BUREAU OF SUGAR EXPERIMENT STATIONS

QUEENSLAND, AUSTRALIA

KEY FACTORS IN CONTROL OF GREYBACK CANEGRUB POPULATIONS

SRDC Final Report, BS 120S

by

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SUMMARY

Greyback canegrub outbreaks with severe losses in sugar production have persisted for more than six years in the Burdekin district, but population densities of the pest have declined at locations in the Herbert Valley, Tully and Innisfail districts over the same period.

Six species of pathogenic organism were found to cause disease and death of greyback canegrubs, with relatively high incidence in grubs collected from the Herbert Valley, Tully and Innisfail study sites, but low incidence in the Burdekin. No deaths from entomopathogenic diseases were recorded in 226 grubs examined from the Burdekin in 1998. Two microorganisms, *Adelina* sp. (Protozoa: Coccidia), and *Metarhizium anisopliae* (Deuteromycetes fungi), were the most prevalent pathogens in far north Queensland grub populations. Incidence of *Adelina* sp. alone accounted for 55 - 64% of the variance in mortality of grubs recorded from samples collected across all locations in 1994 and 1995.

Key factor analysis of population changes between years at sites in the Burdekin, Tully and Innisfail suggested that recruitment rate of young grubs was the best predictor of population changes ($r^2 = 0.74$). This is probably caused by beetle oviposition behaviour, where tall crops are preferentially selected for egg-laying. Key factor analysis suggested that population density of greyback canegrubs at some sites including that at Innisfail and in a recently planted crop at Tully was determined between seasons as a result of immigration. Mortality rate of grubs within a season tended to counteract overall population changes between seasons at all sites apart from the Burdekin, suggesting a possible regulatory effect. In the Herbert Valley, population density of greyback canegrubs declined to zero in two monitored fields, and data were not available for more than two consecutive years at either site to include in key factor analyses.

Relative population estimates of adult greyback canegrubs caught in light traps were not correlated with subsequent numbers of established grubs in tall crops of cane. At all locations, maximum population density of grubs was estimated at 2 - 4 grubs per stool in 1994-1996, despite adult catches ranging from 25,000 beetles per trap-week in the Burdekin to a maximum of 16 beetles per trap-week in the Herbert Valley over this period. A density compensating mechanism to limit the number of grubs establishing on the host plant is suggested from this work and also in experimental studies. Intraspecific competition is known to limit upper population size in related species of melolonthine scarabs. Beetle control therefore may be 'replaceable' mortality, and current attempts in the Burdekin district to control greyback canegrub by insecticidal control of beetles or by using light traps is futile. Grub survival determines adult population size, but adult population density has no influence on density of grubs subsequently established on sugarcane stools, over a wide range of population densities.

Analysis of population changes within and between seasons at locations excluding the Burdekin indicated that incidence of *Adelina* sp. tended to stabilise population changes, with proportionately higher mortality when initial grub population was high. Incidence of *Adelina* sp. was density-dependent, but with a lag effect indicated at the Tully site. Incidence of *Adelina* sp. peaked the year after maximum population density of the host

grub. Disease incidence then declined as the grub population declined. Population density of greyback canegrub increased again at Tully between 1997 and 1998, when incidence of *Adelina* sp. reached a low of 5 - 10% of the grub population.

Incidence of *Metarhizium anisopliae* was highest in greyback canegrubs at the Tully monitored site, but was relatively constant between seasons (23 - 28% of the grubs affected each season). The action of this entomopathogen was to lower the average population density at sites with the disease, but the pathogen had no dynamic effect on population changes of the host grub between seasons, as assessed by k-factor analysis. The method of assessing disease infection only in the early part of the grub development period may have under-estimated the impact of *Metarhizium* on population dynamics of greyback canegrub.

A general model explained population changes in greyback canegrub in terms of density-dependent regulation by *Adelina* sp. at intermediate pest densities, population growth from low densities as a consequence of escape from natural enemies, and population decline from high densities as a result of intraspecific competition for limited food resource. Population densities of grubs in the Burdekin district are high because the populations have escaped natural control from entomopathogens. Observed cycles of outbreaks and subsequent crashes in populations of greyback canegrub are explained by delayed density dependence of infection rate by *Adelina* sp, relatively poor persistence of *Adelina* sp. at low population densities of the host grub, and resurgence of grub populations from low density following decline of disease incidence. A stable point of no nett population change is predicted from the model, and estimated at 0.7 grubs per stool from the density relationships between *Adelina* sp. infection and greyback grubs established under cane early in the season.

Studies to explain the virtual absence of entomopathogens from greyback canegrub in the Burdekin district suggest that ratoon cultivation, short crop cycles between intensive cultivation periods, and burning of trash may be responsible in part or in combination for low persistence of diseases and the maintenance of high densities of the pest in this district. Results from a Tully trial comparing three trash management practices showed lowest levels of *Adelina* sp. infection in grubs under burnt-cultivated ratoons in both years, despite the highest population density of greyback grubs under this treatment. At monitoring sites, *Adelina* sp. infection was shown to be density-dependent, and higher mortality at higher grub densities was predicted in the trash management trial. Greyback grub numbers are suppressed under green trash blankets, even without entomopathogens. Low incidence of *Adelina* sp. despite high population of grubs in burnt-cultivated ratoons therefore indicates suppression of disease in this treatment.

Laboratory studies by CSIRO Entomology showed that the infective oocyst of *Adelina* sp. is fragile and easily destroyed by handling. The implication is that *Adelina* sp. may not persist with intensive cultivation, and disease is suppressed in the Burdekin grubs by current farming practices. Another protozoan, *'Nosema'* sp. (Microsporida), is less commonly found causing death in greyback canegrubs in far north Queensland. Studies have shown that spores of this entomopathogen are relatively robust, and that the disease is readily transmitted to greyback grubs in the laboratory. Young grubs artificially infected with $2x10^6$ spores of '*Nosema'* per gram of diet yielded an average of $5x10^9$

spores per grub on death. *'Nosema'* sp. may be suitable for mass-rearing and release as a biological control agent in areas like the Burdekin which have an ongoing outbreak of greyback canegrub and low levels of natural enemies. Further research is in progress on production of this pathogen as a biological control agent.

Further research and development is needed to determine the impact of different farming practices, including use of pesticides, on the persistence and infectivity of entomopathogens, and subsequently on population dynamics of greyback canegrub, in the Burdekin district in particular. Incidence of pathogens is very low in the Burdekin district, and any increase in pathogen incidence will occur slowly after adoption of minimum-disturbance farming practices. Slow development of natural control must be accommodated within development and extension programs aimed at increasing the adoption of minimum-tillage planting, trash blanketing and no-tillage ratooning by canegrowers. The availability of *Metarhizium anisopliae* (BioCane^{TM}), and perhaps *'Nosema'* sp. as inundative biological control agents, will allow more rapid establishment of natural control.

1.0 INTRODUCTION

Greyback canegrub, *Dermolepida albohirtum* (Waterhouse) (Coleoptera: Scarabaeidae: Melolonthinae) is the most economically damaging of the insect pests of sugarcane in Australia (Robertson *et al* 1995). Losses currently exceed $5 m per annum, largely as a result of a continuing outbreak of the pest in the Burdekin River district in north Queensland. The grub stage feeds on the root system of well-grown sugarcane, causing poor growth, depressed sugar content, lodging, and sometimes death of the plant between February and July each year. In addition, severe damage to the cane stool prevents a subsequent ratoon crop, and removal of stools at harvest contributes to high levels of soil and extraneous matter entering the mill. These indirect costs have not been calculated.

Greyback canegrub is a native insect which has caused damage to sugarcane since the crop was first grown in north Queensland in the 1870s. Early attempts to control the pest were largely unsuccessful until benzene hexachloride (BHC) was introduced in 1948. Damage to sugarcane by greyback canegrub was then restricted to small areas each year as approximately 26,000 ha of canelands were treated each year with BHC (Wilson 1969). Following the removal of organochlorine insecticides, a controlled-release insecticide based on chlorpyrifos (suSCon® Blue) was the sole registered insecticide for control of greyback canegrubs. This product is currently registered to control greyback canegrub in the plant crop, with subsequent ratoons susceptible to infestation by the grub (Robertson *et al* 1997).

Damage by greyback canegrub increased dramatically through the 1990s at locations from Sarina in central Queensland to Mareeba in far north Queensland. Most severe damage has been recorded in the Burdekin delta over the past eight years, with the area of damage increasing each year during that period. Estimates from Cane Protection and Productvity Boards in the Burdekin indicate approximately 4000 ha damaged with about 200,000 tonnes of cane lost in 1998.

Annual records of areas damaged by pests have been kept by the Bureau of Sugar Experiment Stations and Cane Protection and Productivity Boards since early this century. Before the introduction of BHC, the area damaged by greyback canegrub each year fluctuated between several thousand hectares and '…almost complete absence of any noticeable grub damage'. The spectacular decline in area damaged by greyback canegrub in 1922 and 1935 was thought to be caused by dry spring conditions, but this hypothesis is not supported by the evidence (see Robertson *et al* 1997).

Severity of damage by root-feeding whitegrubs (larvae of melolonthine scarabs) is in direct proportion to the numbers of the pest per plant or per unit area (eg see Allsopp *et al* 1995). Population density of greyback canegrub was seldom measured by early observers, but the variable levels of damage by the pest implies considerable fluctuation in numbers of grubs per unit area. No studies have been undertaken on the population dynamics of greyback canegrubs, or indeed on any canegrubs in Australia, despite their economic importance. Most research and development on controls for canegrubs was on insecticides, and insecticides have been used against greyback canegrub for 50 years. Failure in the Burdekin of the only registered product against greyback canegrub as a result of enhanced microbial degradation mediated by high pH (Robertson *et al* 1998)

stimulated a reappraisal of current knowledge and possible controls for this pest (see Robertson *et al* 1995).

Sound knowledge of the population dynamics of any pest is essential to develop cultural and biological controls, and even to improve insecticidal control. Population studies of related whitegrubs resulted in rapid advances in understanding and development of controls in other agricultural systems (eg East *et al* 1986; Robertson and Kettle 1994). The project reported here was initiated as a first step to improve the understanding of population dynamics of greyback canegrub, and to provide a rational base upon which to develop effective management strategies for this major pest of sugarcane.

The project started with support from BSES in November 1993, and was funded by Sugar Research and Development Corporation (SRDC) from July 1994. This report includes the period not formally covered by the SRDC project.

2.0 OBJECTIVES

The initial objectives as outlined in the SRDC-funded study were to:

- study dispersion of greyback canegrubs in the soil profile and devise a sampling program to estimate population density with known precision;
- monitor population density of greyback adults, eggs and larvae;
- identify mortality factors which act on each life stage including climate, natural enemies and farming practices;
- determine key factors which control greyback population changes and suggest ways to suppress population growth;
- develop a model which predicts changes in greyback populations.

Following a review of the project by SRDC in April 1995, the project was expanded to encompass the following additional objectives:

- assess the effects of crop management strategies on frequency of occurrence of diseases in greyback canegrubs;
- demonstrate pathogenicity of selected microorganisms to greyback grubs; determine dose/response relationships (ie infectious dose, time to death, effects on larval feeding and growth);
- attempt small-scale *in vitro* culture of selected microorganisms which display pathogenicity to grubs.

3.0 DISPERSION OF GREYBACK CANEGRUBS WITHIN CANEFIELDS AND DESIGN OF SAMPLING SYSTEM (Objective 1)

The dispersion of organisms affects the estimation of population densities; the populations of individual organisms can range from highly aggregated or clumped, through to randomly dispersed or even uniformly dispersed. With subterranean insects like greyback canegrubs, the individuals cannot be counted directly. A destructive sampling system involves the removal of soil samples and subsequent extraction and counting of canegrubs (eg see Allsopp *et al* 1995).

The precision of population density estimates is a function of dispersion and the number of soil samples taken, and must be balanced by the cost of removing and processing large numbers of samples. Sampling errors of 10 - 20% of the sample mean estimate are considered acceptable in studies of population dynamics of insects (Southwood 1978), and 25% standard errors will detect a doubling or halving of population size between sample estimates.

The methods and results of the studies on greyback canegrub dispersion are presented in Attachments 1 and 2 (Walker *et al* 1998a, 1998b, in prep). The size of the sampling unit was a cube of soil, 30 cm x 30 cm x 30 cm deep, taken with a 18 cm wide spade or shovel (following Allsopp and Bull 1989 and Allsopp *et al* 1995). Samples were taken from the centre of the row and included a cane stool. In addition, samples were taken from the inter-row space, and from the side of the stool (approximately 15 cm from the centre-line of the row of cane).

Greyback beetles lay approximately 26 eggs in clusters, and these clusters were only rarely encountered during field sampling. Beetles lay their eggs anywhere from the centre of the cane row to the centre of the inter-row space, and at depths from 2 – 40 cm from the soil surface. Because a large number of soil samples was required to give population estimates of eggs (several hundred samples to give 25% standard error of mean), population density of eggs could not be monitored with acceptable precision.

First stage grubs are generally found at shallower depths than third stage grubs (Attachment 1, Walker *et al* 1998a). In excess of 80% of grubs were found above 20 cm depth in Tully and Stone River (Herbert Valley) over the period of active grub feeding (January to June). Grubs could be found down to 30 cm over the same period in the Burdekin, where cane is grown in higher ridges than at Tully or the Herbert. First stage grubs were commonly found in the inter-row space as well as on rows of cane, although virtually all third stage grubs were in the row by March. The sampling program adopted for this project thus involved soil samples taken from the cane rows and adjoining side of row early in the period when first and second stage grubs were expected (January - February), and only from the row from March when most grubs were actively feeding close to cane stools. Sample size was standardised to 30 cm^3 in the Burdekin, and 25 cm^3 elsewhere, based on the difference in depth of grubs and shape of row profiles between the Burdekin and elsewhere.

Greyback canegrubs within cane rows tended to be slightly aggregated, and this dispersion was similar under burnt cane and trash blankets (Attachment 2, Walker *et al* 1998b). This

distribution was also similar to that found for southern Queensland canegrub species by Allsopp and Bull (1989). The sample variance was found to be related to the mean over a range of population densities, following Taylor's power law (see Southwood 1978). Transformation of data (by $x^{0.4}$) stabilised the variance sufficiently to allow the use of parametric statistical methods, in a similar way to that found by Allsopp and Bull (1989).

