are reported briefly here as a basis to the design of experiments for 1973.

Comparison of preliminary pre-treatment and post-treatment sample data showed a reduction in larval densities was achieved on only one occasion. On a plot where traps were spaced at 10 m intervals arranged in a grid pattern, numbers of larvae decreased by 69% from one generation to the next compared with a decrease of 55% in the control plot. Where traps were arranged at 20 m intervals, populations increased, but remained relatively unchanged on a plot where traps were arranged around the periphery of damaged areas of pasture. The conclusions drawn from these experiments were that insufficient male beetles had been removed from the plots to have a substantial negative effect on the success of mating and more frequent and intensive population assessment would be desirable.

During the 1973 flight season an attempt was made to remove more males from the population by increasing trap density, since it was considered impracticable to increase trap efficiency. Two criteria were primarily considered in the design of these experiments. First, there was a limit to the number of traps that could be operated by one person and also the number of samples that could be taken to monitor the experiments. Second, there had to be sufficient replication to average the effects of variation in grass grub density, of aspect and exposure of the sites.

The experiments carried out during 1973 are the main content of this thesis. They involved pre-sampling of larval populations during September and October, trapping the male beetles in the three weeks during which the majority of beetles fly, and post-sampling the eggs-first instar larvae (December) and the third instar larvae (September and October, 1974) to assess the effects of trapping. In addition, the adults were sampled at intervals during the flight season to determine their numbers and sex ratios.

PLOTS

Location

The experiments during 1973 were carried out on the properties of R. Clark (hereafter, Site I) and D. Rountree (hereafter, Site II) in the Staveley and Alford Forest districts of the Ashburton County, Canterbury, at

approximately 367 m above sea level and 96 km southwest from Christchurch. Sites I and II were 12.5 km apart by road.*

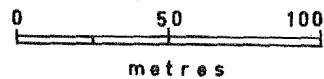
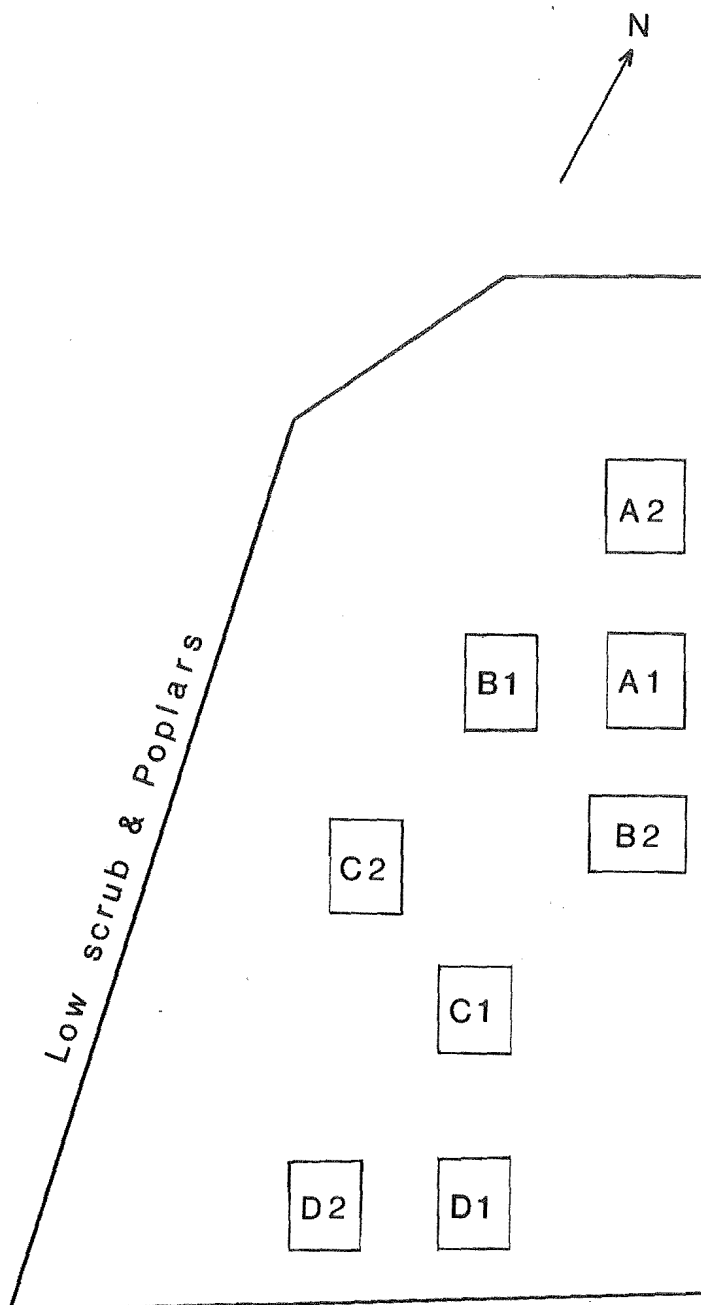
Size and Number of Plots

The plots, 30 x 25 m (0.076 ha) in size, were sited on areas of the paddocks with high and low grass grub populations by visually assessing the degree of pasture damage. Control and treatment plots were kept as far apart as conditions dictated (minimum 20 m) with care being taken to locate control and treatment plots of any one replicate on the same soil type and in an area with the same degree of exposure. Figures 3.1 and 3.2 outline the layout of plots at the two sites. Site I was in an exposed position with only low bushes and a few poplar trees on the western boundary, whereas Site II was more sheltered, being surrounded on all sides by shelter belts of pines and spruces, 6 to 18 m high.

At each site a pair of plots, one with traps and attractant, and the other without attractant as a control, were replicated twice each on areas of high and low grass grub population densities, i.e. a total of eight plots at each site.

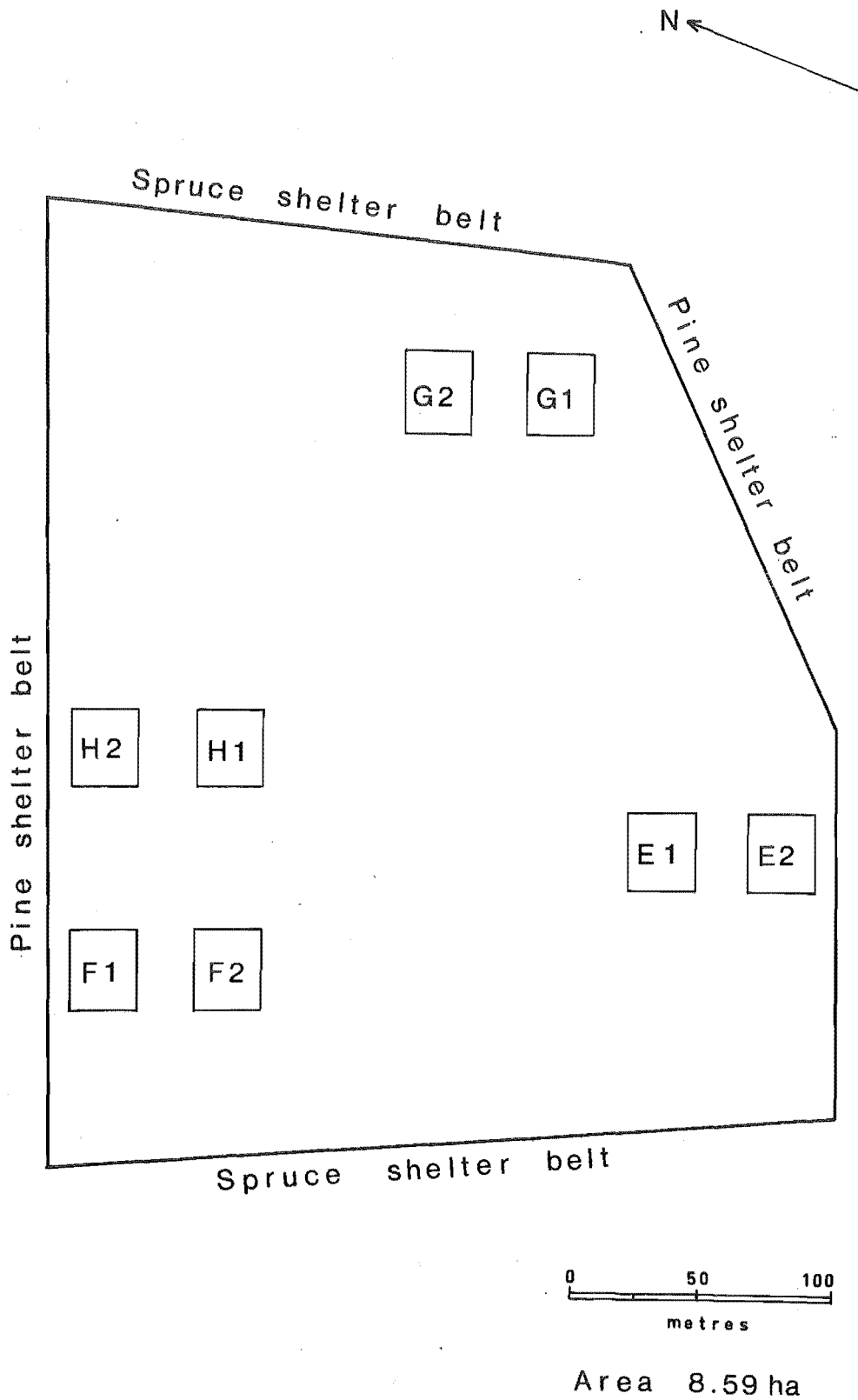
* Footnote: The author lived at Site II during the 1973 flight season and maintained the experiments with an assistant at Site I.

FIGURE 3.1 PLOT LAYOUT SITE I.



Area 4.86 ha

FIGURE 3.2 PLOT LAYOUT SITE II.