Sampling to achieve 25% standard error of the mean generally required 15 - 30 samples, over the range of 1 - 3 grubs per sample (see Figure 1, Attachment 2, Walker *et al* 1998b). Sampling of grubs therefore consisted of taking at least 15 samples per monitoring site at least once per month. Sampling early in the season when grubs were small and more dispersed (December to February) usually involved 30 samples every two weeks. From March, at least 15 samples were taken every four weeks. The samples were randomly selected within a delineated section of a canefield of approximately 0.1 ha. Entire fields were not sampled, to reduce the variability inherent in greyback canegrub infestations across fields.

4.0 MONITORING OF POPULATION DENSITY OF GREYBACK ADULTS, EGGS AND LARVAE (Objective 2)

Four districts were selected to monitor changes in population density of greyback canegrubs, viz Burdekin River delta, Tully, Herbert Valley and Innisfail. Locations within each district were selected on the basis of current damaging infestations of greyback canegrub. Number of samples taken at each location varied in 1993 - 1994, when dispersion of greyback canegrubs was being determined. From the 1994 - 1995 season, number of samples was standardised at 30 per site at each sampling period until February (15 on row and 15 adjoining cane row), and then 15 per site taken on the cane row each month until June.

Greyback canegrubs recovered alive from the samples were placed individually in 200 mL plastic containers with soil from the field in which they were found, and with a piece of cane for food. The grubs were weighed soon after collection, and kept in an incubator at 26^{o} C (plus or minus 1^{o}C) for up to one week before being air-freighted to CSIRO Entomology in Canberra for screening for entomopathogens. Samples from each sampling period were screened for diseases in 1993 - 1994, but only grubs collected before March were screened in following years. Details of screening procedures are given in Dall *et al* (1995).

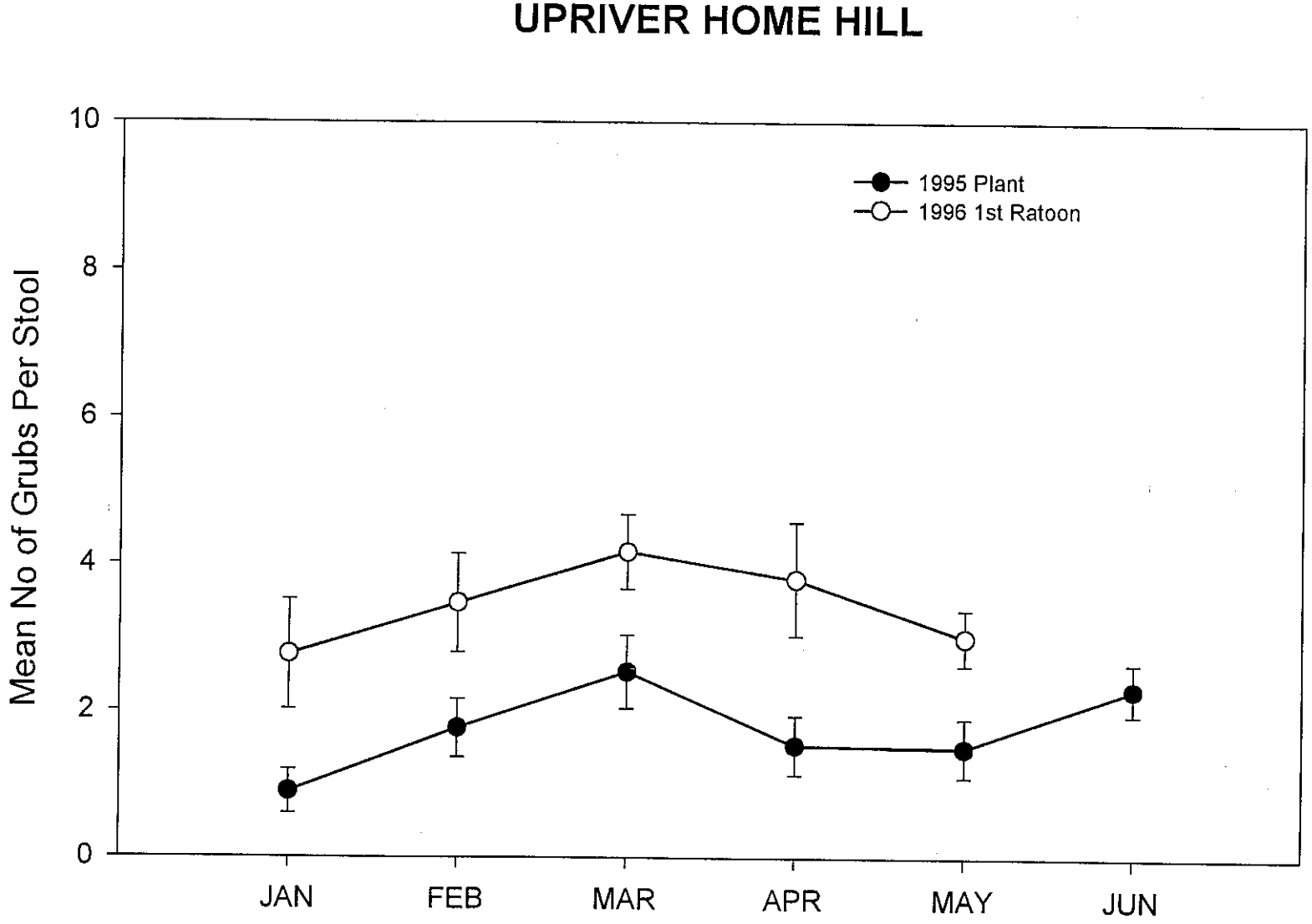

Figure 1. Mean number of greyback canegrubs per stool during the grub development period in one field on the property of K. Fowler, Upriver Home Hill, Burdekin district, 1995 and 1996. (Standard error of mean estimates indicated by vertical bars).

The timing and relative size of flights of adult greyback canegrub beetles were recorded with light traps at Tully, Burdekin and Herbert each year, and at Innisfail from 1994 until 1996. The traps were white fibreglass cones with the apex removed and inverted to funnel greyback beetles downward into a steel drum. A 250 watt mercury-vapour light bulb was suspended with clear perspex vertical baffles over the centre of the cone to attract and intercept flying beetles. Counts of beetles were done at least weekly during the flight period each year, and with the sex of beetles determined from samples in 1993 - 1994 and 1996 - 1997 in the Burdekin, and from 1994 until 1998 at Tully.

4.1 Burdekin

Three sites were monitored in detail in the Burdekin River delta. The sites could not be sampled continually throughout the five-year program because crops were ploughed out and replaced due to damage by greyback canegrub within the study period. All sites were on alluvial sandy silt loam soil (58 - 73% sand fraction) near the Burdekin River, and the soils were alkaline (pH 8 - 9). The insecticide suSCon® Blue was not applied to the study sites for canegrub control because of accelerated breakdown of the active ingredient (see Robertson *et al* 1998).

Several light traps were operated by canegrowers throughout the study period. Greyback beetles began emerging in the first week of October each year. Rainfall was not necessary to initiate emergence because the Burdekin canegrowing district is fully irrigated. Peak trap catches were through November and December each year, with some beetles still caught during January. Beetles were not counted due to the large numbers caught in each light trap, although the volume of beetles caught was recorded from one trap using a 1 L measuring container, with approximately 190 beetles per L. Trap catches reached approximately 23,000 beetles per trap-week in the week ending 1 December 1993, and approximately 25,000 beetles per trap-week in the week ending 4 December 1994, at a riverbank trap adjoining a monitored field on the property of K Fowler, Kirknie Road, Home Hill.

A first-ratoon block of cultivar Q96 was monitored for greyback canegrub population changes on the property of P Cvjetanovic, Kirknie Road, Home Hill through 1994. The site was adjoining that of K Fowler, where the light trap was operated (see above). The highest numbers of greyback canegrub at the site was estimated at 1.3 grubs per stool (0.6 s.e.) on 26 April 1994, although population density varied little through the season (1.2 per stool on 5 April to 1.0 per stool on 7 June 1994). The site was ploughed out after harvest in 1994.

A second site in a field of cultivar Q117 was monitored through 1994-1995 (plant crop) and 1995-1996 (first ratoon) on the property of K Fowler, approximately 200 m from the light trap (see above). In the plant crop, a peak population of three grubs per stool was estimated on 27 March 1995, with 2.3 grubs per stool still present on 6 July 1995 (Figure 2). The field was ratooned, and the population of greyback canegrub reached 4.2 grubs per stool on 5 March 1996. The population was estimated at 2.3 grubs per stool on 27 May 1996 (Figure 1), and the field was ploughed after harvest in June 1996, as a result of severe damage by greyback canegrub.

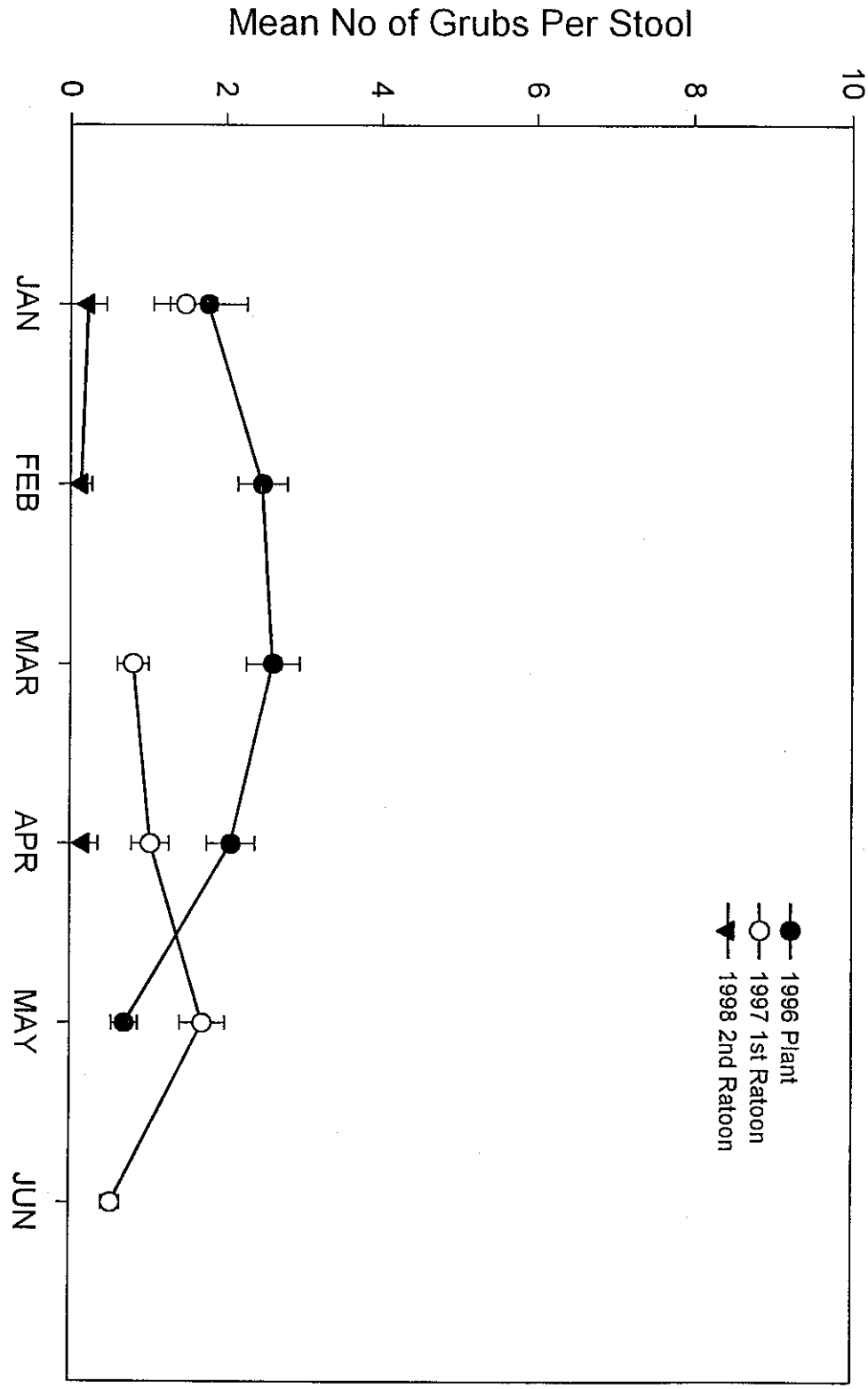

Figure 2. Mean number of greyback canegrubs per stool during the grub development period in one field on the property of J. Stevens, Clare, Burdekin district, 1996-1998. (Standard error of mean estimates indicated by vertical bars).

A third site in the Burdekin was monitored from 1995 - 1996 until 1998. This was located on the property of J Stevens, near Clare. No light trap was operated for beetle flight monitoring, although large numbers of beetles were seen each year, and damage by greyback canegrub was severe in the vicinity since 1990 (see Chandler *et al* 1993). In the plant crop (Q96), numbers of greyback canegrub peaked at 2.6 per stool on 28 March 1996. Numbers declined to an estimated 0.7 per stool on 23 May, and 0.8 pupae per stool were found on 12 September 1996. A further assessment on 14 November 1996 after the beginning of the beetle emergence period recovered 0.65 pupae and teneral adults per stool (Figure 2).

In the first ratoon crop, 1.5 greyback grubs per stool were recovered on 11 January 1997, and an estimate of 1.7 grubs per stool was made on 2 May 1997. In the second ratoon in 1998, low numbers of greyback canegrub established, probably because of the relatively poor crop growth due to grub damage in previous years. Numbers of greyback canegrubs were maintained at 0.4 - 0.6 per stool at the Stevens site through 1998 (Figure 2).

A total of 238 greyback canegrubs were collected from the Burdekin in 1994, and 144 survived to pupation. Of those which died, seven grubs (3% of the total population) were infected with *Adelina* sp. (Protozoa: Coccidia), 33 with fungi including *Metarhizium anisopliae* (Fungi imperfecti) (14%) and three had *Bacillus popilliae* (a bacterium causing 'milky disease'). In 1995, 383 grubs collected from the Burdekin before April were sent to CSIRO Entomology for screening for entomopathogens. Forty-three grubs survived to pupation (94% mortality), but only two showed *Adelina* sp. (0.5% of total population), two developed *Metarhizium*, one died with *B popilliae*, and one with entomopox virus. Thus, primary pathogens accounted for only 1.5% of the total population assessed in 1995.

In 1996, 437 greyback canegrubs collected before April were held at CSIRO Entomology in Canberra, and assessed for pathogens when they died. Of 364 which died before pupation (83% mortality), only one had *Adelina* sp. infection, three had *Metarhizium* and one had an entomopox virus. Thus, microorganisms accounted for 1% of the population of greyback canegrubs collected from the Burdekin in 1996.