SAMPLING

Sampling Plans

A pre-treatment sample taken prior to trapping to determine the population present and two post-treatment samples to assess the effects of trapping were taken from all plots at both sites. Sampling of the adults during the trapping period was confined to plots at Site II. The type of sampling plan and the number of samples per plot for each stage are summarised in Table 3.1

Table 3.1

Summary of Sampling Plans

| STAGE SAMPLED | TYPE OF SAMPLE PLAN | NO. SAMPLES PER PLOT | DATE SAMPLED |
|--|---------------------|----------------------|----------------------|
| Third instars (Pre-treatment) | Random | 40 | Sept. - Nov. 1973 |
| Adults (During treatment) | Random | 20 | Nov. - Dec. 1973 |
| Eggs-first instars (Post-treatment) | Stratified | 15 | Dec. 1973 |
| Third instars (Post-treatment) | Random | 40 | Sept. - Oct. 1974 |

Sample Unit Size

Because of the stoney nature of the soils, particularly at Site II, soil corers commonly used for sampling soil insects were unable to be used, therefore 15 x 15 cm spade square

samples taken to a depth of 15-25 cm were used for sampling all stages throughout the experiments.

Selection of Sample Positions

(i) Third Instars - Forty randomly selected samples per plot at each sample period were located by pacing out two randomly selected co-ordinates.

(ii) Adults - Adult samples were similarly selected by pacing out two randomly selected co-ordinates, however, samples were only taken from the central portions of the plots within a 20 x 15 m rectangular area on all plots at Site II, this being the area covered by the traps on the treatment plots (See Fig. 3.3). This procedure reduced the number of samples and allowed all the plots to be sampled in one day. Also, the area around the traps but within the boundaries of the plot was considered to be a buffer zone, thus the adults sampled within the area of the traps are more likely to give a truer indication of the effect of trapping.

(iii) Eggs-First Instars - As the sorting of soil samples is an extremely time consuming process [East (1972) records 17.9 minutes per spade square sample using a flotation, wet sieving technique] a sampling plan with fewer samples and greater precision than a random plan was considered necessary as time and labour were limiting factors.

Grass grub populations generally have clumped distributions because the female lays most of her eggs close to the point of initial emergence. As the areas of grass grub damage were readily detected in the field in December, the following sampling plan described by French (pers. comm.) was used for eggs-first instars. A transect of five samples across each of two areas of grass grub damage with an additional five random samples were taken from each plot. The transect of five samples across a damaged area included two samples from just inside the area and one sample from the centre of the damaged area. The five random samples were taken anywhere in the plot where there was no visible damage.

Collection and Sorting of Samples

(i) Third Instars - All pre-treatment larval samples were collected into pre-labelled plastic bags and transported to Lincoln College for hand sorting. Hand sorting was usually completed within five days of sampling thus enabling the damaged larvae to be readily detected. Numbers of second instars, third instars and pupae were recorded. To check on the efficiency of hand sorting, as a number of persons were sorting samples, 5% of all pre-treatment samples were re-checked by the flotation, wet sieving technique described for the egg-first instar samples. East (1972) found that 99.6% of undamaged and 96.4% of damaged larvae could be recovered by this method. As the hand sorting of soil samples with a number of operators proved satisfactory

(only 3.7% of the total number of larvae were missed), post-treatment third instar samples were all hand sorted in the field.

(ii) Adults - All samples were sorted in the field and the adults placed into pre-labelled plastic bags, sealed and placed in a freezer immediately at the conclusion of sampling for that day. Their numbers and sex were to be determined at a later date.

(iii) Eggs-First Instars - All samples were collected into pre-labelled plastic bags and transported to Lincoln College for sorting by the flotation, wet sieving technique described briefly here but in detail by East (1972). Eggs and first instars were floated from the soil in a saline solution (density 1.2 - 1.3) and separated from the soil organic matter by a series of sieves. Soil samples were held in a cool store at 5.6 - 7.2°C and processed within six weeks. Numbers of eggs and first instars were recorded and in addition, any third instars or adults from the preceding generation.

TRAPPING

Trap Design

Traps used were of the type designed by Osborne and Hoyt (1968) consisting of a 20 cm diameter tin, 8 cm deep with a single vane 20 x 9 cm having a slot to accommodate a

25 ml glass jar that contained the attractant (see Frontispiece). The traps were half filled with water and detergent (a few drops of detergent per 20 l water) to trap and kill the beetles. All traps were painted red, a colour previously shown to have no influence on the beetles.

Trap Placement

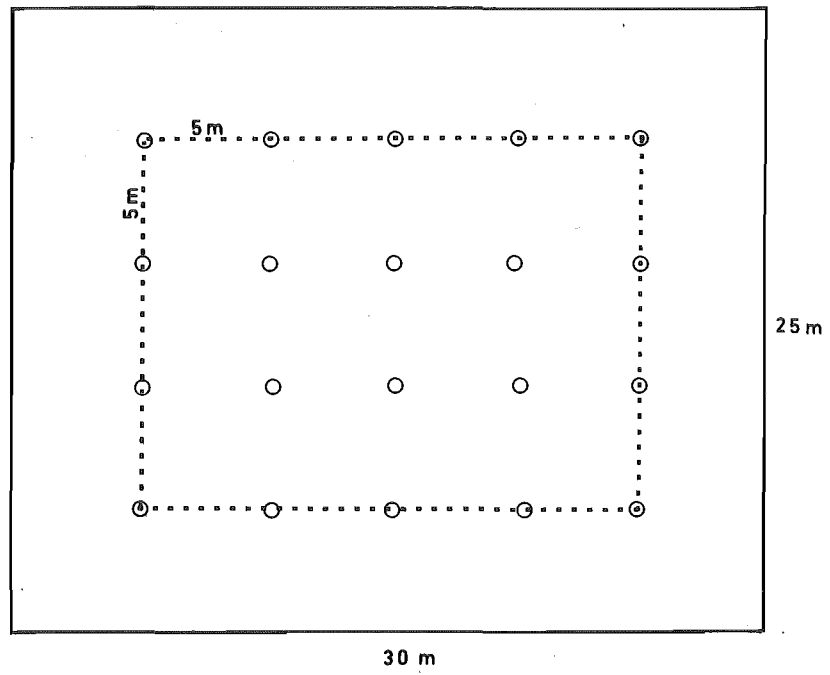
Twenty traps per treatment plot and four traps per control plot were allocated. This is a total of 192 traps and the maximum number that could be operated by one person with an assistant. Traps were placed on the pasture surface at 5 m intervals in a 5 x 4 grid arrangement (Figure 3.3) on the treatment plots A1, B1, C1, D1, E1, G1 and H1 and four in a single row in the centre of the control plots A2, B2, C2, D2, E2, F2, G2 and H2.

The Attractant

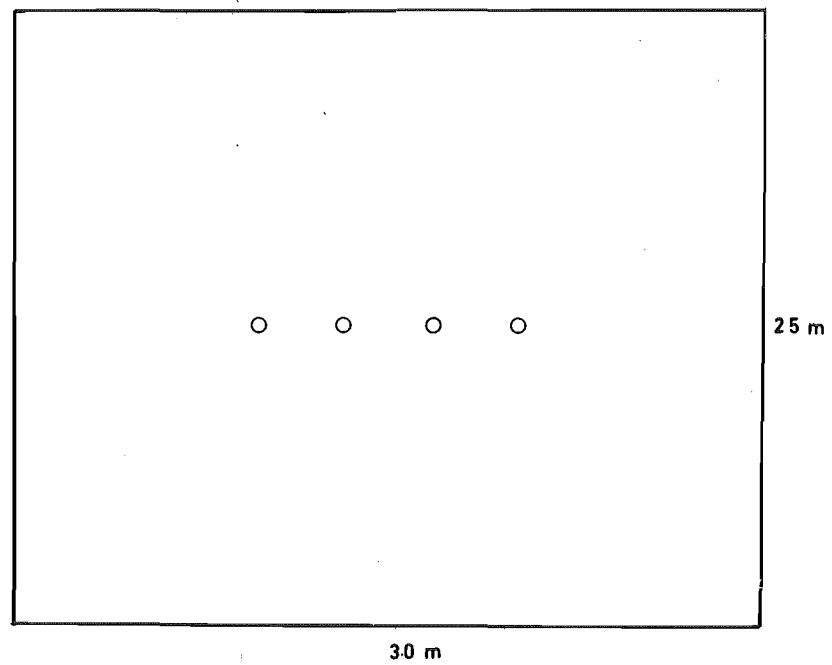
Approximately 100 mg of the phenolic resin, Durez 12687, moistened with 0.3 - 0.4 ml water was placed in the glass jars in the traps on treatment plots each evening. The amount of water is fairly critical as Boyd (pers. comm.) found that 0.3 ml water per 100 mg resin caught more beetles than wetter or dryer resin. The resin, a fine brown powder, was measured out into each jar with a shallow spatula and water added from a graduated 2.0 ml plastic syringe. By this method the amounts of water and resin could be measured out relatively quickly. The jars were then capped and remained so until just prior to the beetle flights each night.

FIGURE 3.3 TRAP LAYOUT ON PLOTS.

Treatment



Control



Trap Operation

Traps were replenished with water and the jars containing attractant placed in the traps in the late afternoon of each day. Half to one hour before the start of the beetle flights the lids on the attractant jars were removed. After the flights each night the beetles were collected by tipping the contents of each trap into a plastic food cullender suspended over a 10 l plastic bucket to separate the beetles from the water. The beetles were transferred into labelled plastic bags with the contents of each trap being placed in a separate bag. The water and the jars containing the attractant were returned to the trap and left set to determine if any male beetles were flying later in the night, and to this end, the traps were inspected on occasions in the early morning.