Similar results were recorded in 1997, when 58% of 458 greyback canegrubs collected died before pupation. Three individuals were infected with *Adelina* sp. at death (0.7% of total population), one was assessed with *Metarhizium* and one with entomopox virus. This gave a total of 1% infection by entomopathogens in the greyback canegrubs from the Burdekin in 1997.

Twenty-two percent of 226 grubs died before pupation, in the sample sent from the Burdekin in 1998. None showed symptoms on any entomopathogen.

4.2 Tully

A first-ratoon block of cultivar Q138 was selected for monitoring on the Tully Sugar Experiment Station in 1993-1994 (field 26B). This location had a history of damage to cane by greyback canegrub, with an adjoining field suffering damage as a first-ratoon crop in 1992 (see Chandler *et al* 1993, Trial EN90-9). The monitored field had been planted in 1992 without insecticide. The site was on recent alluvium (28% sand, 31% silt and 41%

clay) and had grown sugarcane since 1974. The crop was harvested early each year (June or July), with all trash retained, and fertilised to maintain good crop growth. Early harvested ratoons generally attract disproportionately higher numbers of egg-laying beetles of greyback canegrub (Ward and Cook 1996).

A light trap was sited on the Tully Sugar Experiment Station, approximately 300 m from the monitored field. Greyback beetles were caught in the light trap after at least 40 mm rainfall in October or November each year (following the dry season). First catches in each season were 16 November 1993, 1 November 1994, 5 November 1995, 20 October 1996 and 12 October 1997. Peak flights were generally through November and December, and catches ceased by late January. A maximum of 190 beetles were caught per trap-night (November 1995 and November 1996) with little difference in numbers caught between years. The sex ratio of beetles caught in traps seldom varied from 1:1. All ages of beetles were caught, as determined by dissection and examination of female reproductive condition (see Illingworth and Dodd 1921) and general condition of scales on the elytra.

Greyback grubs were first found in soil in January each year. Highest numbers were recovered on 27 January 1994 with a mean of 9.4 per stool (1.4 s.e.). Numbers of greyback canegrub steadily declined through 1994, and a mean of 1.2 grubs per stool remained in June (87% decline in numbers during the grub growth period in 1994) (Figure 3).

Of 282 greyback grubs collected from the site in 1994 and held for observation at CSIRO Entomology in Canberra, 60% died before pupation. Forty-five grubs (16% of the total) were characterised as being infected with *Adelina* sp. while 64 (23% of the total) were infected with *Metarhizium anisopliae* (including isolate FI 1045). In addition, ten grubs were infected with *Bacillus popilliae*, and four with a bacteria resembling *B sphaericus*. The organism resembling *B sphaericus* was cultured in the laboratory but did not reinfect healthy greyback grubs (Dall *et al* 1995) and was therefore not confirmed as a primary pathogen of this pest species. Of the grubs which died with infections of primary pathogens, most were collected early in the season. Grubs collected after April generally did not express infections of microorganisms, and therefore entomopathogens were thought to act early in the life cycle of greyback canegrub. Subsequently, only grubs collected before April were sent to CSIRO Entomology for screening.

Lower numbers of greyback grubs established at the site in January 1995 (now second ratoon) compared to the previous year. A maximum of 2.9 grubs per stool (1.1 s.e.) plus 21 greyback eggs in a cluster in one sample were recovered on 23 January 1995. Numbers declined throughout the grub growth period in 1995, to give 0.1 grubs per stool in June 1995 (Figure 3), a decline of 97% (excluding unhatched eggs from calculation).

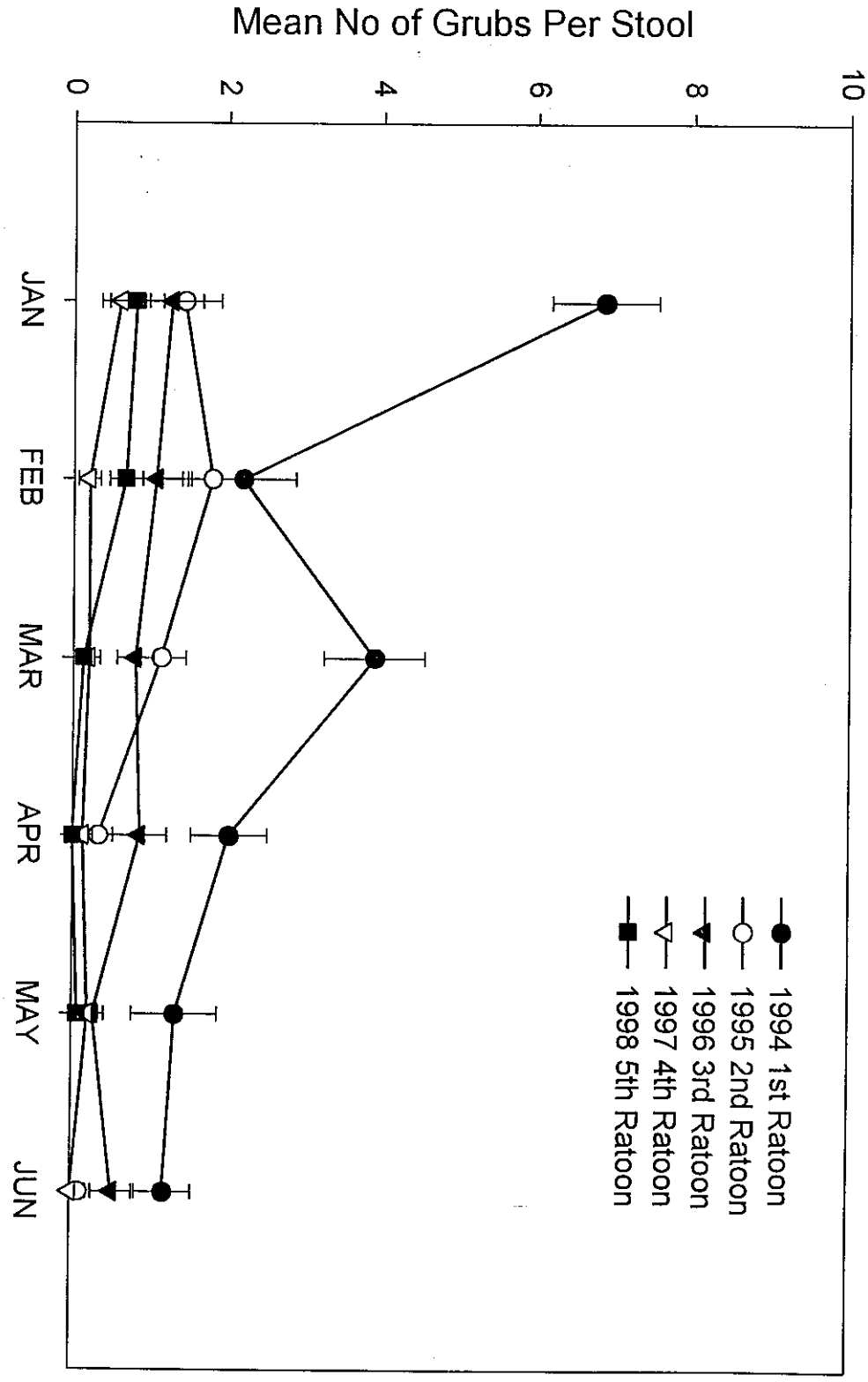

Figure 3. Mean number of greyback canegrubs per stool during the grub development period in field 26B on the Tully Sugar Experiment Station, 1994-1998. (Standard error of mean estimates indicated by vertical bars).

A total of 135 greyback grubs from this site was sent to CSIRO Entomology in Canberra, and 132 (98%) of these died before eclosion. Of these, 54 were identified with *Adelina* sp. infection (40% of the total population), 38 died with *Metarhizium* (28% of total), one had *B popilliae* and one had an entomopox virus.

In the third ratoon, a maximum of 1.3 greyback grubs per stool (0.4 s.e.) was recovered on 10 January 1996. The population density per stool declined throughout the grub growth period to 0.5 grubs per stool in June 1996 (78% decline) (Figure 3). An estimate of 0.2 grubs per stool was made in May 1996 (91% decline). Standard errors of mean density estimates increase as grub populations decline.

In 1996, 84 greyback canegrubs were held at CSIRO Entomology for examination. Seventy-four of these died, 11 were infected with *Adelina* sp. (13% of the total population) and 19 died with *Metarhizium* (23% of total). No other pathogens were recorded from greyback grubs from this site in 1996.

In the fourth ratoon, the population density of greyback canegrub peaked at this site in the samples taken on 2 February 1997 when a mean of 0.6 grubs per stool (0.2 s.e.) were recovered. The population density declined to undetectable levels by June, with 0.2 grubs per stool recorded in May (Figure 3).

Thirty-nine grubs were sent to CSIRO Entomology from this site in 1997, and 23 of these died before pupation. Four were identified with *Adelina* sp. (10% of the total population) and 10 were infected with *Metarhizium* (26% of total). No other diseases were recorded from greyback grubs from this site in 1997.

In the fifth ratoon, a mean of 0.8 (0.3 s.e.) grubs per stool was established on 14 January 1998, and this declined through the season to less than 0.1 per stool on 28 May 1998. A total of 22 grubs were sent for examination of pathogens, and 17 of these died before pupation (77%). Only one individual was infected with *Adelina* (5% of total), and six were infected with *M anisopliae* (27% of total).

4.3 Herbert

Beetle activity was assessed by examining trees favoured by greyback beetles on the banks of Stone River in 1993 - 1994. Beetles were first recorded feeding on foliage of sandpaper fig trees at Stone River on 17 December 1993 following 36 mm of rain, and were present in low numbers until 26 January 1994. Infestations of greyback canegrub in sugarcane fields in the vicinity of Liborio's Road were not found until 8 March 1994, when 1.6 grubs per stool (0.9 s.e.) were recovered from a fourth ratoon field of Q115 adjacent to Stone River, on the property of R Romano, Liborio's Road. The sampling undertaken on 9 May 1994 gave an estimate of 3.2 grubs per stool. At the next sampling in July 1994, no live grubs were recovered from this site. The site was cultivated and replanted in 1995.

Of 54 greyback canegrubs sent to CSIRO Entomology in May 1994, 40 died before pupation (74% mortality) and all of these were infected with *Adelina* sp. One individual also had dual infection with *Bacillus popilliae* and *Adelina.*

A light trap near the house of R Romano began catching greyback beetles on 23 November 1994, with peak catch of 16 beetles per trap-week between 21 December 1994 and 6 January 1995. Beetle flights ceased in late February 1995. No infestations of greyback canegrubs could be found on the property of R Romano, but a field of Q115 on the opposite bank of the river was found to be infested. This field of third ratoon was on the property of S Leonelli, Liborio's Road, approximately 200 m from the field monitored in 1994. A mean of 3.3 grubs per stool was recovered on 9 March 1995, and this declined to 1.1 grubs per stool by 15 June 1995. The field was ploughed out after harvest in July 1995, and replanted.

A total of 216 greyback canegrubs was sent to CSIRO Entomology from the location in 1995, and 193 of these died before pupation (89% mortality). Four of the dead grubs were found to be infected with *Adelina* sp. and a further two with *Metarhizium.*

Greyback canegrub beetles were caught in low numbers in the light trap at R Romano from 6 December 1995 until February 1996. No infestations of greyback canegrub could be found in 1996 in the vicinity of Liborio's Road, Stone River, on properties belonging to R Romano, S Leonelli, A D'Urso and R Seri. Monitoring of greyback populations was shifted in 1996 to Gap Creek Road, Bambaroo, where damage by greyback grubs was seen on the property of B Benassi in May 1995. No light trap was operated at Bambaroo, but large numbers of greyback beetles were found feeding on foliage of a cluster fig from 6 December 1995, near the site where cane was damaged in early 1995.

An infestation of greyback canegrub estimated at 3.4 grubs per stool was found in a fourth ratoon of Q124 at Gap Creek Road, Bambaroo on 22 March 1996. This field was ploughed out after harvest in June 1996. A total of 208 greyback grubs from this location were held at CSIRO Entomology in 1996 and 139 died before pupation (67% mortality). Forty-eight individuals were infected with *Adelina* sp. (23% of the total population sampled), and no other pathogens were identified.

In 1996 - 1997, few greyback canegrub beetles were caught in the light trap at R Romano, Stone River. Extensive searching indicated very low populations of greyback canegrubs in the Liborio's Road area in February 1997. The highest population encountered was 0.3 grubs per stool in a small area of one canefield at R Romano, found on 24 February 1997. Sampling was abandoned at this locality in 1997.

A second ratoon of cultivar Q124 on Gap Creek Road, Bambaroo (B Bonassi) was found to be infested with 1.3 grubs per stool on 28 January 1997 and 1.7 grubs per stool on 24 February 1997. The population density at this location declined to 0.4 grubs per stool in March, and less than 0.1 per stool by April and May. No grubs were detected at the site in June 1997 (Figure 4). Of 43 grubs collected from the location in 1997, 40 died in the laboratory of CSIRO Entomology before pupation (93% mortality), and 34 of these were infected with *Adelina* sp. No other pathogens were recorded.