Estimation of Beetle Numbers

The numbers of beetles caught in the traps were estimated by weight. Each day, bags containing beetles were weighed on a quick weigh balance, the beetles tipped out and 3 out of 20 bags weighed for an average bag weight as the amount of water in the bags was variable. Similarly, three 10 g samples of beetles from the four plots at each site were counted each day to obtain an average number of beetles per unit weight. Numbers of beetles per 10 g varied, at maximum, \pm 5% of the means.

Method of Estimating Trap Efficiency

In previous attempts at trapping grass grub beetles with traps of this design, numerous beetles were often observed around the traps on the pasture surface. An attempt to quantify the numbers of beetles in the immediate vicinity of the traps was carried out by the following method.

Twelve, 10 x 10 cm by 7.5 cm deep plastic pots were arranged around a single trap on control and treatment plots at Site II as detailed in Plate 3.1. Pots were set into the ground level with the soil surface and 10 cm apart in each of four directions. Each pot or pit trap was half filled with water and detergent to trap the beetles. Each night after the flights, lids were placed on the pots to prevent birds from eating the beetles in the early morning. The numbers of beetles caught were counted the following day.

METEOROLOGICAL DATA

Air temperature, humidity and barometric pressure were recorded continuously throughout the duration of the trapping period on a recorder positioned in a Stevenson screen adjacent to plot E1 at Site II. In addition air temperature, grass temperature and soil temperature at a depth of 5 cm were recorded on individual thermometers at 8.30 p.m. each night. Per cent relative humidity was also recorded at 8.30 p.m. by a whirling hygrometer. Wind speed was recorded by a hand held Birams' vane anemometer at 1 m



Plate 3.1

Arrangement of pit traps around a trap to estimate trap efficiency.

above ground level and wind direction noted. Wind speed and direction were recorded at Site I as this was the more exposed site. Three one-minute recordings taken between 8.30 and 9.00 p.m. were averaged. A rain gauge was also operated adjacent to the continuous recorder at Site II. Meteorological data recorded for the 1973 trapping period is summarised in Appendix XI.

SEXING ADULT BEETLES

Grass grub beetles can, with practice, be sexed relatively quickly by the method described by Kelsey (1965) using the following characters.

| | <u>Male</u> | <u>Female</u> |
|--|-------------|---------------|
| Shallow depression in the centre of the 6th sternite | Present | Absent |
| Sulcus each side of centre; markedly convex posterior margin at the centre of the 6th sternite | Present | Absent |

Kelsey (1965) concluded that the first of these characters is the most conspicuous and reliable. Beetles in this study were sexed by this method with the first few beetles examined being dissected to confirm their sex.

Fenemore (1971) claims that the reproductive status of the adults can also be determined by dissection and assessment of the reproductive structures, however, as the internal

condition of the beetles stored in the freezer had deteriorated over several months, meaningful analysis of the mated status of adults was not possible in this study.

STATISTICAL ANALYSIS OF RESULTS

Test for statistical differences between treatments, i.e. trap and control plots, were carried out by analysis of variance. A split plot design was used because an analysis of this type permits more precise information to be obtained on the factor allocated to sub-plot treatments at the expense of losing some information on the factor assigned to main-plot treatments (Sokal and Rohlf, 1969).

In these experiments high and low population levels are designated as main-plot treatments and trap and control plots designated as sub-plot treatments. Therefore, in an analysis of this type, differences between high and low population areas as well as between trap and control plots and their interaction can be determined statistically. Where the analysis of variance (F-test) indicated the means of the variables were significantly different, a least significant difference (LSD) test was performed to determine which pairs of means differed significantly.

Pre-treatment third instar counts, post-treatment third instar counts and egg-first instar counts were all analysed in this way by computer. The programme held in the Lincoln College library is designated L/SPLIT.

CHAPTER IV

RESULTS

TRAPPING

Trapping started two or three days after the first few beetles had started to fly (November 20, 1973) and continued for three weeks. Trapping ceased when catches in the traps were approximating those at the start of the flight season. Flights commenced on most evenings between 8.30 and 9.00 p.m., i.e. when it was almost totally dark, and finished 20-30 minutes later. Beetles were caught on all nights during the trapping period except on November 25 at both sites and December 1 at Site II. On these occasions heavy rain was falling and air temperatures were the lowest recorded, being 7.5°C and 8.8°C respectively (see meteorological data in Appendix XI).

Mean numbers of beetles caught and the mean catch per trap, per night are recorded in Table 4.1. Total catches from each plot on each night appear in Appendices I and II.

Inspection of Table 4.1 shows that more beetles were caught in traps on treatment plots at Site II despite the higher initial populations at Site I (see Tables 4.4 and 4.5). Catches in traps placed on areas of high population were not markedly higher from those on low population areas at either site, once again despite differences in initial populations and presumably emergent adult males.

Traps on control plots without attractant caught very few beetles with catches being fairly similar at both sites and on high and low population areas.

Catches in the traps at any one plot were usually not uniform on any one night as the outer row traps tended to catch more beetles than did inner row traps. The mean catch per trap, per night over the whole trapping period for inner and outer row traps are recorded in Table 4.2. A comparison of paired means by Student's t-test showed that the differences between inner and outer row traps to be significant at the 0.05 level at Site I and the 0.01 level at Site II.

ESTIMATION OF TRAP EFFICIENCY

Per cent trap efficiency may be defined as follows.

$$\% \text{ Efficiency} = \frac{\text{Nos. caught in trap}}{\text{Nos. caught in trap plus nos. landing in area around trap}} \times \frac{100}{1}$$

Mean numbers caught in pit traps and numbers caught in traps surrounded by pit traps from six nights and four replicates each night on control and treatment plots at Site II are presented in Appendix III. To determine nett numbers, i.e. to discount those beetles which emerge in the immediate vicinity of the baited traps, pit trap catches from control plots were subtracted from treatment plot pit trap catches.

Calculated mean numbers of beetles landing in the pit trap zones around the traps appear in Table 4.3. From Table 4.3, per cent trap efficiency is estimated to be 33%. Thus, on average, only 33% of the beetles coming to within 0.5 m of the attractant source were caught or conversely, 67% escaped.

ASSESSMENT OF THE EFFECTS OF TRAPPING

Pre-treatment Third Instar Counts

Mean numbers of third instars per m² for Sites I and II are presented in Tables 4.4 and 4.5 respectively. Sample means for individual plots and analysis of variance summaries appear in Appendices IV and V.

As pairs of control and treatment plots were selected by assessing the degree of pasture damage, it was necessary to determine if there were any differences between population densities prior to trapping. Ideally, pairs of control and treatment plots should have comparable populations, i.e.

$$\bar{x}_1 = \bar{x}_2.$$

At Site I, treatment plot means slightly exceeded control plot means at both population levels but the contrary was found at Site II. The F-test showed a significant difference occurred between control and treatment plots at Site II when data from the two population levels are pooled, however, this difference is not significant when the population levels are considered separately (inspect LSD values). There was also

a significant F-test between control and treatment plots at Site I, however, the LSD values indicate this difference was significant only between high population plots.

Egg-First Instar Counts

Mean numbers of eggs-first instars per m² sampled on December 19 and 20 are presented in Tables 4.6 and 4.7. Sample means for individual plots and analysis of variance summaries appear in Appendices VI and VII.

The significant aspects of these tables are the greater numbers of eggs-first instars found on treatment plots compared with control plots and the higher overall count at Site I compared with Site II.

As the raw data invalidated the assumptions of analysis of variance, e.g. variance was not independent of the mean, tested by plotting variances vs. mean (Snedecore and Cochran, 1969), the data was transformed as recommended by Bartlett (1947) where $y = \sqrt{x + \frac{1}{2}}$. A significant difference between control and treatment plots was demonstrated only for Site II, the LSD test indicating that this difference was significant only at the high population level.

Post-treatment Third Instar Counts

Samples taken in Spring the following year include both second and third instar grubs as most would have resulted from eggs laid by the adults of the previous generation.

Mean numbers of third instars per m^2 for Sites I and II are presented in Tables 4.8 and 4.9 respectively. Sample means for individual plots and analysis of variance summaries appear in Appendices VIII and IX.

Greater numbers of third instars were found on treatment plots compared with control plots at both population levels, but the difference was only found to be significant at Site I. The LSD test indicates the difference was significant only between high population plots.

Adults Sampled at Intervals During the Flight Season

At Site II, 20 samples per plot were randomly selected and beetles counted on November 20, 26 and 29, and December 3 and 6. These beetles were later sexed and the mean numbers of males and females recorded in Appendix X. Figure 4.1 outlines the trend of mean numbers of males and females on high and low population areas.

Populations of males were maintained at relatively low numbers throughout the trapping period ($10-20/m^2$), whereas females reached peak numbers ($40/m^2$) around November 29. There was very little difference in numbers between high and low population areas or between control and treatment plots.

To determine by what factor number of males and females differ, ratios of males to females on control and treatment plots and on high and low population areas are recorded in Table 4.7. From these ratios no large differences are

apparent, but there are slightly fewer males compared with females on high population areas, whereas on low population areas, fewer males compared with females occur on control plots.