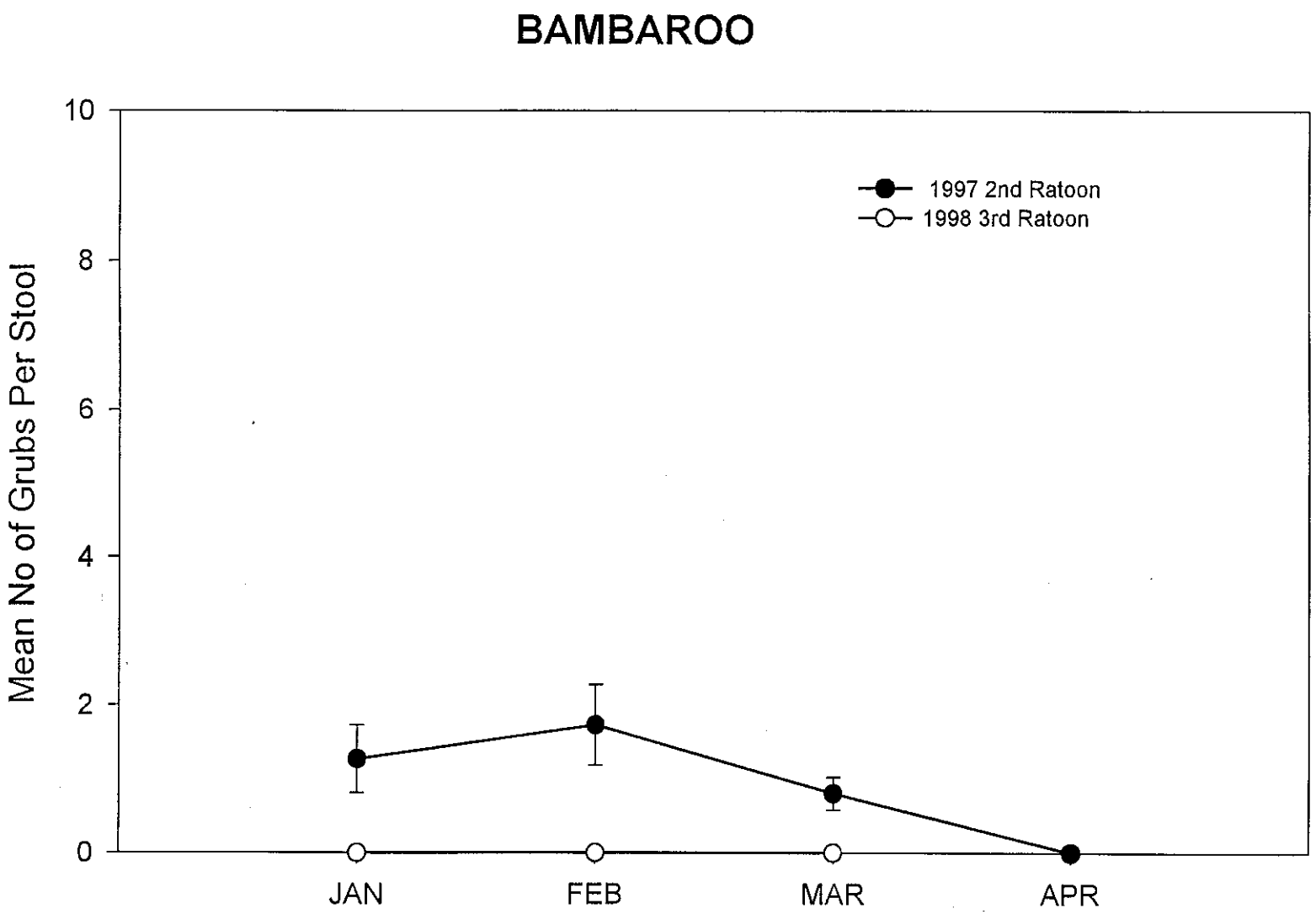

Figure 4. Mean number of greyback canegrubs per stool during the grub development period in one field on the property of B. Bonassi, Bambaroo, Herbert district, 1997 and 1998. (Standard error of mean estimates indicated by vertical bars).

The crop was harvested in July 1997 and ratooned. This third ratoon crop was assessed for greyback canegrubs in January, February and March 1998 without grubs being detected (Figure 4). A small portion of another field of second ratoon Q124 on the same farm, approximately 250 m from the field monitored in 1997, was found to be infested with greyback canegrub at 0.8 grubs per stool (0.3 s.e.) on 6 March 1998. This population declined to less than 0.1 grubs per stool in April and May, and no grubs were detected at the site when monitoring in June 1998. Fifty-eight percent of 38 grubs collected at the site in 1998 died before pupation in the laboratory at Canberra. Thirteen of these (34% of total) were infected with *Adelina* and another one had *M anisopliae* (3% of total sample).

The light trap operated at Liborio's Road, Stone River caught greyback beetles from 16 December 1997 after 48 mm of rain the previous week. A maximum of 102 beetles per trap-week was recorded on the week ending 22 December 1997. No beetles were caught after 5 January 1998. Greyback beetles were common on the foliage of a cluster fig on Gap Creek, Bambaroo, over the same period.

4.4 Innisfail

Systematic sampling of greyback canegrub began at Nicholson Road, Mundoo, in the Innisfail district in 1995-1996, after aborted attempts at Silkwood in 1993 - 1994 and Boogan in 1994-1995. Populations of greyback canegrub declined to undetectable levels at the site at Silkwood, and the Boogan location was planted to bananas. Limited data on greyback canegrub populations were collected from both locations before moving the monitoring to Mundoo.

Greyback canegrub beetles were trapped at Nicholson Road, Mundoo, from 14 November 1995 until mid January 1996. The peak greyback beetle activity was during the week ending 25 November 1995, when 1,700 beetles per trap-week were recorded. Greyback canegrubs were recorded from 11 January 1996 in a first ratoon crop of cultivar Q152 on the property of B Torrisi. The field had been treated with suSCon® Blue insecticide at planting, but the grower had been getting no apparent control of greyback canegrubs in first ratoons after using the insecticide in the plant crop (Chandler and Erbacher 1997). The site was on red volcanic (krasnozem) soil with approximately 70% clay content.

A maximum density of 3.3 grubs per stool (0.5 s.e.) was recorded on 15 March 1996, and numbers declined through the season to less than 0.1 per stool by 27 June 1996 (Figure 5). A total of 91 greyback canegrubs from this site were held at CSIRO Entomology in Canberra and 86 died before pupation (95% mortality) and were examined for entomopathogens. Sixty-two individuals were infected with *Adelina* in the 1996 collection (68% of the total population collected), six were infected with *Metarhizium*, and one with a microsporidian resembling *Nosema* sp. (Protozoa: Microsporida).

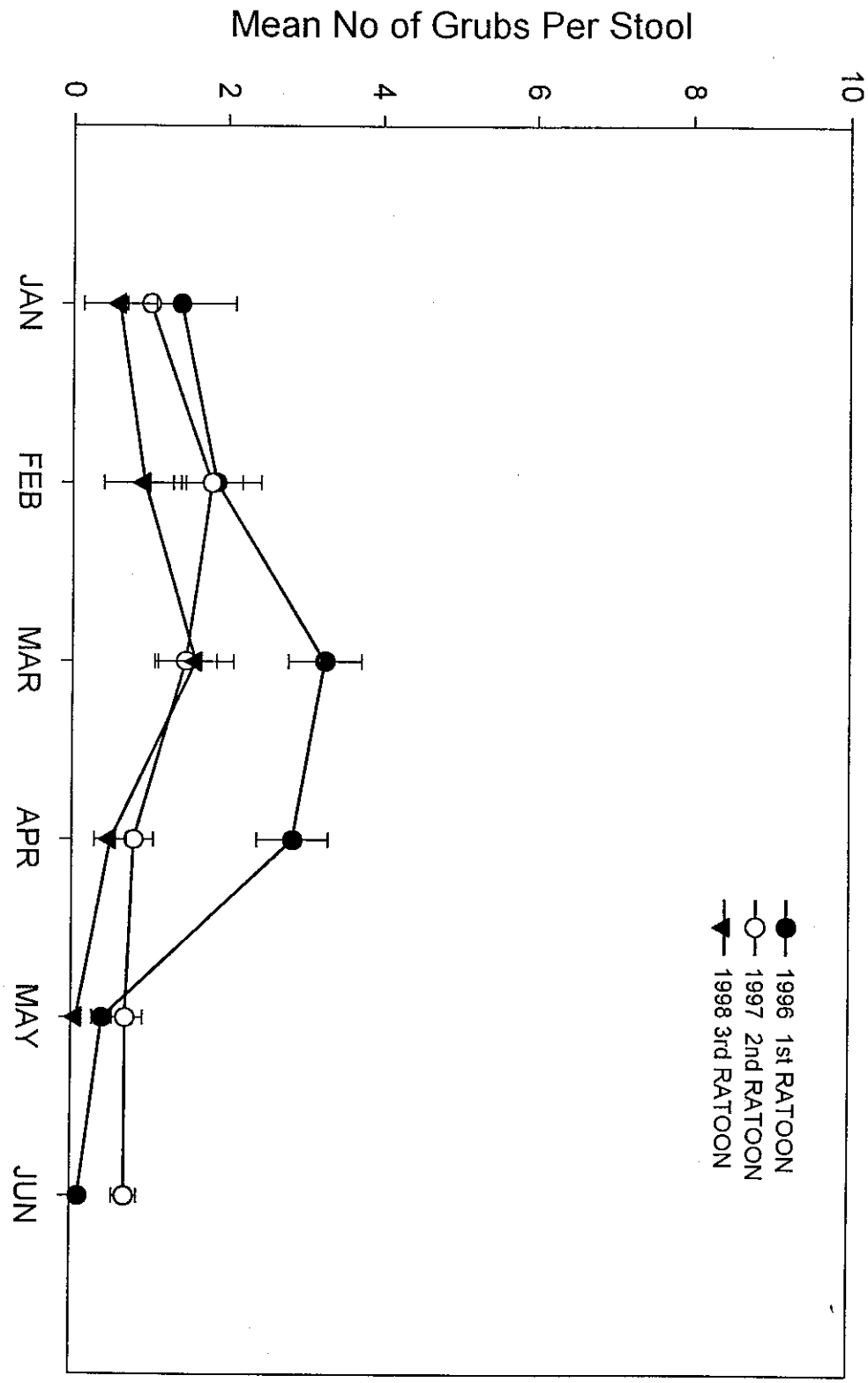

Figure 5. Mean number of greyback canegrubs per stool during the grub development period in one field on the property of B. Torrisi, Mundoo, Innisfail district, 1996-1998. (Standard error of mean estimates indicated by vertical bars).

The crop was ratooned after harvest in July 1996. Greyback canegrub beetles were caught in the light trap on Nicholson Road from 12 November 1996 until trapping ceased in late December 1996. Peak numbers were caught in the week ending 18 November (170 per trap-week). Greyback grubs were detected at the site (now second ratoon) on 3 January 1997 when 1.1 per stool were recovered. Maximum density was recorded on 21 February 1997 with 1.8 grubs per stool (0.4 s.e.). The population density declined through the 1997 season, to less than 0.1 per stool on 11 August 1997 (Figure 5). Sixty-five greyback canegrubs from this site were held at CSIRO Entomology, and 54 of these (83%) died before pupation. Forty-one individuals were identified with *Adelina* sp. (63% of the total population collected from the site in 1997). In addition, one specimen was infected with *Metarhizium* and one with '*Nosema'* sp.

Following harvest in July 1997, the crop was ratooned and again monitored for greyback canegrubs. A light trap was not operated at Nicholson Road over the 1997-1998 greyback canegrub emergence period due to relocation of the equipment to a new infestation at Sarina. A population density of 0.6 greyback canegrubs per stool was estimated from the same site on 14 January 1998, and the maximum population of 1.6 grubs per stool (0.5 s.e.) was reached on 24 March 1998. Population density declined from that date, to reach a density of less than 0.1 grubs per stool on 28 May 1998 (Figure 5).

A total of 43 greyback grubs were sent to CSIRO Canberra in 1998. Forty of these died before pupation, and 34 were found to be infected with *Adelina* (79% of total).

5.0 MORTALITY FACTORS OPERATING ON GREYBACK CANEGRUB (Objective 3)

5.1 Climate

Climatic conditions including rainfall and maximum and minimum temperatures were recorded from the Burdekin, Herbert, Tully and Innisfail. In addition, maximum and minimum soil temperatures were recorded from soil at 15 cm depth at Tully Sugar Experiment Station, one of the grub monitoring sites. Gravimetric soil moisture was assessed from the upper 15 cm of soil at every sampling period.

Greyback canegrub populations were monitored over a wide range of climatic conditions, including the dry tropics of the Burdekin where cane is fully irrigated throughout the year, the humid tropics of Stone River and Bambaroo with dry periods generally extending over several months and approximately 2,000 mm annual rainfall, and the wet tropics at Tully and Innisfail with mean annual rainfall of approximately 4,000 mm.

Greyback canegrub beetles emerged after rainfall of 40 mm or more in November or December, following the relatively dry spring period in the Herbert, Tully and Innisfail. With irrigation throughout the year in the Burdekin, greyback beetles emerged during the first week of October each year, with large numbers active during November. In the Herbert Valley, the driest non-irrigated location, below-average rainfall was recorded in 1993, 1995 and 1996 (Table 1). Large-scale population decline

in greyback canegrub was attributed to below-average rainfall and dry soil over spring in early literature (Buzacott 1947; Volp 1947), but this hypothesis is not supported by correlating rainfall records with patterns of grub damage in far north Queensland early this century (Robertson *et al* 1997). Spring conditions were particularly dry in the Herbert district in 1993 to 1995 (Table 1), which immediately preceded an outbreak of greyback canegrub at Bambaroo; this further negates the hypothesis of population decline triggered by dry conditions.

Table 1 Monthly rainfall total (mm) for Ingham from January 1993 to May 1998. The mean annual rainfall from 1968 at this location is 2071 mm. (Data supplied by Bureau of Meteorology).

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual Total
'93	365	487	113	99	70	50	58	41	62	12	27	110	1495
'94	594	1222	87	113	36	57	33	7	8	42	27	121	2347
'95	121	603	202	90	109	52	4	95	5	67	41	38	1427
'96	346	128	323	100	27	96	26	10	30	110	15	102	1312
'97	325	441	620	137	95	70	13	125	53	30	72	469	2450
'98	916	244	204	382	263								(2009 to May)

Greyback canegrub beetles emerge at the start of the wet season, and lay eggs into wet soil. Greyback grubs develop in wet soil. Soil moisture levels were generally at field capacity (44%) or higher throughout the grub development period each year at Tully, and did not decline below 30% moisture by weight between January and June each year. Similarly, soil moisture in the Burdekin exceeded field capacity for a few days every 1 - 2 weeks with the furrow irrigation regime practiced in that district. The driest soils were in the Herbert, and tended to be below field capacity particularly late in the grub development period. Monthly rainfall totals for Ingham are given in Table 1.

The Tully grub monitoring site was inundated with floodwater for five days in early February 1994 following 750 mm of rain on 29 - 31 January. Grub population density was estimated at 9.4 grubs per stool (1.4 s.e.) on 27 January 1994, and 5.0 grubs per stool (1.5 s.e.) on 1 March 1994. The decline in density of greyback grubs could not be attributed to the flooded soil, because 60% of grubs collected from Tully and held at CSIRO Entomology died before pupation that season (see section 4.0, above) despite not being subjected to inundation.