Table 4.1

Beetles trapped and the mean catch/trap/night during
the 1973 grass grub beetle flight season.

| SITE | POPULATION LEVEL | TREATMENT (20 traps) | | CONTROL (4 traps) | |
|------|---------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| | | \bar{x} season total | \bar{x} /trap/ night | \bar{x} season total | \bar{x} /trap/ night |
| I | High ^a | 41,173 | 98 | 117 | 1.4 |
| | Low ^a | 38,802 | 93 | 58 | 0.7 |
| | \bar{x} | 39,988 | 96 | 87 | 1.1 |
| II | High ^a | 58,519 | 140 | 61 | 0.8 |
| | Low ^a | 53,410 | 127 | 46 | 0.5 |
| | \bar{x} | 55,965 | 134 | 54 | 0.7 |

^a 2 replicates

Table 4.2

Comparison of trap catches from
inner and outer rows.

| SITE | POPULATION LEVEL | MEAN CATCH/TRAP/NIGHT | |
|------|---------------------|-----------------------|------------|
| | | Outer Rows | Inner Rows |
| I | High ^a | 101 | 91 |
| | Low ^a | 96 | 85 |
| | \bar{x} | 98 | 88* |
| II | High ^a | 147 | 123 |
| | Low ^a | 132 | 118 |
| | \bar{x} | 139 | 120** |

^a 2 replicates

* significantly different at 0.05 level for a paired t-test

** significantly different at 0.01 level for a paired t-test

Table 4.3

Beetles landing in and around traps on
treatment plots at Site II.

| MEAN CATCH/TRAP | MEAN NUMBERS IN ZONES AROUND TRAPS | | | |
|-----------------|------------------------------------|--------|--------|-------|
| | Inner | Middle | Outer | Total |
| 136 | 154 | 72 | 50 | 412 |
| 33.00% | 37.38% | 17.48% | 12.14% | 100% |

Table 4.4

Pre-treatment third instar counts, Site I.

| POPULATION LEVEL | TREATMENT \bar{x}/m^2 | CONTROL \bar{x}/m^2 |
|---------------------|----------------------------|--------------------------|
| High ^a | 175 | 132 |
| Low ^a | 39 | 31 |
| \bar{x} | 107 | 81* |

^a 2 replicates, 40 samples per replicate

* significantly different at 0.05 level

LSD values ($/m^2$) for treatment means

S.E. = 0.281
p 0.05 = 34.5
p 0.01 = 46.1

Table 4.5

Pre-treatment third instar counts, Site II.

| POPULATION LEVEL | TREATMENT \bar{x}/m^2 | CONTROL \bar{x}/m^2 |
|---------------------|----------------------------|--------------------------|
| High ^a | 112 | 141 |
| Low ^a | 39 | 66 |
| \bar{x} | 75 | 103* |

^a 2 replicates, 40 samples per replicate

* significantly different at 0.05 level

LSD values ($/m^2$) for treatment means

S.E. = 0.281
p 0.05 = 33.3
p 0.01 = 44.4

Table 4.6

Egg-first instar counts, Site I.

| POPULATION LEVEL | TREATMENT \bar{x}/m^2 | CONTROL \bar{x}/m^2 |
|---------------------|----------------------------|--------------------------|
| High ^a | 273 | 196 |
| Low ^a | 338 | 281 |
| \bar{x} | 306 | 239 ^b |

^a 2 replicates, 15 samples per replicate

^b not significantly different at 0.05 level

LSD values ($/m^2$) for treatment means

Non-significant F-test

Table 4.7

Egg-first instar counts, Site II.

| POPULATION LEVEL | TREATMENT \bar{x}/m^2 | CONTROL \bar{x}/m^2 |
|---------------------|----------------------------|--------------------------|
| High ^a | 217 | 78 |
| Low ^a | 98 | 69 |
| \bar{x} | 158 | 73* |

^a 2 replicates, 15 samples per replicate

* significantly different at 0.05 level

LSD values ($/m^2$) for treatment means (from transformed data)

S.E. = 0.190
p 0.05 = 23.3
p 0.01 = 31.1

Table 4.8

Post-treatment third instar counts, Site I.

| POPULATION LEVEL | TREATMENT \bar{x}/m^2 | CONTROL \bar{x}/m^2 |
|---------------------|----------------------------|--------------------------|
| High ^a | 188 | 127 |
| Low ^a | 105 | 95 |
| \bar{x} | 146 | 111** |

^a 2 replicates, 40 samples per replicate

** significantly different at 0.01 level

LSD values ($/m^2$) for treatment means

S.E. = 0.301
p 0,05 = 37.0
p 0.01 = 49.4

Table 4.9

Post-treatment third instar counts, Site II.

| POPULATION LEVEL | TREATMENT \bar{x}/m^2 | CONTROL \bar{x}/m^2 |
|---------------------|----------------------------|--------------------------|
| High ^a | 112 | 102 |
| Low ^a | 192 | 149 |
| \bar{x} | 152 | 125 ^b |

^a 2 replicates, 40 samples per replicate

^b not significantly different at 0.05 level

LSD values ($/m^2$) for treatment means

Non-significant F-test

FIGURE 4.1 TRENDS OF MEAN NUMBER BEETLES PER M², SITE II.

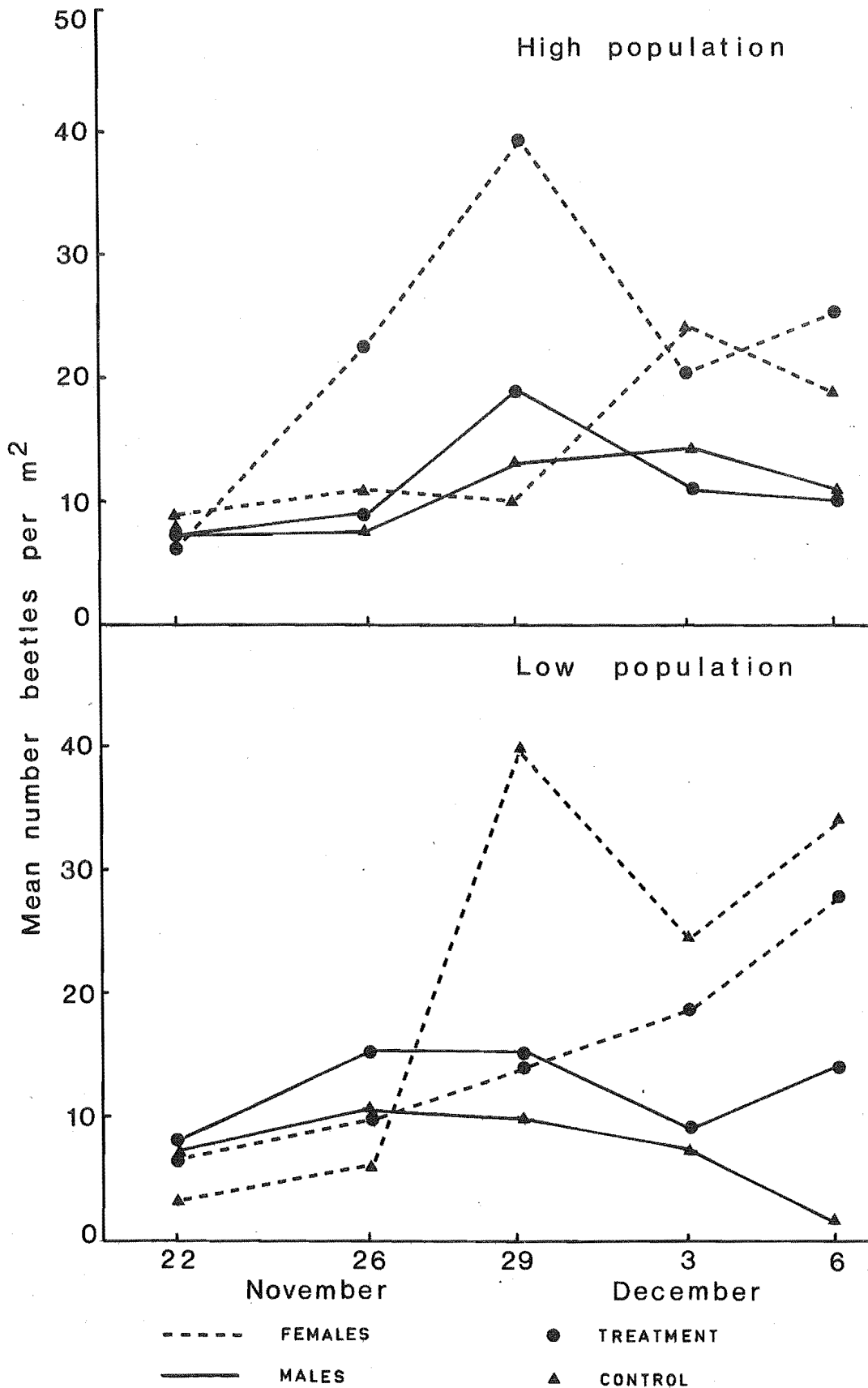


Table 4.10

Ratios of males to females on control and treatment plots on high and low population areas at Site II.

| POPULATION LEVEL | DATE SAMPLED | TREATMENT M : F | CONTROL M : F |
|------------------|--------------|-----------------|---------------|
| High | 22.xi.74 | 1 : 0.87 | 1 : 1.33 |
| | 26.xi.74 | 1 : 2.50 | 1 : 1.39 |
| | 29.xi.74 | 1 : 2.00 | 1 : 0.77 |
| | 3.xii.74 | 1 : 1.92 | 1 : 1.67 |
| | 6.xii.74 | 1 : 3.00 | 1 : 3.00 |
| Low | 22.xi.74 | 1 : 1.00 | 1 : 0.50 |
| | 26.xi.74 | 1 : 1.00 | 1 : 0.37 |
| | 29.xi.74 | 1 : 1.43 | 1 : 2.66 |
| | 3.xii.74 | 1 : 2.50 | 1 : 3.22 |
| | 6.xii.74 | 1 : 20.00 | 1 : 2.42 |

CHAPTER V

DISCUSSION AND CONCLUSIONS

INTRODUCTION

Fenemore and Perrott (1970) considered attempted control of grass grub adults as a practical possibility because:

- (a) Adults are theoretically more vulnerable to attack than any other stage because they are the only stage to emerge above the surface.
- (b) Populations are at their lowest numbers.
- (c) Adults are significantly more susceptible to insecticides than are larvae, with males being more susceptible than females (Perrott, 1964).