The same site was inundated by floodwater for five days in March 1996. Population density of greyback canegrubs was estimated at 1.1 per stool (0.4 s.e.) on 1 February 1996 before the flood, and 0.8 per stool (0.2 s.e.) on 9 March 1996 after the flood. Over the same period, high mortality due to disease was recorded in grubs collected from this in 1996. It was concluded that submersion for five days as a result of flooded soil did not increase the mortality of greyback canegrub.

Greyback canegrubs developing in soil are buffered from extremes of temperatures, and mortality is probably not affected by short periods of high or low air temperatures. Several days of abnormally high temperatures were recorded during the week 25 - 31 December 1995, including a record high air temperature for December of 40.5^{o}C at

Cairns airport. Greyback canegrub beetles were active during this period, and a sample taken on 2 January 1996 from Silkwood showed that 45% of dissected females were gravid (n = 11). Grub populations were successfully established at Innisfail and Tully monitoring sites by January 1996 (see section 4.0, above). There was no evidence, *a priori*, that population density of canegrubs was affected by high temperatures.

5.2 Natural enemies

Six entomopathogenic diseases were recorded from greyback canegrubs over the course of this study. These were *Metarhizium anisopliae*, including isolate FI 1045 collected at Tully in 1993, and an entomopox virus also previously known from greyback canegrub (see Goodwin and Filshie 1975). In addition a second entomopox virus was isolated from greyback canegrubs at Stone River, Herbert Valley. The protozoan *Adelina* sp. was found in greyback canegrubs from all districts monitored, and a microsporidian (‘*Nosema’* sp.) was found in canegrubs in Tully and Innisfail districts. The bacterium *Bacillus popilliae,* isolates of which are known to cause ‘milky disease’ in a number of whitegrub species (eg Fowler 1974), was recovered from a few grubs throughout the range (see section 4.0 above).

Incidence of entomopathogens was recorded from each monitored site each year between 1994 and 1998 (see section 4.0, above). In addition, samples of greyback canegrubs were collected from sites between Mackay and Mareeba on a less systematic basis, and screened for entomopathogens at CSIRO Entomology in Canberra, in the same way as for grubs from monitored sites. These data gave an indication of general infection levels in greyback canegrub each year across several districts, and were used to supplement data from intensively-monitored sites. A summary of the disease incidence data is given in Table 2. Onstad (1993) advocated sampling of many sites and regressing disease incidence against host population density to evaluate the importance of pathogens and the threshold densities at which pathogens exerted an effect on host population dynamics.

Table 2 Summary of the incidence of two entomopathogenic diseases recorded from greyback canegrub collected from sugarcane fields monitored for grub population changes. Grubs were held at CSIRO Entomology, Canberra, until death or pupation each year between 1994 and 1998.

Location	Year	Pathogen (% incidence)	
		Adelina sp.	*M anisopliae*
Tully			
Tully 26B[1]	1994	16	23*
	1995	40	28
	1996	13	23
	1997	10	26
	1998	5	27
Innisfail			
Silkwood	1994	10	7
Boogan	1995	17	13
Mundoo[1]	1996	68	10
	1997	63	2
	1998	79	0

Table 2 continued

Location	Year	Pathogen (%incidence)	
		Adelina sp.	*M anisopliae*
Herbert Valley			
Stone River 1	1994	74	0
Stone River 2	1995	2	1
Bambaroo 1	1996	23	0
Bambaroo 2	1997	79	0
Bambaroo 3	1998	34	2
Burdekin District			
Pooled sites	1994	30	0
Pooled sites	1995	0.5	0.3
Pooled sites	1996	0.2	0.7
Pooled sites	1997	0.7	0.2
Pooled sites	1998	0	0

[1] same field monitored in consecutive years
[*] may include fungi other than *M anisopliae* in 1994

The lowest incidence of entomopathogens was recorded each year at the Burdekin monitoring sites. Overall mortality caused by entomopathogens declined throughout the study period, from 18% infection of total population sampled in 1994, to 1% infection of total population screened in 1996 and 1997. No individuals were detected with entomopathogens in the sample from the Burdekin in 1998. Although continuous monitoring of the population density of greyback canegrubs could not be maintained at the same site due to continual ploughout and replacement of crops, numbers of grubs were generally maintained at relatively high levels within each year. Area of cane damaged by greyback canegrub in the Burdekin increased substantially over the period of monitoring (Cane Protection and Productivity Boards Annual Reports, unpublished), and therefore area-wide population density of greyback canegrub undoubtedly also increased across this district between 1993 and 1998.

Conversely, incidence of entomopathogens was relatively high in the monitored sites in the Herbert, Tully and Innisfail, compared to the Burdekin. Population density generally declined within years, and between years, at each of the monitored locations outside of the Burdekin. There were significant correlations between percent mortality of greyback canegrubs measured by CSIRO Entomology, and incidence of *Adelina* infection, across all sites, in both 1994 and 1995 (Robertson *et al* 1996); viz:

1994; mortality = 28.6 + 0.58 (*Adelina*); $P = 0.03$, $R^2 = 0.64$;
1995; mortality = 64 + 2.55 (log *Adelina* + 1); $P = 0.04$, $R^2 = 0.55$,

(where mortality and *Adelina* percentages are arcsin-transformed before analysis).

These results suggest that 55 - 64% of the variance in mortality of greyback canegrubs is explained by incidence of *Adelina* alone. Monitoring of population changes in the field support the laboratory assessments, with relatively high survival within seasons in the Burdekin, coincident with invariably low incidence of *Adelina.* Conversely, sites with a high incidence of *Adelina* showed a decline in populations within the same season, and also between seasons (see section 4.3 Herbert, and section 4.4 Innisfail in particular).

Incidence of *Metarhizium anisopliae* was relatively constant between years within sites, with relatively high incidence each year at the Tully site (23 - 28%), low incidence each year at Innisfail, the Herbert and the Burdekin (0.2 - 13%). Adding mortality caused by *M anisopliae* to incidence of *Adelina* across all sites did not increase the regression coefficients. The other entomopathogens were recorded too infrequently to have a significant impact on the overall mortality of greyback canegrubs at any location.

Infection with *Adelina* sp. occurred early in the life stage of greyback canegrub, as grubs collected after April seldom expressed the disease. *Metarhizium* however, can kill grubs at any stage of their development, and restricting collections to the early part of the grub development period may have led to under-estimation of the total mortality caused by *Metarhizium* in the field. This could have affected interpretation of the relative impact of *Adelina* and *Metarhizium* at the Tully site, where incidence of both diseases was relatively high early in the season.

Unexplained mortality was high in grub samples held at CSIRO Entomology, particularly in grubs collected at an early stage in development. This mortality may have been caused by handling associated with collection in the field and subsequent handling in BSES or CSIRO laboratories. Within each season, the level of unexplained mortality did not vary greatly between similar-sized grubs collected from different locations, indicating that unrecognised disease was not the cause of high mortality.

The scoliid wasp *Campsomeris* sp. was occasionally recorded parasitising late stage larvae of greyback canegrub in the Burdekin and Innisfail districts. The highest population densities of *Campsomeris* sp. recorded were estimated at two cocoons per stool in one field in the Burdekin in July 1993, and a similar number in a field at Boogan, Innisfail in August 1995. Greyback canegrubs parasitised by *Campsomeris* sp. and *Campsomeris* cocoons were infrequently recovered from intensively-monitored sites, and the parasitoid was therefore thought to have little effect on population dynamics of the pest.

Several vertebrate predators including bandicoots and insectivorous birds preyed on greyback canegrub, but there was no evidence that these had any significant impact on population size of their prey.

5.3 Effect of farming practices on greyback canegrub population size

Farming practices differed between the districts where greyback canegrub population dynamics were monitored. The most obvious differences were in irrigation and trash management. Burdekin crops were furrow irrigated, and no other study site was artificially irrigated. All ratoon crops in the Burdekin monitored for greyback canegrub population changes were burnt before harvest, and the ratoons cultivated. In the Herbert,

Tully and Innisfail, all monitored sites were in ratoons in which cane had been harvested without burning, all trash was retained, and the inter-row space was not cultivated (ie green-cane trash blanket ratoon, GCTB). The Burdekin crops were also generally ploughed out and replaced more frequently than ratoons at the Herbert, Tully and Innisfail locations (see section 4.0).

Interpretation of farming practice differences and population dynamics of greyback canegrub suggests that the burnt-trash, frequently cultivated crops of the Burdekin have more stable populations of greyback canegrub than the trash-blanketed, uncultivated ratoons with longer ratoon cycle crops in the Herbert, Tully and Innisfail. Monitoring of greyback canegrub population densities in trials comparing GCTB and burnt trash/cultivated ratoons in the Burdekin and Tully showed that more grubs survived under burnt trash ratoons, provided all crops were kept free from grass weeds (Robertson and Walker 1996). Greyback beetles apparently laid similar numbers of eggs under GCTB and burnt ratoons, but grubs developed more slowly under GCTB compared to burnt and cultivated ratoons (Robertson and Walker 1996).

In a parallel study to this one, Ward and Cook (1996) showed that greyback canegrub populations tended to be higher in crops of cane which were tallest at the time of beetle oviposition (ie early-planted, and early-harvested crops). The implication was that greyback canegrub beetles preferentially selected tall crops in which to lay eggs, a phenomenon observed early this century (Illingworth 1918). Variation in planting dates and harvesting dates only affects local population density, and not area-wide population density, because the distribution of grubs is affected without influencing survival.

Maintenance of high populations of greyback canegrubs in the Burdekin could not be attributed to the practice of furrow irrigation. Outbreaks of greyback canegrub have been recorded in the wet tropics, as well as in the humid tropics without irrigation. Soils were saturated for long periods during the growth period of greyback canegrub in the wet tropics, as well as in the irrigated Burdekin, and yet the population dynamics of grubs differed between these two areas.

6.0 KEY FACTORS CONTROLLING POPULATION SIZE OF GREYBACK CANEGRUB (Objective 4)

As determined in section 5.0 (above), two factors that were demonstrated to influence population size and changes in population density of greyback canegrubs were entomopathogens and farming practices. The preferential selection of tall crops of cane for oviposition and subsequent development of greyback canegrubs does not influence population dynamics over large areas, but rather dictates the distribution of individuals within that population. In our study, early-planted and early-harvested crops were monitored to ensure that there was a high probability of encountering the highest possible densities of greyback canegrubs. However, lower numbers of greyback grubs and suspected higher mortality of grubs under GCTB compared to burnt and cultivated ratoons, were factors which could plausibly result in significant depression of population densities between seasons.

The mechanism leading to lower densities of greyback canegrub under GCTB compared to burnt trash and cultivated ratoons is unknown (Robertson and Walker 1996). Slower development under trash blankets, due to cooler and perhaps more moist conditions, may expose greyback canegrubs to additional natural mortality. Entomopathogens are not necessarily involved in increasing the mortality under GCTB. Burdekin trials comparing trash management treatments had very low incidence of disease in greyback grubs, but there were fewer grubs under GCTB compared to burnt trash in those trials (provided grass weeds were controlled in all treatments) (Robertson and Walker 1996).

To identify the factors which most influence population change in greyback canegrub, the changes in numbers between the start and end of each stage were plotted sequentially, at sites from which there was at least three full years monitoring data. The data for Tully are tabulated in life-table format as an example (Table 3). Population densities, calculated as numbers per m^2, were $\log_{10}(x+1)$ transformed. The differences between log numbers at the beginning and end of stages (k_i, a measure of mortality or disappearance) were summed to give a measure of total mortality (K_{total}), and changes in k within each stage correlated with K_{total} (following Podoler and Rogers 1975, and Varley and Gradwell 1960).

Table 3 **Life-table for greyback canegrub at Tully BSES, field 26B, based on population estimates for each complete generation. Data are $\log_{10}(x+1)$ transformed from mean number per m^2 to facilitate k-factor analysis (see Figure 6). Estimates of mortality due to *Adelina* and *Metarhizium* diseases are calculated from laboratory-held samples of grubs (see text).**

Life stage	No./m^2	Log_{10}	k-value	k-*Adelina*	k-*Met*
1994					
C. Grubs in June	19.2				
(estimated females	9.6)				
1995					
A. Potential natality	250	2.4			
B. Grubs established	46.4	1.68	0.72 (k_{0+1})		
C. Grubs in June	1.6	0.41	1.27 (k_2)	1.29 (k_a)	1.15 (k_m)
(estimated females	0.8)				
1996					
A. Potential natality	20.8	1.34			
B. Grubs established	36.8	1.58	-0.24 (k_{0+1})		
C. Grubs in June	8.0	0.95	0.63 (k_2)	0.76 (k_a)	0.98 (k_m)
(estimated females	4.0)				

Table 3 continued

Life stage	No/m^2	Log_{10}	k-value	k-*Adelina*	k-*Met*
1997					
A. Potential natality	104	2.02			
B. Grubs established	9.6	1.03	0.99 (k_{0+1})		
C. Grubs in June	3.2	0.62	0.41 (k_2)	0.29 (k_a)	0.54 (k_m)
(estimated females	1.6)				
1998					
A. Potential natality	41.6	1.63			
B. Grubs established	12.8	1.14	0.49 (k_{0+1})		
C. Grubs in May	0	0	1.14 (k_2)	0.21 (k_a)	0.65 (k_m)

The absolute population density of adults, and subsequent numbers of eggs laid into monitored fields could not be assessed. For the purposes of analysis, it was assumed that all late-stage grubs produced emergent beetles, 50% of which were female, and that these laid an average of 26 eggs each. This potential population recruitment (potential natality) was then used in analyses to complete the k-factor correlations.

Plots of the log population changes at four sites are shown in Figure 6. Changes in k_{0+1} (includes potential number of eggs, and mortality of eggs and neonate larvae) is the factor which most closely resembles changes in K_{total}, and is therefore deemed to be the 'key factor' of population change (see Varley and Gradwell 1960). Negative values of k_{0+1} and K_{total} (see Figure 6) indicate that recruitment was higher than expected in some years and sites, based on surviving grubs in the previous season. This is explained by immigration of egg-laying adults to tall crops, a factor already identified as driving the dynamics of greyback canegrub at the field level (Ward and Cook 1996). Populations at Innisfail, for example, may have been maintained between seasons because of immigration (Figure 6).

Regression of K_{total} against k_{0+1}, and against k_2 (change in grub density within a season), following Podoler and Rogers (1975), indicated that changes in natality and establishment rate of young grubs, was the key factor of population change between seasons, with 74% of the variance in K_{total} explained by the following regression on k_{0+1}:

$$K_{total} = 0.82 + 0.83(k_{0+1}); \quad r^2 = 0.74, P < 0.002.$$

There was no significant correlation between K_{total} and k_2, indicating that larval mortality did not influence overall population change. Examination of Figure 6, however, shows that k_2 tended to counteract changes in K_{total} and k_{0+1} (except at Stevens in the Burdekin), indicating that k_2 acted to dampen the population changes. The action of k_2 therefore was density-dependent and regulatory.