Further reasons that may be considered include:

- (d) Most beetles emerge over a very short period of time, therefore being ideally suited to the use of transient control agents.
- (e) Adult populations are highly mobile, especially male beetles, and therefore suited to manipulation by aggregating agents, e.g. sex attractants.
- (f) Control agents directed at the adult have an influence on the reproductive capacity of the population.

The above points support the hypothesis that attempted control of the adult stage of the life cycle may be a practical proposition and in the case of grass grub would limit the damage caused by the larvae of the subsequent generation.

However, in the past, attempts at controlling adults have not always been successful. Kelsey (1951) records light traps and fires are of little practical value because too few numbers are attracted. Fenemore and Perrott (1970) also experienced failure with insecticides applied to the pasture surface during the flight season and concluded, unless increased contact between beetles and insecticide could be achieved, this method was not likely to be effective. More recently, though, Wightman (1972) claims to have achieved control on a small scale by several passes of a heavy roller over the infested pasture and also by the multiple application of a foam, insecticide formulation. The success of these small plot techniques must however be reviewed in the context of their practicability for field scale use, and also, repeated applications of such treatments would no doubt limit their appeal.

The conclusion drawn from these records is that little success has been experienced in attempting to control grass grub adults largely because too few beetles are destroyed.

EVALUATION OF SAMPLING METHODS AND ESTIMATION OF POPULATIONS

Sampling Methods

As the analysis of these experiments relied solely on the assessment of population densities, critical evaluation of the sampling methods used is desirable to indicate the reliability of the data.

Sampling third instars by spade square samples and hand sorting proved to be an adequately reliable method of estimating larval populations. Re-sorting 5% of pre-treatment larval samples showed only 3.7% of the total numbers of larvae recorded were missed in the first sorting. This element of error could satisfactorily be accounted for by variation between the various sorters and their ability to detect larvae in such samples. A similar error could also be expected in sorting soil samples for adults, although they are more readily detected because of their movements when disturbed. The success of detecting eggs and first instars by the flotation, wet sieving technique was not determined in this study, but East (1972) records 98.9% and 97.6% recovery for undamaged eggs and first instars respectively with slightly lower values for damaged individuals. The equipment used in both cases was identical.

Plot Selection and Estimation of Populations

Pairing of control and treatment plots was not satisfactorily achieved by visually assessing pasture damage as some pre-treatment third instar counts differed significantly.

More even pairing of plots may have been achieved had treatments been allocated to plots subsequent to the pre-treatment third instar sample. However, this would counter the initial criterion to locate pairs of control and treatment plots in a similar situation, although, in hindsight, this may not be overridingly important.

The number of samples required per plot to give a good estimate of the populations of third instars was derived from sample data collected the previous year. The number of sample units necessary for a 10% standard error of the mean, a level arbitrarily suggested by Morris (1955), was determined by,

$$N_s = \frac{(CVn.)^2}{p}$$

where: N_s = number of samples required
CVn. = coefficient of variation
 p = per cent standard error required.

Forty samples per plot for third instars resulted in per cent standard errors of the means within the range of 12-15%, although on a few low population plots these values were considerably exceeded.

Variability was also relatively high between adult samples, a result no doubt due to the lower numbers being sampled. As sampling was carried out on five occasions during the trapping period, the total number of samples had to be limited because:

- (a) Sampling of all eight plots at Site II had to be completed within one day.
- (b) The percentage of the plot destroyed should ideally be retained at a minimum level.

Twenty samples per plot on these five occasions removed only 0.77% of the total area of each plot.

Egg-first instar counts exhibited extreme variability despite sampling in areas where they are most likely to be found, i.e. close to the periphery of areas of pasture damage. Low precision of these counts is usually attributed to the highly clumped distributions of eggs, therefore sampling of the second or early third instars when some lateral movement has taken place may be more desirable. Fenemore (1965) stated that larvae may move up to 30-60 cm laterally thereby reducing to some extent, their clumped distribution. Transforming raw data for analysis successfully reduced the dependence of variance on the mean.

ANALYSIS OF COMPONENTS OF THE TRAPPING REGIME

Factors which influence events when attempting to trap beetles using this attractant can be divided into two groups:

- (a) Those that are extrinsic to the system being treated, i.e. the attractant, the traps, trap density, etc.

- (b) Those that are intrinsic to the system being treated, i.e. the behaviour of beetles, influence of weather, etc.

Extrinsic Factors

(i) Influence of the Attractant - Adult grass grub populations are highly mobile and males can cover considerable distances over the pasture surface in search of a mate. Males normally emerge before females each night so females are usually mated as soon as they emerge. A review of the events under the influence of this attractant reveals a modification of this normal behaviour.

Detection and orientation to the attractant was typically by random weaving flight and the observation of direct "bee-liners" was not common. The majority of beetles approached the traps from downwind although upwind approaches were also observed. The upwind approach is likely to be a result of male beetles being attracted to the multiple odour source and locating a single trap from an upwind position as a consequence of random flight within the plot. If beetles were not caught in the trap, they invariably landed on the pasture within a 0.5 m radius of the attractant source and commenced searching activity, with their lamellate antennae outstretched. These activities continued until the cessation of flights for that night and very few beetles were observed leaving the vicinity of the traps once they had been attracted. Night activity culminated with the majority of

beetles burrowing into the sward or under the traps, although some remained on the pasture surface for several hours, and, on occasions, feeding was observed. Traps left set over night sometimes caught a few beetles, but then only in the early part of the flight season.

The facet of normal behaviour which is modified by the influence of this attractant is further searching flight by males is suppressed. Male beetles are known to normally indulge in a random weaving flight and do so in a series of take-offs and landings. The impetus for successive flights by males is possibly the failure to find a mate and conversely, the suppression of flight could either be the location of a female or due to exhaustion or some other factor. Therefore, Durez 12687 appears to act as an arrestant of subsequent searching flights by males. Whether it is a specific component of this attractant or the steady source of phenol that suppresses flight is at this moment speculation, although Osborne and Boyd (1975) have recently reported on the activities of various components of this attractant but their influence on beetle behaviour has still to be investigated.

The immigration of male beetles into treatment plots indicates the potency of this attractant as on most plots the total number of beetles caught exceeded what might be expected to emerge from that plot (estimated from third instar numbers and assuming a 1:1 sex ratio, with no subsequent mortality). Additionally, there was very little difference between catches in traps on high and low population areas,

and outer row traps on all plots caught significantly greater numbers of beetles, factors which further support considerable immigration into treatment plots.

(ii) Efficiency of Traps and Trapping - When the efficiency of traps in a trapping programme are considered, the use of two distinct definitions emerge: trapping efficiency and trapping performance. Trapping efficiency, as previously defined, is the ability of traps to capture insects that are attracted, whereas trapping performance is a measure of success or failure of traps and attractant at a given density to remove insects from a prescribed area. Elaborate methods of determining these values are described by Hartstack *et al.* (1971) and Wolf, Kishaba and Toba (1971), but were beyond the bounds of these experiments.

Three main factors appear to have influenced the performance of this trapping operation:

- (a) The potency of the attractant.
- (b) The efficiency of the traps.
- (c) The density of the traps employed.

The attractant in its present form and method of utilisation is highly attractive, a fact adequately supported by the large numbers of beetles attracted. It therefore appears not to be a limiting extrinsic factor.

The efficiency of traps has emerged as probably the single most important extrinsic factor to have influenced the outcome of these experiments. Even though this trap design had earlier been observed to have only moderate efficiency, it possessed a number of advantageous attributes which supported its continued use:

- (a) It was cheap to construct.
- (b) It was durable and would withstand maltreatment from stock.
- (c) It was easily maintained.

Table 4.3 gives an estimate of trap efficiency and on average 67% of those attracted escaped. The fate of adults escaping was not determined in this study, however, what is probable, is they are again able to contribute to the overall mating success of the population. Knowledge of the number of times beetles emerge during their life obviously has significance in determining what chance of success these individuals have in finding a mate. Increasing the trap's surface area is one solution to gain increased efficiency, but compounds the problems of cost, construction and maintenance.

Estimates of trapping performance cannot be concluded from these experiments. The method of expressing the number of males caught as a percentage of total numbers of males which might be expected to emerge from a particular plot is meaningless in the case of small plots open to the

surrounding population, as immigration would seriously bias the results.

Trap density also effects the probability of an individual being caught, and to cover all points in a given area, the trapping patterns of individual traps must overlap (Wolf, Kishaba and Toba, 1971). The density of traps employed in this study was chosen arbitrarily, but influenced to some extent by:

- (a) Observations of Osborne and Harrison (pers. comm.) that beetles may be directly attracted for distances up to 12 m.
- (b) Traps on a 10 m grid spacing in the previous year's experiment resulted in some small reduction of larva numbers.

The density of traps used in these experiments, whatever their performance, is obviously beyond the realm of practical feasibility. Also, considering the random weaving flights of male beetles a greater number of traps would probably be required to reduce the probability of locating a female, a factor which would be complicated by higher population densities.

Intrinsic Factors

(i) Influence of Weather - The conditions under which grass grub beetles fly are well documented. Lesser numbers of beetles fly under windy conditions and practically no

beetles fly when it is raining. Two peaks of flight activity were recorded during the trapping period on November 27 and December 3 at Site II and November 28 and December 3 at Site I. Both peaks coincide with declining barometric pressures associated with approaching cold fronts and wind changes from NW to S or SW, factors similarly linked to the flights of Argentine stem weevil (Pottinger, 1961) and porina moth (French, 1973) in Canterbury.

(ii) Effect of Exposure - Exposure to weather, especially wind, reduces flight activity and a fact possibly reflected in the much lower catches at Site I, despite higher initial populations of third instars and presumably emergent adults. However, it may also be as a result of the lower total population present in the particular field (compare areas in Figs. 3.1 and 3.2). The effect of exposure was however, explicitly demonstrated when 10% more beetles were caught on the more sheltered of the two high population plots at Site II.

ASSESSMENT OF THE EFFECTS OF TRAPPING

Adults Sampled During the Flight Season

The sampling of adult populations throughout the trapping period as described in the Method section was an attempt to determine the effect of trapping on the numbers of beetles and their sex ratios on the base populations of each plot. Subsequent sexing of these beetles revealed

lesser numbers of males compared with females occurred in both control and treatment plots, a result synonymous on both high and low population areas. At both population densities the numbers of males per m^2 on treatment plots exceeded those found on control plots whereas the number of females presented a mixed result. Higher numbers were found on treatment plots in high population areas, but the opposite occurred on low population areas. Overall, numbers of males and females on control and treatment plots did not greatly differ from 1:2 at either population level.

A possible explanation of this result is as follows. Assuming an overall 1:1 ratio of males to females were initially present, East (1972) found this ratio repeatedly in several Canterbury populations, then the resultant 1:2 ratio indicates that approximately half of the male population has been removed from areas close to the vicinity of the traps. The fact that control plots exhibit similar ratios might also suggest this effect has extended up to 20 to 30 m from the treatment plots. The lack of any large differences between high and low population areas also reflects the mobility of grass grub beetles. Despite the large initial differences in larval populations, males, and presumably females once they have laid their first batch of eggs, are free to fly at random over the pasture and become more evenly distributed.

Plausible explanation of the pattern of events with respect to females is difficult to reconcile without knowledge of their mated status because females are generally

considered to fly only after they have laid most of their eggs. As sampling for tenereal adults was confined to the trapping period the development and emergence of female beetles may have only been in its initial phases. Variation in the periods of development and emergence can occur between paddocks of the same locality (East, 1972) and different soil types (Kain, 1968) and could account for the apparent lack of pattern.

Post-treatment Egg-First Instar Counts

The variability exhibited by egg-first instar counts makes concise conclusion as to the immediate effects of trapping difficult. However, the one trend evident is the number of occasions egg-first instar counts from treatment plots exceed those from control plots. Although the difference was significant at Site II, the trend was not repeated at Site I.

Three possible explanations could account for the observed result:

- (a) With the influx of males into the treatment plots, the probability of a female being mated must be increased and assuming males are immigrating from areas immediately outside, the probability of a female being mated in these areas may be reduced.

- (b) Females may be immigrating into treatment plots. The occurrence of up to a dozen or more mating couples in close vicinity to the traps were often observed in the latter half of the trapping period. Although no counts were made, they appeared to be more numerous than elsewhere in the plots.
- (c) The sampling plan does not give an adequate estimate of the population present.

However, when initial populations of third instars are comparable on control and treatment plots, (plots A and C at Site I; plot F at Site II) differences in excess of two times the numbers of eggs-first instars recorded on treatment plots suggest there may have been some effect attributable to factors (a) and/or (b).

Post-treatment Third Instar Counts

The third instars are undoubtedly the most suitable stage for assessing grass grub populations, because their initial highly clumped distribution becomes more random as the larvae mature. The aim of carrying out a post-treatment count of third instars was to substantiate the trends indicated by the less reliable egg data. However, sampling at this late stage, with respect to the treatment, leaves the population subject to the modifying effects of other density dependent and independent mortality factors which may mask the result.

A significant difference between treatments was only recorded at Site I with this post-treatment count, a difference not apparent when the eggs-first instars were sampled. At Site II, the occurrence of winter snow and damage caused by sheep treading are suspected to have influenced the result. The effect of sheep treading in slushy snow would reduce the higher densities of third instars to lower levels, more so than among lower population areas, because of the higher probability of a larva being trodden on and being damaged. Therefore, at Site II, the occurrence of a significant difference between treatments may have been masked. A converse argument may however be as equally valid, in that the egg-first instar counts were not representative of the populations present on each plot as some post-treatment third instar counts exceed these counts.

From this discussion it is evident that sampling at this stage did not adequately substantiate the trends exhibited by the egg-first instar data and indicated the numbers of third instars present had risen only slightly overall from the previous year.

A SUMMARY OF THE EFFECTS OF TRAPPING

The first section of the discussion reasons that control of the adult stage of the life cycle may be possible, although methods tried in the past have proven to be unsatisfactory. The use of an attractant probably has some advantage

over these other techniques in that the population may be aggregated for the purposes of destroying them. From these experiments it has become clear by using traps and attractant as described the main objective was not fulfilled, i.e. sufficient males were not removed from the population to reduce the frequency of mating and result in a decline in numbers of the subsequent generation. Although large numbers of male beetles were attracted, approximately two-thirds of those escaped capture each night. Whether they would be attracted again later in the flight season can only be speculation at present. However, despite low trap efficiency, the effect of large numbers of beetles immigrating onto treatment plots must have also influenced the outcome. This conclusion is supported by evidence from the egg-first instar data where, in the majority of cases, treatment plot counts exceeded those of control plots. The fact that sufficient males were not removed from the population is also probable as there were no large differences between the numbers of adult males present on control and treatment plots as determined by regular sampling throughout the trapping period.

A RETROSPECTIVE ASSESSMENT OF SEX ATTRACTANT TRAPPING FOR GRASS GRUB CONTROL

The results of these experiments show sex attractant trapping for grass grub control cannot be achieved on small plots by the methods described. However, it does not

conclude the concept of this technique is unsound when the consequences of low trap efficiency and immigration of beetles into plots are considered in perspective. Thus, a small scale assessment of this nature may not have indicated the true worth of this method of control for grass grub.

An important aspect overlooked in this study, and others related to the assessment of this sex attractant for control, is contained in the statement by Knipling (1972):

"A uniform suppressive pressure applied against a total population of the pest over a period of generations will achieve a greater degree of suppression than a higher level of control on part of the population."

The importance of total population suppression is explicitly demonstrated by the large scale mass trapping programmes reported for the red banded leaf roller and boll weevil, a condition also simulated by Read (1975) with grass grub in large field cages.

It is also clear any further consideration of this technique should be directed at more discriminatory assessments of the factors involved. For example, the efficacy of the attractant must be divorced from the efficiency of the traps or any other control agent employed. In these experiments the attractant acted as a population aggregator, but it was the traps which failed to eliminate all the beetles attracted. It therefore becomes apparent a more intimate knowledge of the factors operating under such conditions need to be known.

A mathematical model predicting the outcome of a set of hypothetical parameters has shown sex attractant trapping for grass grub control is theoretically possible (Henzell, 1973). The per cent control achieved by such computations largely depends on how competitive the synthetic attractant source is to the virgin females in the field. This model, based on those of Knipling and McGuire (1966) show sex attractant trapping is more successful on lower than higher populations. For example, Henzell (1973) calculates that 90% control could be expected if 178 traps per hectare which were each 400 times more attractive than a virgin female and used on a population containing 12,000 females per hectare (or $2/m^2$). But by using the same number of traps on a population of 200 females per m^2 would result in only 10% control. Therefore with a higher population density, a greater incidence of traps or a higher competition ratio would be required to achieve a similar degree of control.

In the case of grass grub, it would probably be more expedient to increase the number of point sources of attractant so a male, on emergence, will have a greater probability of detecting the source of the synthetic attractant than a newly emergent, virgin female. Obviously the traps used in these experiments have no place in this concept of control and therefore, the development of an entirely new approach would be required if this attractant is to be employed to advantage for suppression of grass grub.

SUMMARY

- I. The aim of this study was to determine if sufficient numbers of males could be removed from a population of grass grub beetles using the synthetic sex attractant, Durez 12687, and traps to reduce the frequency of mating and result in a decline in numbers of the subsequent generation.
- II. The experiments were carried out on two sites in the Staveley and Alford Forest districts of the Asbhurton County, Canterbury, during 1972 and 1973.
- III. Control and treatment plots, 30 x 25 m (0.076 ha) in size, were replicated twice each on areas of high and low population densities at both sites.
- IV. The effects of trapping were assessed by pre-sampling third instars, post-sampling the eggs-first instars and third instars the following year. Additionally, tenereal adults were sampled on five occasions during the flight season.
- V. Trapping was carried out over a period of three weeks from November 20 to December 10 using simple water traps, 20 per plot in a 5 x 4 grid arrangement, each 5 m apart.

- VI. Catches in the traps baited with the attractant varied little between high and low population areas, although greater numbers were caught at the more sheltered of the two sites.
- VII. The efficiency of the traps was determined by assessing the number of beetles landing in zones around the traps, the result indicating approximately two-thirds of the beetles being attracted were escaping on average each night.
- VIII. Sampling the adults at intervals throughout the flight season revealed male beetle populations to be comparable on control and treatment plots with very little difference between numbers on high and low population areas. Overall there were approximately twice as many females present.
- IX. Estimates of egg-first instar populations showed their numbers in treatment plots to exceed those in control plots, although the difference attained significance at only one site.
- X. Estimates of third instar populations did not adequately substantiate the above trends.

XI. The conclusion apparent from these experiments is that trapping in this manner did not satisfy the aim because:

- (a) Immigration of beetles into treatment plots negated the effect of removing males from the plots.
- (b) The efficiency of the traps was so low a significant proportion of those attracted, escaped.