Changes in density of grubs within a season (k_2) are mirrored closely by mortality due to *Adelina* sp. (k_a) (as measured in samples sent to CSIRO in Canberra) at all sites except the Burdekin (Figure 6). Mortality due to *Metarhizium anisopliae* (k_m) also mirrors population changes in grub density at the two Tully sites. This suggests regulation of density within season. These analyses were compromised by lack of sequential data covering more than three years at most sites. In addition, incidence of *Metarhizium* at the Tully site may have been under-estimated by restricting the collection of grubs for analysis of disease to the first half of the grub development period (see section 5.2).

K-factor analysis has statistical and conceptual problems (eg see Sibly and Smith 1998) but the method is useful as a tool to analyse population changes, provided other techniques are used to confirm or disprove hypotheses generated.

Absolute population density of greyback canegrub adults was not estimated. Attempts to count emergence holes in the soil surface were unsuccessful because these openings did not persist long with irrigation or heavy rainfall. Nevertheless, a relative population estimate of beetle numbers was recorded from light traps of a similar design operated in each district. Catches of greyback beetles were 10 - 100 times greater in the Burdekin compared to other districts, with maximum catches of 23,000 to 25,000 beetles per trap-week in the Burdekin compared to a maximum of 1,700 beetles per trap-week at Mundoo, Innisfail, and a maximum of approximately 200 beetles per trap-week at Tully and Stone River.

Despite the wide variation in trap catches between districts, the population density of subsequently established greyback grubs was remarkably constant. There were 2 - 4 grubs per stool at Fowler's property in the Burdekin when catches in the light trap 200 m distant peaked at 23,000 - 25,000 beetles in 1993 and 1994, respectively. Similar grub densities were recorded early in the season at Innisfail when 3.3 grubs per stool were established in 1996 after a maximum of 1,700 beetles per trap-week were recorded in November 1995. At Tully, density of greyback canegrub in January 1995 was estimated at 2.9 per stool, but beetle numbers peaked at 20 per trap-week in December 1994.

The relatively constant population of greyback canegrubs established early in the season in tall crops in each district, despite a large difference in peak catches (and total catch) of beetles, suggests a powerful density-compensating mechanism between beetle emergence and grub establishment. Although the population density of greyback eggs could not be assessed in this study, there may be high mortality in eggs or neonate larvae in the soil. The difference between potential number of eggs (inferred from beetle numbers) and realised establishment of young greyback grubs is particularly great in the Burdekin. The stool, as a finite resource for larval feeding, may be a limiting factor to maximum population size of establishing greyback canegrub larvae.

Studies of the dispersion of greyback canegrub (Attachment 1,Walker *et al* 1998) showed that early-stage grubs rapidly disperse from the highly-aggregated egg-cluster. The neonate larvae may have an innate capacity and mechanism to space themselves out, perhaps by avoidance of conspecifics. When held in confined conditions, greyback grubs commonly inflict damage on each other, resulting in death. Ward (1998) introduced a range of densities of neonate greyback larvae to cane stools enclosed by sheet-metal, and found that survivorship was lowest with the highest initial population, and conversely, highest with low initial density. He attributed this density-dependent mortality to intraspecific competition. More research is needed on the mechanisms operating to limit numbers of greyback canegrubs establishing on cane stools. Ward’s (1998) results suggest that density-dependent differences in beetle survival or oviposition rate need not be invoked as a possible hypothesis to explain relative stability in establishment rate of grubs.

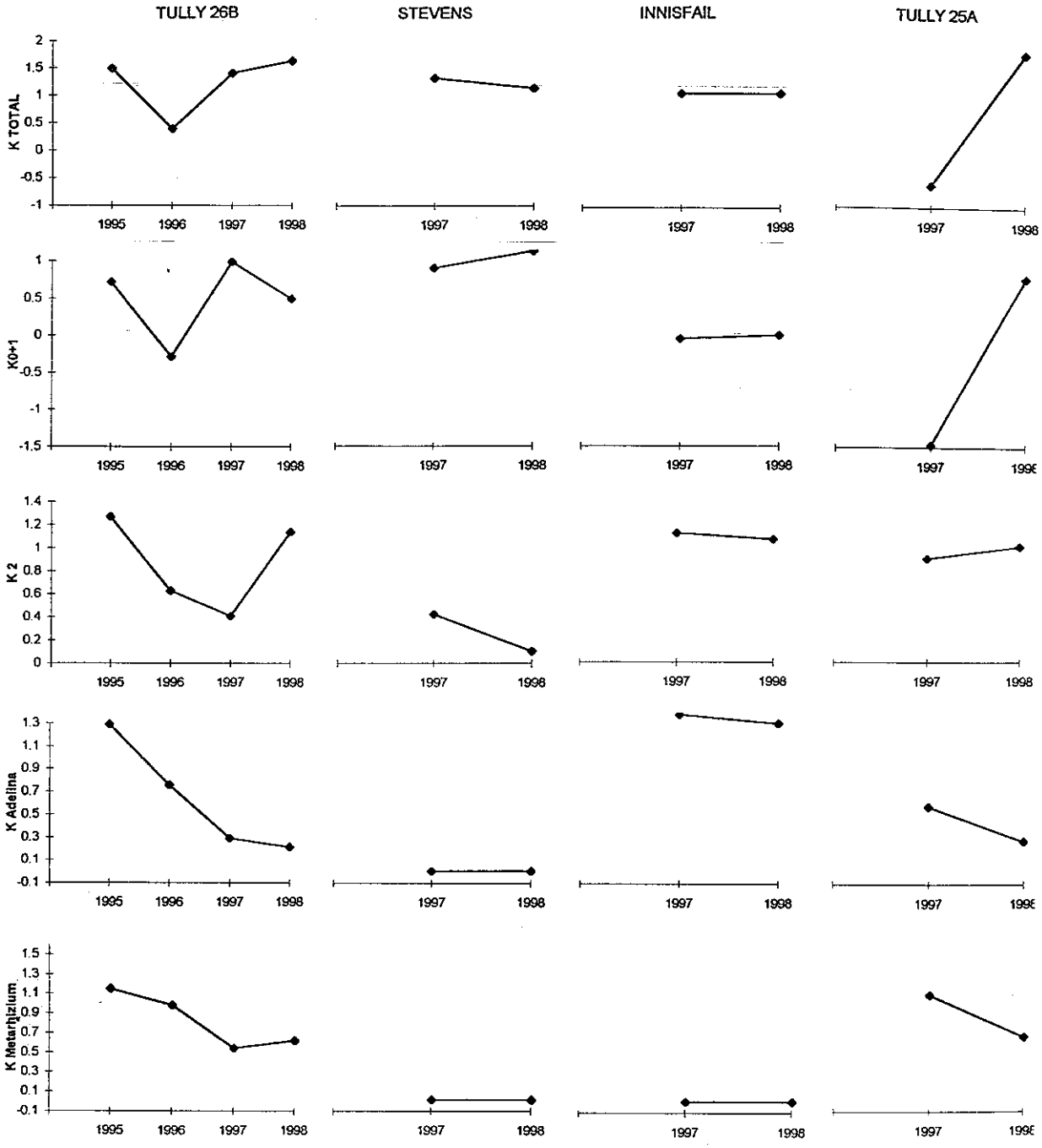

Figure 6. Graphs of k-values (see section 7.0) and K_{total} from monitoring population density of greyback canegrub in consecutive years at Tully BSES (fields 26B and 25A), Stevens (Clare, Burdekin district) and Torrisi (Innisfail). The $\log_{10}$ density of grubs which died from entomopathogens (*Adelina* and *Metarhizium*) as calculated from percent mortality in samples held at CSIRO Entomology, are also plotted as k-values estimated as $\log_{10}$(initial no./m^2 + 1) - $\log_{10}$(survivors/m^2 + 1). Changes in K_{total} were correlated with k_{0+1} (potential natality plus mortality of eggs and neonate grubs) ($r^2 = 0.74$, $P < 0.002$).

Ward (1998) showed that there was no correlation between numbers of greyback canegrub beetles caught in intercept traps in Burdekin canefields, and subsequent numbers of grubs established on cane stools in those fields. Those results support the hypothesis that there are strong density-stabilising effects between egg-laying and establishment of greyback canegrubs. In addition, canegrub numbers declined more rapidly when established at high densities compared to lower densities in the Burdekin district (Figures 1 and 2), in the absence of entomopathogenic diseases. This suggests intraspecific interactions operating in a density-dependent way.

Grubs of other scarab species show density-stabilising mechanisms at high densities. Carne (1956) showed that *Acrossoides tasmaniae (=Aphodius howitti)* grubs had high mortality at high densities as a result of intraspecific competition and aggression ('larval combat'), and similar behaviour and effects are seen with New Zealand grassgrub (*Costelytra zealandica*) (East 1979) and the garden chafer (*Phyllopertha horticolla*) in Britain (Milne 1984). Harari *et al* (1997) measured the population density of newly-established grubs of the melolonthine *Maladera matrida*, and found a constant population density of three grubs per 25 cm^2 in five different crops in Israel, although subsequent survival rates differed between crops. Competition for resources early in the life cycle was shown to stabilise population density of the weevil *Listronotus bonariensis*, despite large variation in the population size of egg-laying adults (Barker *et al* 1989).

The indications of density-stabilising mechanisms between beetle emergence and subsequent grub establishment on cane stools has implications for pest management. In the Burdekin, current attempts to control greyback canegrub with insecticide applications against adults have not been successful in preventing subsequent damage by grubs (Robertson, unpublished). Growers in the Burdekin district have also expended considerable effort to mass-trap greyback beetles using light traps. In light of the apparent density-compensating mortality, with largest effects on potential natality in the Burdekin where beetle population was largest, control of beetles with light traps or spraying is unlikely to result in reduction in density of establishing grubs. In other words, beetle control is 'replaceable' mortality (eg see Jones 1982; Knutson and Gilstrap 1989) whereas mortality acting later in the grub life cycle is more likely to reduce the population size of the damaging stage.

Once grubs are established on sugarcane stools, the factor controlling grub populations within a season was incidence of entomopathogens, and incidence of *Adelina* sp. in particular. Over half of the variance in annual mortality could be explained by the level of incidence of *Adelina* alone (see section 5.2 above). Sites with a high incidence of *Adelina* (40% incidence or greater, as assessed by CSIRO Entomology in samples held in Canberra) had declining numbers of greyback canegrubs in the field over time, both within and between seasons. Local population density declined to undetectable levels where incidence of *Adelina* exceeded 70% within a season, at Stone River in 1994, and Bambaroo in 1997. Population density was relatively constant through the season where incidence of *Adelina* sp. in greyback canegrub was low, ie in the Burdekin each year.

A Morris plot of log density of greyback grubs established each January against log density established in the following January for sequential data (Morris 1963) suggested that population change between seasons at the Tully site (field 26B) was influenced by a

density-dependent process (ie rate of grub recruitment declines as population increases). The slope of the regression of N_{t+1} on N_t (Figure 7) was 0.53, which did not differ significantly from b = 1 (P = 0.1). The strength of prediction of subsequent population size based on numbers established in the previous year was high, with 81% of the variance explained by the regression of N_{t+1} on N_t, viz:

$$N_{t+1} = 0.455 + 0.53(N_t); \quad (r^2 = 0.81, P = 0.1)$$

(where N_t is log number per m^2 established in January in year 1, and N_{t+1} is the log number per m^2 established in the following January).

Plotting log density of survivors against log initial density for life stages within generations is used to determine the stages where density dependent mortality acts on populations (Varley *et al* 1973). For serial data collected at Tully field 26B, density in March correlated well with density in the previous January, viz:

Log no/m^2 in March = -0.54 + 1.045(Log no/m^2 in January)
($r^2 = 0.84$, $P < 0.03$) (Figure 8).

A regression slope of approximately 1.0 indicates no density-dependent factors acting over this period.

Log density in the month between mid-April and mid-May was correlated with log density in the previous March, with the form of the regression line suggested increasing mortality as density increased:

Log no/m^2 in April/May = -0.01 + 0.63 (Log no/m^2 in March)
($r^2 = 0.88$, $P < 0.02$) (Figure 9).

The slope (0.63) just failed to be statistically different from b=1 ($0.05 < P < 0.1$).

Plotting the difference between log density of grubs in March and April/May each year and log density in March (ie k_i) at the Tully site (following East *et al* 1986) gave a significant relationship which indicated that mortality increased as population density increased, viz:

k_i = 0.12 + 0.29 (Log no/m^2 in March) (Figure 10)
($r^2 = 0.88$, $P < 0.02$; b = 0.29 is significantly greater than b = 0, $P < 0.05$).

Observations of grub mortality in samples held at CSIRO Canberra revealed significant mortality caused by *Adelina* sp. from early April each year (approximately 120 days post-hatching, see Lai-Fook *et al* 1997), which coincides with the period of change in form of the regression slope suggesting density-dependence of mortality. In addition, the observed mortality in samples within seasons across all sites was correlated with incidence of *Adelina* sp. (see section 5.2, above). Obviously, a high degree of correlation does not prove a cause-and-effect relationship, but the correlations do support a hypothesis that density-dependent mortality caused by *Adelina* infection is the mechanism for population change between seasons.

Insufficient serial data were available from other sites to test density relationships between seasons.

Adelina sp. infection appears to be influenced strongly by the density of greyback canegrub larvae, with high incidence following high population density of the host grub, and low incidence in years following low population density of the host. This is seen most clearly at Tully, where incidence increased from 16% in 1994 when population density of greyback canegrub was relatively high, to 40% in 1995, then declined to 13% in 1996, 10% in 1997, and 5% of the grub population in 1998, as the grub population declined to low densities.

Adelina sp. appears to behave in the delayed density-dependent way characteristic of other pathogens which depend on host density for the spread of infection (see review by Anderson and May 1981). Decline in incidence after the host population density declines suggests that the pathogen may have a limited period of persistence in the soil. Studies at CSIRO Entomology showed that the oocyst of *Adelina* sp. is fragile and easily destroyed by handling. This indicates that levels of the infective stage of *Adelina* sp. may decline rapidly with cultivation of soil. In addition, in the absence of grub hosts, the population of infective oocycts may decline over time as activity of soil fauna including earthworms disturbs the soil.