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APPENDICES

APPENDIX I

Beetles trapped at Site I.

| DATE | PLOT | | | |
|----------------|--------|--------|--------|--------|
| | A1 | B1 | C1 | D1 |
| 20.xi.73 | 9 | 4 | 8 | 4 |
| 21.xi.73 | 2 | 1 | 3 | 2 |
| 22.xi.73 | 1,653 | 2,799 | 2,047 | 2,186 |
| 23.xi.73 | 2,501 | 2,160 | 4,112 | 1,206 |
| 24.xi.73 | 360 | 315 | 706 | 379 |
| 25.xi.73 | 0 | 0 | 0 | 0 |
| 26.xi.73 | 4,079 | 3,794 | 3,303 | 3,008 |
| 27.xi.73 | 2,623 | 2,847 | 3,448 | 2,821 |
| 28.xi.73 | 6,876 | 6,533 | 8,748 | 5,305 |
| 29.xi.73 | 4,995 | 5,217 | 4,868 | 4,343 |
| 30.xi.73 | 1,752 | 1,754 | 1,485 | 1,628 |
| 1.xii.73 | 261 | 312 | 0 | 6 |
| 2.xii.73 | 624 | 487 | 1,198 | 388 |
| 3.xii.73 | 6,600 | 6,072 | 6,186 | 5,452 |
| 4.xii.73 | 1,938 | 3,326 | 942 | 749 |
| 5.xii.73 | 3,837 | 4,093 | 3,967 | 437 |
| 6.xii.73 | 0 | 0 | 0 | 0 |
| 7.xii.73 | 1,270 | 1,148 | 1,613 | 1,224 |
| 8.xii.73 | 278 | 460 | 245 | 201 |
| 9.xii.73 | 279 | 245 | 504 | 281 |
| 10.xii.73 | 345 | 398 | 330 | 270 |
| Totals | 40,382 | 41,965 | 43,713 | 33,890 |
| Total Controls | 160 | 74 | 67 | 48 |

APPENDIX II

Beetles trapped at Site II.

| DATE | PLOT | | | |
|-------------------|--------|--------|--------|--------|
| | E1 | F1 | G1 | H1 |
| 20.xi.73 | 22 | 1,296 | 44 | 16 |
| 21.xi.73 | 800 | 472 | 602 | 358 |
| 22.xi.73 | 4,425 | 2,359 | 4,274 | 1,572 |
| 23.xi.73 | 1,646 | 3,359 | 4,872 | 1,548 |
| 24.xi.73 | 5,387 | 3,522 | 3,295 | 4,536 |
| 25.xi.73 | 0 | 0 | 0 | 0 |
| 26.xi.73 | 2,465 | 2,161 | 1,769 | 3,990 |
| 27.xi.73 | 11,060 | 9,088 | 7,898 | 11,060 |
| 28.xi.73 | 3,829 | 6,513 | 5,448 | 7,155 |
| 29.xi.73 | 5,408 | 8,233 | 2,709 | 7,740 |
| 30.xi.73 | 5,511 | 6,278 | 6,767 | 4,393 |
| 1.xii.73 | 0 | 0 | 0 | 0 |
| 2.xii.73 | 303 | 259 | 312 | 411 |
| 3.xii.73 | 9,587 | 7,282 | 6,593 | 8,741 |
| 4.xii.73 | 427 | 2,879 | 180 | 875 |
| 5.xii.73 | 3,717 | 5,546 | 2,068 | 4,552 |
| 6.xii.73 | 512 | 1,286 | 239 | 635 |
| 7.xii.73 | 333 | 454 | 512 | 546 |
| 8.xii.73 | 73 | 308 | 29 | 450 |
| 9.xii.73 | 149 | 12 | 178 | 53 |
| 10.xii.73 | 53 | 51 | 213 | 146 |
| Totals | 55,707 | 61,330 | 48,042 | 58,777 |
| Total Controls | 74 | 43 | 48 | 50 |

APPENDIX III

Catches in traps surrounded by pit traps on six nights on 4 treatment plots at Site II.

| NIGHT | PLOT | | | |
|-------|------|-----|-----|-----|
| | E1 | F1 | G1 | H1 |
| 1 | 89 | 466 | 29 | 353 |
| 2 | 6 | 10 | 3 | 25 |
| 3 | 362 | 282 | 292 | 316 |
| 4 | 12 | 96 | 2 | 35 |
| 5 | 23 | 237 | 81 | 247 |
| 6 | 22 | 41 | 5 | 35 |

$$\bar{x}/\text{trap/night} = 136 \text{ beetles}$$

Mean catch per pit trap in zones around traps on control and treatment plots at Site II.

| PLOT | ZONE | | |
|-----------------------------------|-------|--------|-------|
| | Inner | Middle | Outer |
| Treatment | 13.14 | 3.98 | 3.13 |
| Control | 3.78 | 2.34 | 2.39 |
| Nett (T-C) | 9.36 | 1.64 | 0.74 |
| Area of each zone cm ² | 1649 | 4369 | 6708 |

APPENDIX IV

Pre-treatment third instar larvae, Site I.

| Plot | Date Sampled | Samples ^c / plot | \bar{x} /Sample |
|-----------------|--------------|--------------------------------|-------------------|
| A1 ^a | 4.ix.73 | 40 | 4.53 |
| A2 ^b | 4.ix.73 | 40 | 4.86 |
| B1 | 11.ix.73 | 40 | 3.60 |
| B2 | 11.ix.73 | 40 | 1.20 |
| C1 | 18.ix.73 | 40 | 1.23 |
| C2 | 18.ix.73 | 40 | 1.20 |
| D1 | 18.ix.73 | 40 | 0.58 |
| D2 | 18.ix.73 | 40 | 0.28 |

a 1 = treatment plot

b 2 = control plot

c = 15 x 15 cm spade square

Anova summary for pre-treatment third instar data, Site I.

| Source | Df. | Sums of sqs. | Mean sq. | F. |
|---------------|-----|--------------|----------|-------|
| Replicates | 1 | 190.653 | 190.653 | |
| Main-plot tr. | 1 | 613.278 | 613.278 | 14.83 |
| Error a | 1 | 41.328 | 41.328 | |
| Main plots | 3 | 845.259 | | |
| Sub-plot tr. | 1 | 28.203 | 28.203 | 4.46* |
| Sub X Main | 1 | 12.403 | 12.403 | 1.96 |
| Error b | 314 | 1984.256 | 6.319 | |
| Total | 319 | 2870.121 | | |

* significant at 0.05 level

APPENDIX V

Pre-treatment third instar larvae, Site II.

| Plot | Date Sampled | Samples ^c / plot | \bar{x} /Sample |
|-----------------|--------------|--------------------------------|-------------------|
| E1 ^a | 27.ix.73 | 40 | 2.05 |
| E2 ^b | 27.ix.73 | 40 | 3.18 |
| F1 | 13.ix.73 | 40 | 3.13 |
| F2 | 13.ix.73 | 40 | 3.35 |
| G1 | 11.ix.73 | 40 | 0.55 |
| G2 | 11.ix.73 | 40 | 1.05 |
| H1 | 11.ix.73 | 40 | 1.25 |
| H2 | 11.ix.73 | 40 | 2.03 |

a 1 = treatment plot

b 2 = control plot

c = 15 x 15 spade square

Anova summary for pre-treatment third instar data, Site II.

| Source | Df. | Sums of sqs. | Mean sq. | F. |
|---------------|-----|--------------|----------|----------|
| Replicates | 1 | 43.512 | 43.512 | |
| Main-plot tr. | 1 | 231.199 | 231.199 | 288.99** |
| Error a | 1 | 0.800 | 0.800 | |
| Main plots | 3 | 275.512 | | |
| Sub-plot tr. | 1 | 33.800 | 33.800 | 5.74* |
| Sub X Main | 1 | 0.012 | 0.012 | 0 |
| Error b | 314 | 1847.162 | 5.882 | |
| Total | 319 | 2156.487 | | |

* significant at 0.05 level

** significant at 0.01 level

APPENDIX VI

Eggs-first instar larvae, Site I.

| Plot | Date Sampled | Samples ^c / plot | \bar{x} /Sample |
|-----------------|--------------|--------------------------------|-------------------|
| A1 ^a | 20.xii.74 | 15 | 9.33 |
| A2 ^b | 20.xii.74 | 15 | 4.66 |
| B1 | 20.xii.74 | 15 | 3.33 |
| B2 | 20.xii.74 | 15 | 4.40 |
| C1 | 20.xii.74 | 15 | 14.47 |
| C2 | 20.xii.74 | 15 | 4.46 |
| D1 | 20.xii.74 | 15 | 1.20 |
| D2 | 20.xii.74 | 15 | 8.60 |

a 1 = treatment plot

b 2 = control plot

c = 15 x 15 spade square

Anova summary for transformed ($y = \sqrt{x + \frac{1}{2}}$)
Eggs-first instar larvae data, Site I.

| Source | Df. | Sums of sqs. | Mean sq. | F. |
|--------------|-----|--------------|----------|----------|
| Replicates | 1 | 1.857 | 1.857 | |
| Main-plots | 1 | 4.638 | 4.638 | 0.03 |
| Error a | 1 | 136.013 | 136.013 | |
| Main-plots | 3 | 142.507 | | |
| Sub-plot tr. | 1 | 12.772 | 12.772 | 0.54 ns. |
| Sub X Main | 1 | 21 | 21 | 0 |
| Error b | 114 | 2651.095 | 23,255 | |
| Total | | 2806.395 | | |

ns. not significant at 0.05 level

APPENDIX VII

Eggs-first instar larvae, Site II.

| Plot | Date Sampled | Samples ^c /plot | \bar{x} /Sample |
|-----------------|--------------|----------------------------|-------------------|
| E1 ^a | 19.xii.74 | 15 | 1.86 |
| E2 ^b | 19.xii.74 | 15 | 2.14 |
| F1 | 19.xii.74 | 15 | 8.20 |
| F2 | 19.xii.74 | 15 | 1.47 |
| G1 | 19.xii.74 | 15 | 2.73 |
| G2 | 19.xii.74 | 15 | 1.80 |
| H1 | 19.xii.74 | 15 | 1.80 |
| H2 | 19.xii.74 | 15 | 1.40 |

a 1 = treatment plot

b 2 = control plot

c = 15 x 15 spade square

Anova summary for transformed ($y = \sqrt{x + \frac{1}{2}}$)
Eggs-first instar larvae data, Site II.

| Source | Df. | Sums of sqs. | Mean sq. | F. |
|---------------|-----|--------------|----------|-------|
| Replicates | 1 | 22.413 | 22.413 | |
| Main-plot tr. | 1 | 17.617 | 17.617 | 0.52 |
| Error a | 1 | 33.668 | 33.668 | |
| Main-plots | 3 | 73.698 | | |
| Sub-plot tr. | 1 | 47.203 | 47.203 | 4.35* |
| Sub X Main | 1 | 20.228 | 20.228 | 1.86 |
| Error b | 114 | 1235.295 | 10.836 | |
| Total | 119 | 1376.424 | | |

* significant at 0.05 level

APPENDIX VIII

Post-treatment third instar larvae, Site I.

| Plot | Date Sampled | Samples ^c /plot | \bar{x} /Sample |
|-----------------|--------------|----------------------------|-------------------|
| A1 ^a | 25.ix.74 | 40 | 4.63 |
| A2 ^b | 25.ix.74 | 40 | 2.68 |
| B1 | 25.ix.74 | 40 | 4.05 |
| B2 | 25.ix.74 | 40 | 3.18 |
| C1 | 25.ix.74 | 40 | 2.40 |
| C2 | 25.ix.74 | 40 | 2.15 |
| D1 | 3.x.74 | 40 | 2.45 |
| D2 | 3.x.74 | 40 | 2.23 |

a 1 = treatment plot

b 2 = control plot

c = 15 x 15 cm spade square

Anova summary for post-treatment third instar larvae Data, Site I.

| Source | Df. | Sums of sqs. | Mean sq. | F. |
|---------------|-----|--------------|----------|--------|
| Replicates | 1 | 0.049 | 0.049 | |
| Main-plot tr. | 1 | 125.000 | 125.000 | 400.00 |
| Error a | 1 | 0.312 | 0.312 | |
| Main-plots | 3 | 125.362 | | |
| Sub-plot tr. | 1 | 57.800 | 57.800 | 7.95* |
| Sub X Main | 1 | 27.612 | 27.612 | 3.80 |
| Error b | 314 | 2280.612 | 7.263 | |
| Total | 319 | 2491.387 | | |

* significant at 0.01 level

APPENDIX IX

Post-treatment third instar larvae, Site II.

| Plot | Date Sampled | Samples ^c / plot | \bar{x} /Sample |
|-----------------|--------------|--------------------------------|-------------------|
| E1 ^a | 11.ix.74 | 40 | 2.90 |
| E2 ^b | 11.ix.74 | 40 | 2.48 |
| F1 | 11.ix.74 | 40 | 2.30 |
| F2 | 11.ix.74 | 40 | 2.23 |
| G1 | 17.ix.74 | 40 | 3.83 |
| G2 | 17.ix.74 | 40 | 4.53 |
| H1 | 17.ix.74 | 40 | 5.08 |
| H2 | 17.ix.74 | 40 | 2.40 |

a 1 = treatment plot

b 2 = control plot

c = 15 x 15 cm spade square

Anova summary for post-treatment third instar larvae at Site II.

| Source | Df. | Sums of sqs. | Mean sq. | F. |
|---------------|-----|--------------|----------|----------|
| Replicates | 1 | 15.753 | 15.753 | |
| Main-plot tr. | 1 | 172.578 | 172.578 | 55.238 |
| Error a | 1 | 0.003 | 0.003 | |
| Main-plots | 3 | 188.334 | | |
| Sub-plot tr. | 1 | 29.403 | 29.403 | 3.49 ns. |
| Sub X Main | 1 | 11.628 | 11.628 | 1.38 |
| Error b | 314 | 2643.881 | 8.420 | |
| Total | 319 | 2873.246 | | |

ns. not significant at 0.05 level

Mean nos. male and female beetles per sample on five sample dates at Site II.

| PLOT | POPULATION LEVEL | 22.xi.74 | | 26.xi.73 | | 29.xi.73 | | 3.xii.73 | | 6.xii.73 | |
|----------------------|---------------------|----------|------|----------|------|----------|------|----------|------|----------|------|
| | | M | F | M | F | M | F | M | F | M | F |
| E1 | High | 0.02 | 0.02 | 0.10 | 0.25 | 0.25 | 0.35 | 0.45 | 0.35 | 0.25 | 0.10 |
| E2 | | 0.15 | 0.05 | 0.05 | 0.35 | 0.15 | 0.25 | 0.40 | 0.50 | 0.30 | 0.40 |
| F1 | | 0.10 | 0.05 | 0.30 | 0.75 | 0.65 | 1.45 | 0.05 | 0.60 | 0.15 | 0.10 |
| F2 | | 0.15 | 0.35 | 0.30 | 0.15 | 0.45 | 0.20 | 0.25 | 0.60 | 0 | 0.50 |
| \bar{x} Treatments | | 0.15 | 0.13 | 0.20 | 0.50 | 0.45 | 0.90 | 0.25 | 0.48 | 0.20 | 0.60 |
| \bar{x} Controls | 0.15 | 0.20 | 0.18 | 0.25 | 0.30 | 0.23 | 0.33 | 0.55 | 0.15 | 0.45 | |
| G1 | Low | 0.05 | 0.25 | 0.20 | 0.25 | 0.20 | 0.45 | 0.15 | 0.10 | 0 | 0.50 |
| G2 | | 0.10 | 0.10 | 0.25 | 0.15 | 0.35 | 1.35 | 0.20 | 0.55 | 0.15 | 0.45 |
| H1 | | 0.25 | 0.05 | 0.25 | 0.20 | 0.25 | 0.20 | 0.20 | 0.80 | 0.05 | 0.80 |
| H2 | | 0.20 | 0.05 | 0.45 | 0.10 | 0.35 | 0.50 | 0.15 | 0.60 | 0.50 | 1.10 |
| \bar{x} Treatments | | 0.15 | 0.15 | 0.23 | 0.23 | 0.23 | 0.33 | 0.18 | 0.45 | 0.03 | 0.65 |
| \bar{x} Controls | 0.15 | 0.08 | 0.35 | 0.13 | 0.35 | 0.93 | 0.18 | 0.58 | 0.33 | 0.80 | |

APPENDIX XI

Meteorological Data

| DATE | AIR TEMPS. (°C) | | | | GROUND TEMPS. °C | | % RH | RAINFALL (mm) | | WIND 8:30 P.M. | |
|--------|-----------------|------------|------------|------------|------------------|---------|------|---------------|-------|----------------|-----------|
| | 8:30 p.m. | Daily Max. | Daily Min. | Daily Mean | 5 cm | Surface | | Daily | Total | Velocity m/sec | Direction |
| 20 Nov | 14.5 | - | - | - | 12.5 | 9.5 | 54 | - | - | 7.0 | NW |
| 21 | 11.1 | 20.7 | 4.9 | 11.9 | 16.2 | 9.4 | 40 | - | - | 9.6 | NW |
| 22 | 12.2 | 19.8 | 9.4 | 11.2 | 19.6 | 11.4 | 61 | - | - | 1.7 | NE |
| 23 | 11.1 | 18.7 | 9.4 | 13.4 | 15.0 | 10.5 | 62 | - | - | 2.8 | NE |
| 24 | 15.0 | 19.8 | 6.6 | 12.0 | 19.5 | 13.5 | 38 | - | - | 2.9 | NW |
| 25 | 7.5 | 20.9 | 1.1 | 9.2 | * | * | 100 | 10.8 | 10.8 | 0 | SW |
| 26 | 10.5 | 23.1 | 2.5 | 11.7 | 16.9 | 8.3 | 56 | - | 10.8 | 2.1 | NW |
| 27 | 13.5 | 21.4 | 6.0 | 12.9 | 18.9 | 11.1 | 89 | - | 10.8 | 0.3 | S |
| 28 | 10.8 | 16.5 | 6.0 | 10.7 | 19.8 | 11.0 | 75 | - | 10.8 | 2.6 | E |
| 29 | 11.5 | 15.9 | 2.2 | 10.3 | 17.8 | 11.2 | 88 | - | 10.8 | 1.4 | S |
| 30 | 12.2 | 18.2 | 8.2 | 12.7 | 18.0 | 11.6 | 63 | - | 10.8 | 5.1 | S |
| 1 Dec | 8.8 | 18.2 | 7.7 | 12.2 | * | * | 100 | 1.3 | 12.1 | 1.8 | NE |
| 2 | 9.3 | 12.6 | 7.2 | 8.6 | 16.8 | 8.5 | 90 | - | 12.1 | 3.1 | NE |
| 3 | 22.0 | 24.8 | 2.2 | 16.3 | 22.1 | 17.5 | 42 | - | 12.1 | 3.1 | NW |
| 4 | 15.5 | 22.5 | 12.1 | 19.7 | 22.0 | 16.0 | 75 | - | 12.1 | 8.3 | SW |
| 5 | 13.5 | 16.5 | 9.9 | 12.3 | 18.2 | 12.5 | 95 | - | 12.1 | 2.2 | SW |
| 6 | 13.0 | 13.2 | 8.3 | 10.9 | 16.0 | 12.5 | 100 | 0.3 | 12.4 | 0 | NE |
| 7 | 17.7 | 21.4 | 10.4 | 14.8 | 17.0 | 12.5 | 95 | - | 12.4 | * | S |
| 8 | 15.5 | 23.1 | 10.4 | 15.8 | 23.0 | 15.5 | 90 | - | 12.4 | * | SW |
| 9 | 14.4 | 15.4 | 7.2 | 9.0 | 17.3 | 12.4 | 78 | - | 12.4 | * | NE |
| 10 | 9.4 | 20.9 | 7.2 | 12.0 | 15.1 | 13.2 | 100 | Trace | 12.4 | * | SE |

* Not recorded