Incidence of *Metarhizium* was relatively constant between years at the Tully monitoring site, despite a change in population density between years. Incidence of *Metarhizium* was estimated at 23 - 28% of the grub population each year between 1994 and 1998, although the starting population density each year varied from nine grubs per stool in 1994, to 0.6 grubs per stool in 1997. Constant mortalities within a population have no dynamic effect on population changes (Richards 1961) although the absence of the mortality may result in higher average population densities, for example as seen in the Burdekin district.

Metarhizium may infect a constant proportion of the greyback grub population each year irrespective of host population density, because the spores persist and maintain their infectivity for long periods of time. In a concurrent study on *Metarhizium* isolate FI 1045 from greyback canegrub, Milner, Robertson and Samson (unpublished) showed that numbers of viable spores declined rapidly within the first six months to about 25% of initial levels, and then stablilised at this lower level for at least two years in the absence of grub hosts. This persistence at reduced levels may help explain the relatively constant proportion of infections each year irrespective of host density.

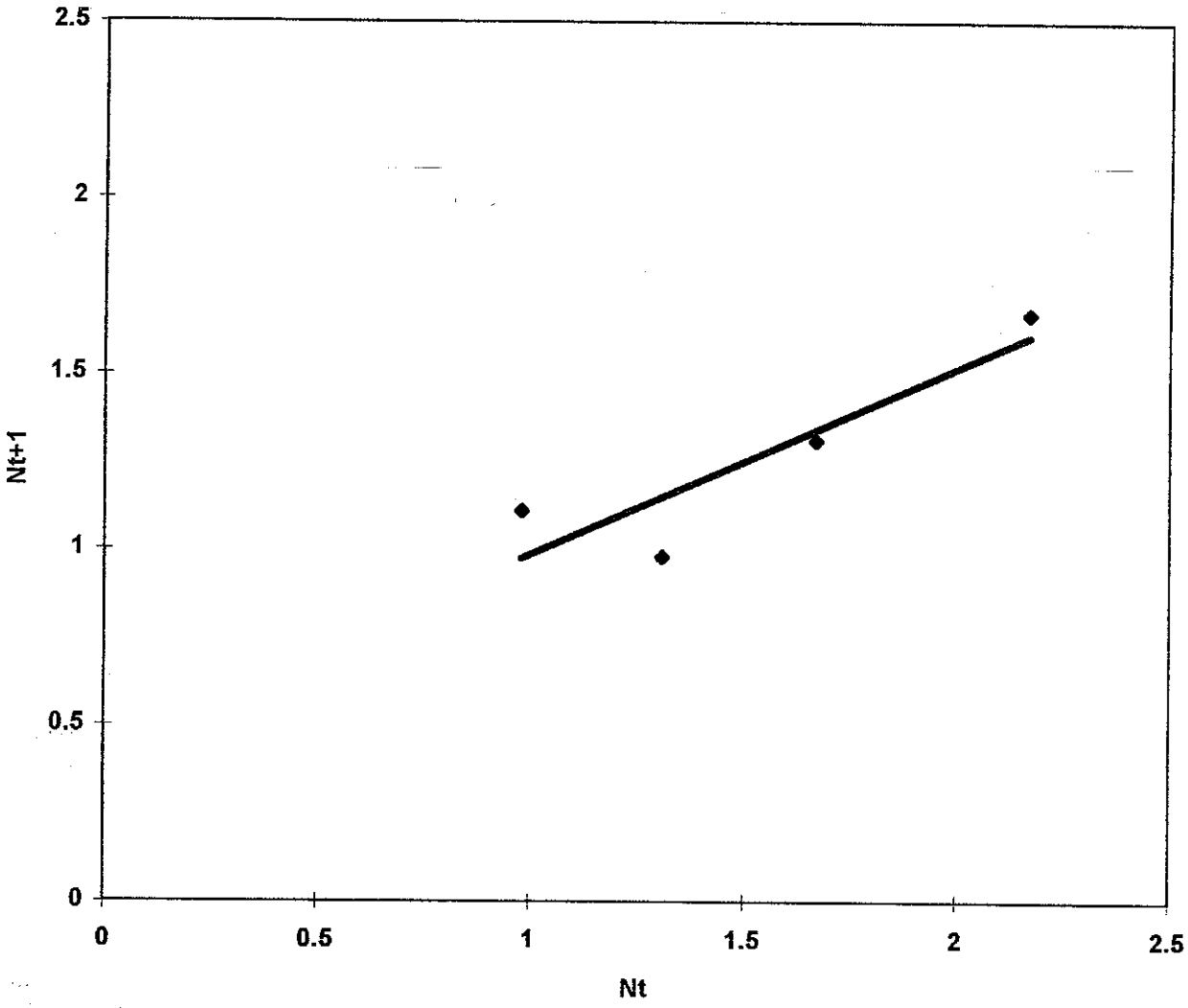

Figure 7. Log number of greyback grubs per m^2 established in each year (N_t), plotted against log no./m^2 in the succeeding year (N_{t+1}) for successive years at the Tully monitoring site; $N_{t+1} = 0.455 + 0.53(N_t)$; $r^2 = 0.81$, $P = 0.1$.

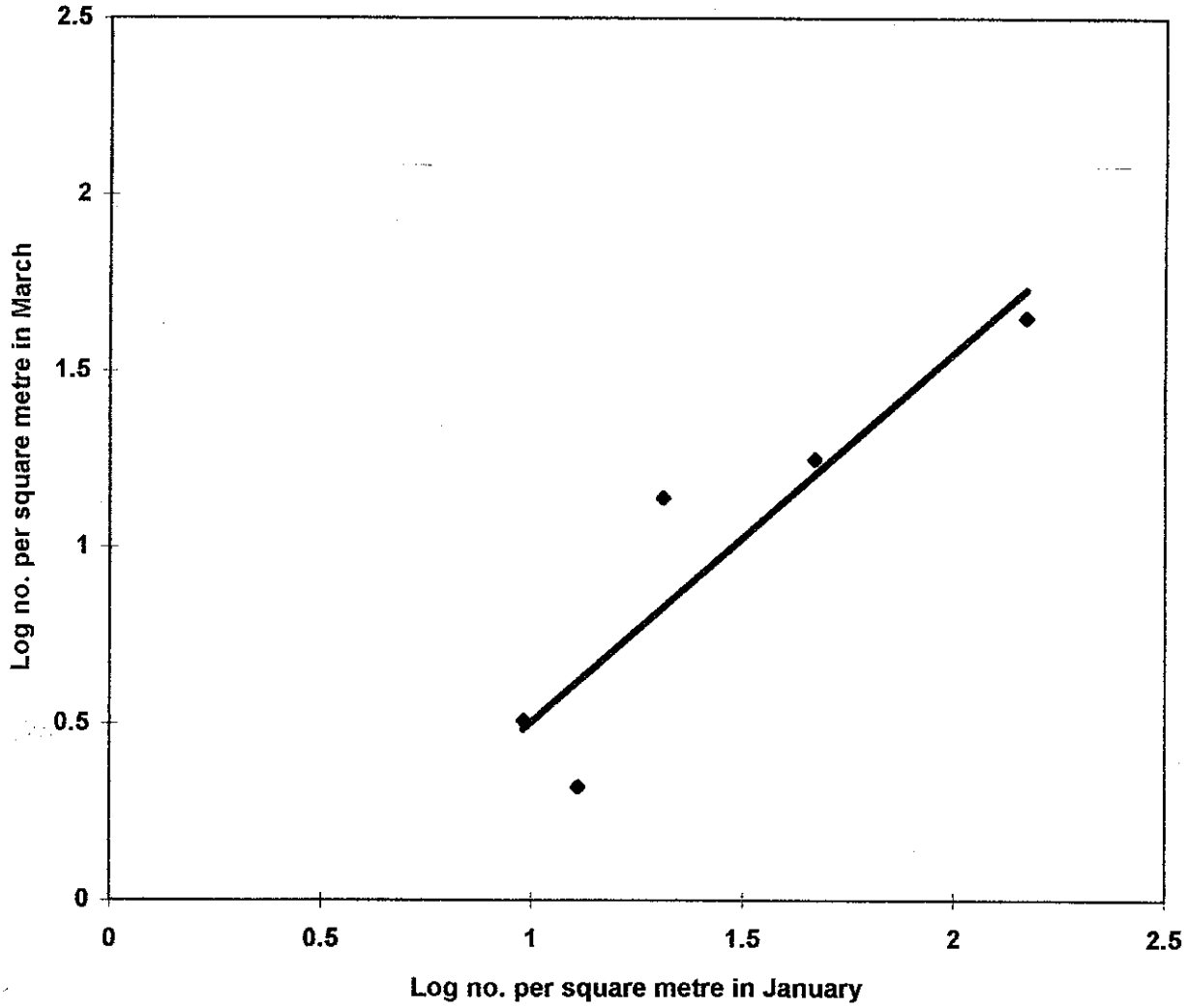

Figure 8. Log number of greyback grubs per m^2 established in January plotted against log no./m^2 surviving in March of the same year over successive years at the Tully monitoring site;
Log no/m^2 in March = -0.54 + 1.045(log no/m^2 in January); $r^2 = 0.84$, $P < 0.03$.

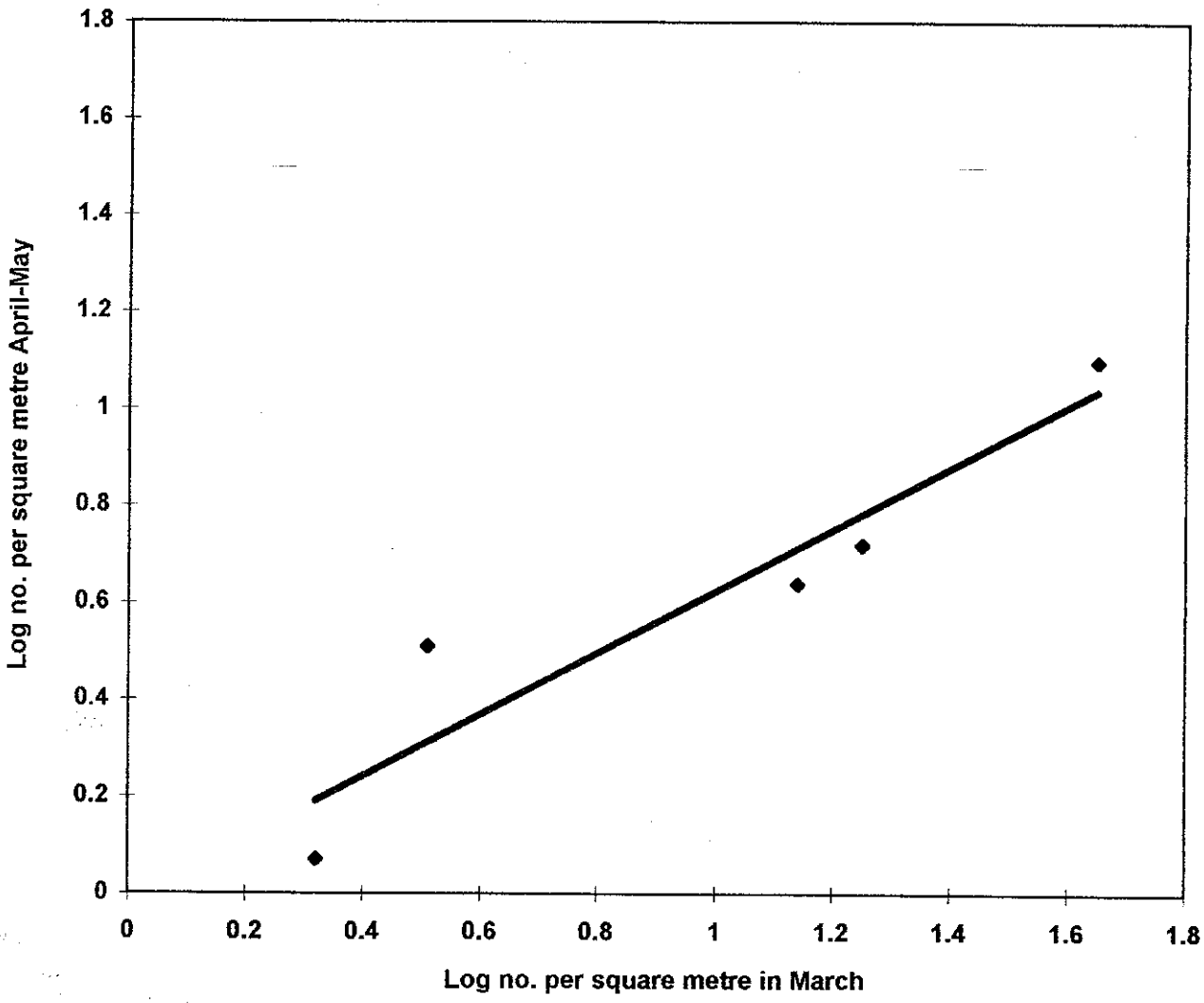

Figure 9. Log number of greyback grubs per m^2 established in March plotted against log no./m^2 surviving in April/May of the same year over successive years at the Tully monitoring site;
Log no/m^2 in April/May = -0.01 + 0.63(log no/m^2 in March); $r^2 = 0.88$, $P < 0.02$.

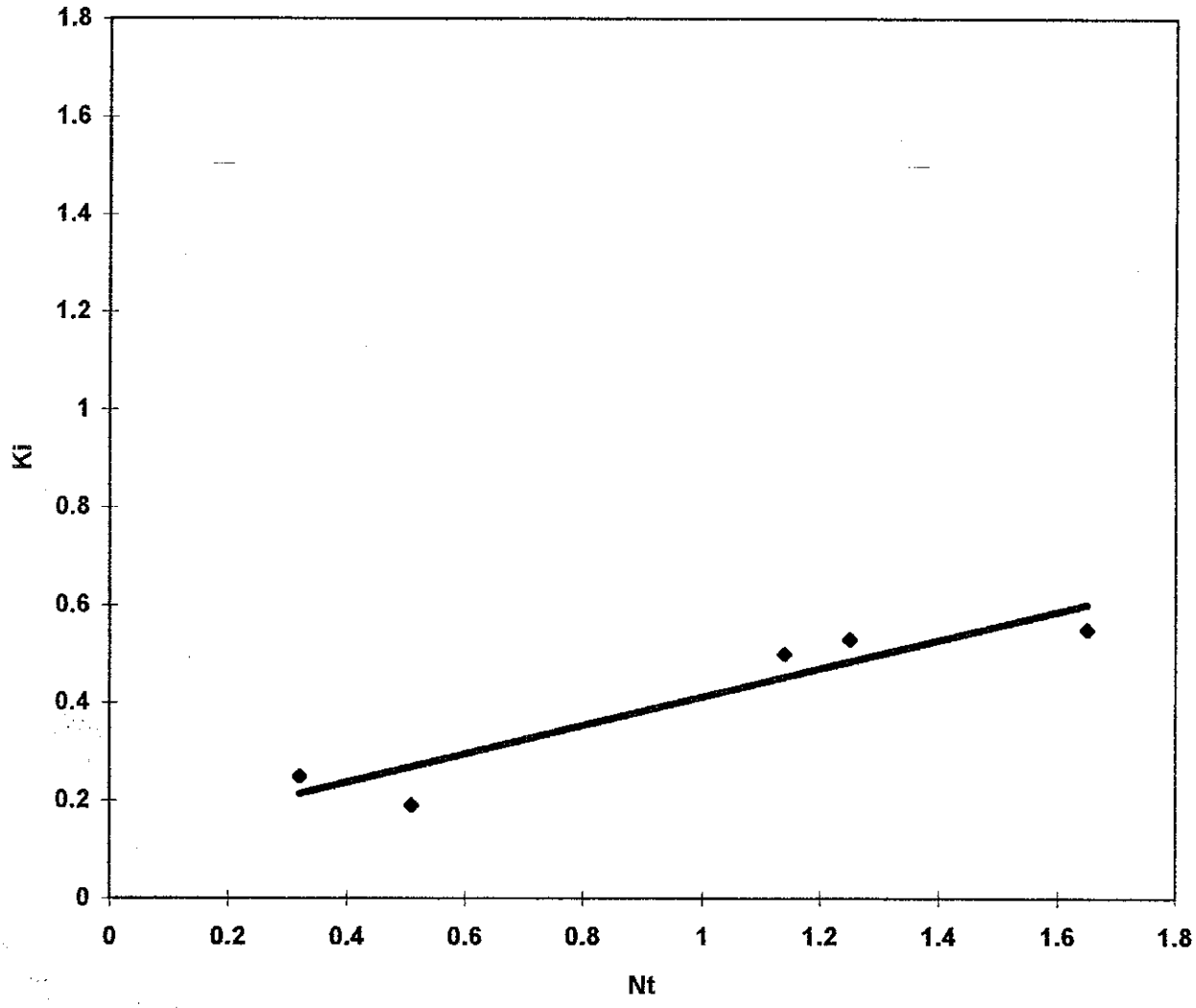

Figure 10. Log number of greyback grubs per m^2 established in March plotted against the difference between log density in March (N_t) and log density in April/May (disappearance or mortality, k_i) within the same year, for successive years at Tully monitoring site;

$k_i = 0.12 + 0.29$ (log no/m^2 in March); $r^2 = 0.88$, $P < 0.02$.

The description of greyback population dynamics emanating from this study is remarkably similar to that of other melolonthine scarabs which have been well studied overseas. Popay (1992) summarised several decades of research on grassgrub (*Costelytra zealandica*) in the North Island of New Zealand, and showed that entomopathogens affecting the grub stage acted as delayed density-dependent regulators of population size. Four species of protozoa and the milky disease bacterium *B popilliae* apparently cause oscillations in the population density of grassgrub, with upper population density also constrained by intraspecific competition between grubs (see also Miln 1979). In the South Island of New Zealand, another bacterium, *Serratia entomophilia,* acts in a similar way on the same grassgrub (Jackson 1984; Villalobos *et al* 1997).

Population cycles of grassgrub were disrupted by severe summer drought, cultivation or insecticides, all of which reduced the population of grassgrub and subsequently reduced the incidence of entomopathogens, resulting in outbreaks (East 1979; Jackson 1984; Popay 1992). Frequent use of organophosphate insecticides also resulted in maintenance of higher average population densities of grassgrub compared to untreated populations, through suppression of disease incidence (Miln and Carpenter 1979; Thomson *et al* 1985). The effect of insecticide use against the larval stage of greyback canegrub was not studied in this project. However, reducing the population density of hosts may reduce the incidence of pathogens such as *Adelina* which act in a density-dependent way. Carpenter (1981) argued that pesticides acting early in the life cycle could limit increase in pathogen incidence and lengthen the time required for pathogens to control the pest.

We postulate that frequent cultivation of soil in the Burdekin suppresses entomopathogens of greyback canegrub, and consequently results in the maintenance of high population densities of the pest in that district. Resurgence of greyback canegrub populations after stopping the use of persistent insecticides also may have been due to the virtual absence of entomopathogens. Cycles of outbreak and crash of greyback canegrub populations were seen prior to the use of persistent insecticides (Robertson *et al* 1997).

Cycles in population size are well known for melolonthine scarabs in Europe, where *Melolontha* spp outbreaks are recorded every 20 - 30 years (Zimmermann 1992). Several entomopathogens are known from *Melolontha* spp including entomopox virus, milky disease bacteria, fungi, *Adelina* and *Nosema* (Zimmermann 1992) but no population dynamics studies have been conducted to determine the role of these pathogens or other factors in the population cycles of the grubs.

In Britain, garden chafer (*Phyllopertha horticola*) reaches damaging levels in cultivated soils but seldom in undisturbed grasslands (Milne 1984). Population dynamics of garden chafer are influenced by entomopathogens operating in an 'imperfectly' density-dependent way, and by intraspecific competition and adverse weather (Milne 1984).

The population dynamics of other melolonthine scarabs are influenced by type and availability of host plants. Morgan and Bungey (1979) recorded consistently higher densities of *Heteronyx obesus* in pastures containing sorrel, a favourable host plant for beetle feeding, compared to areas without sorrel. Similarly, Robertson and Kettle (1994) showed that the presence of *Parthenium* weed increased the population density of *Pseudoheteronyx* sp. by increasing the fecundity of beetles. This group of scarabs have

relatively long-lived adults, and eggs are matured and laid over an extended oviposition period, unlike greyback canegrub and *C zealandica*. Davidson *et al* (1979) believed that *Sericesthis* and *Rhopaea* spp increased in density when high-producing grass species were introduced to northern New South Wales pastures.

In our present study, there was no evidence that greyback canegrub population dynamics was influenced by relative availability of adult food, or quality of larval food. Trees suitable for adult feeding (Illingworth and Dodd 1921) were close to all monitored sites. The planting of large areas to sugarcane has undoubtedly increased the carrying capacity of habitats for greyback canegrub, but this does not explain the difference in population dynamics we observed between different canegrowing areas. Indigenous insects often become pests in agricultural habitats because natural enemies are displaced and cannot persist despite the abundance of hosts (Bartlett and van den Bosch 1964). Price (1991) argued that natural enemies could be effective pest controls in monocultures, and that the level of control increased with increasing stability of the habitat. Control of greyback canegrub in sugarcane is possible with entomopathogens, but minimum disturbance by cultivation will increase the infection levels and persistence of the natural controls.

7.0 DESCRIPTIVE MODEL OF GREYBACK CANEGRUB POPULATION DYNAMICS (Objective 5)

The observed high infectivity by *Adelina* sp. in response to high population density of greyback canegrubs, but limited persistence at low densities, helps explain the recorded fluctuations in area damaged by greyback canegrub early this century, reflecting outbreaks and population crashes of the pest (Robertson *et al* 1997). Observations during the course of our study of outbreak and rapid decline of greyback canegrub populations at Tully, Innisfail, Babinda and the Herbert Valley, and isolation of *Adelina* sp. at high incidence during the 'crash' period of greyback densities, lends strong support to a model of greyback canegrub cycles driven by this pathogen acting in a delayed density-dependent way. Cyclical oscillations in population density of insect pests have been well documented. The related grassgrub also shows oscillations in population density driven by pathogens operating in a delayed density-dependent manner (Popay 1992). Myers (1988) believed that numbers of forest-defoliating Lepidoptera fluctuated in response to delayed density-dependent mortality caused by entomopathogens which were highly virulent but had limited persistence outside the host caterpillars.

Cappuccino *et al* (1995) and Hunter (1995) reviewed current knowledge about insect species which regularly show outbreaks to pest status, compared to pests which are always present at relatively constant densities. Outbreaking species tend to have aggregated dispersion, and often lay most of their eggs in one place. Outbreaks generally occur from limited focal areas and spread to surrounding areas as population density rises. Insects which tend to be clumped also tend to be prone to diseases, as pathogen transmission is density-dependent (Cappuccino *et al* 1995). Greyback canegrub fits the general pattern as an outbreaking species. Greyback eggs are deposited in clusters in the fields with the tallest cane, and canegrubs tend to be slightly aggregated as larvae. Outbreaks of greyback canegrub can start from localised areas, and spread to surrounding farms, as seen recently in the Innisfail and Burdekin districts.

In far north Queensland, the decline of greyback grub numbers and subsequent decline in *Adelina* sp. incidence may lead to eventual escape of the host from biological control, and contingent increase in population density of greyback canegrub. This reemergence of populations to outbreak levels following a pathogen-induced crash and subsequent decline in pathogen incidence, has yet to be followed but is predicted as a consequence of the above hypothesis. The cycles of outbreak and crash in greyback canegrub populations, measured as area of cane damaged, had a period of 12 - 13 years between peaks in far north Queensland (outbreaks in 1921, 1934 and 1946, see Robertson *et al* 1997).

If *Adelina* sp. is the driving variable responsible for observed changes in populations of greyback canegrub, then this natural enemy is both a regulatory and destabilising factor in the population dynamics of the pest. For prediction of greyback canegrub population changes in a pest management program, the level of infection by *Adelina* needs to be assessed because over 50% of the variance in population density within a season was explained by incidence of this natural enemy (see section 5.2 above).

The other density-dependent effect operating to regulate greyback population size is the apparent stabilisation of numbers of grubs establishing on cane stools (see section 6.0, above). Population density commonly reaches 2 - 4 grubs per stool regardless of the numbers of beetles available for breeding, and the potential fecundity of this beetle population. The population-stabilising mechanism operating between beetle emergence and subsequent grub establishment is unknown, although intraspecific competition between young grubs at high densities is known to cause similar results in other scarab species (eg see East 1979).

The model of greyback population dynamics thus would incorporate delayed density-dependent mortality of grubs, primarily driven by *Adelina* pathogen infection, and density-stabilising establishment rate when population density is high. Insufficient data are available on natality and associated mortality to construct a predictive mathematical model. Adult flight and oviposition behaviour are also important in determining distribution of grub populations, through preferential selection of tall crops for oviposition (see Ward and Cook 1996).

The ongoing outbreak of greyback canegrub in the Burdekin, in the absence of significant levels of entomopathogens, also supports the hypothesis that disease is a key factor in the population dynamics of greyback canegrub, and a driving variable in any predictive model of population dynamics of the pest.

The virtual absence of *Adelina* sp. from greyback canegrub in the Burdekin may be explained by the differences in farming systems between the Burdekin and far north Queensland. Soils are more intensively disturbed by cultivation in the Burdekin, where trash is burnt and ratoons cultivated, and crops are ploughed out and replaced more frequently than in far north Queensland. Burnt and cultivated ratoons at Tully had a lower incidence of *Adelina* sp. compared to GCTB sites in the same district (Table 4), although a small-plot trial comparing trash management methods had similar percentage infection across the range of grub densities. The infective oocyst of *Adelina* sp. is fragile, and a high proportion of this soil-borne stage may be damaged by intensive cultivation of soil as

in the Burdekin, leading to the observed low levels of infection of grubs. More research on the effect of cultural practices on persistence and infectivity of *Adelina* sp. and other pathogens of greyback canegrub is needed.

Table 4 Relationship between density of greyback canegrubs estimated from insecticide-free plots in March 1997 and 1998 and incidence of *Adelina* sp. from samples collected in the same season in a trash management trial in field 35A on Tully Sugar Experiment Station (Robertson and Bakker, unpublished).

Year	Treatment	Grubs/stool	% *Adelina*
1996	Burnt/cultivated	2.75	38
	Incorporated trash	1.1	53
	Trash blanket	0.54	42
1997	Burnt/cultivated	1.03	33
	Incorporated trash	0.61	50
	Trash blanket	0.14	47

Alternative hypotheses to explain persistently high population density of greyback canegrub across the Burdekin district may involve unknown effects of alkaline soils, furrow irrigation or tillage regimes on canegrub natality or mortality rates, but we have insufficient information to formulate such hypotheses.

To test the prediction that disease is responsible for population change in greyback canegrub, and that frequent cultivation suppresses disease incidence, experiments need to be conducted over relatively large areas. The Burdekin in particular is a suitable environment to evaluate reduced tillage effects on pathogen incidence. Small-plot studies involving trash management at Tully did not show differences in incidence of entomopathogens, although fewer greyback canegrubs occurred under GCTB compared to burnt cane. Frequency of cultivation and replacement of crops may suppress disease incidence more than ratoon treatments, and this needs to be examined. Minimum-tillage planting after no-tillage (herbicide) fallow resulted in higher levels of viable *Metarhizium anisopliae* in the soil in the subsequent crop (Samson and Phillips 1997; see also Sosa-Gomez and Moscardi 1994). The effect of minimum tillage on persistence and infection levels of other entomopathogens of greyback canegrub, particularly *Adelina,* needs study.

A general model which describes the pattern of population changes of greyback canegrub between seasons across all sites (Figure 11) is that of Southwood and Comins (1976). Their model predicts positive population growth at high densities, until an

upper limit set by intraspecific competition and the carrying capacity of the habitat. Measurable cane damage results from populations of 2 - 4 per stool in the period from March to June, indicating this density range as close to the carrying capacity for sugarcane. In the model, population growth is also positive at very low densities as a result of escape from natural enemies. The density-dependent response of *Adelina* sp. (see section 6.0 above), and the occurrence of outbreaks following periods of low population density (inferred from lack of cane damage) suggests that this escape from natural control at low densities can occur. At intermediate densities, the model predicts negative population growth as a result of control by natural enemies responding in a density-dependent way. The action of natural enemies is to keep the population fluctuating about a stable point between low densities where populations escape natural control, and intermediate densities where natural enemies suppress population growth. Examination of Figure 11, a summary of all population monitoring of greyback canegrub, suggests a stable point (no nett population change) of 0.7 per stool (ca 10 grubs/m^2).

The general model of Southwood and Comins (1976) may best describe insect species such as greyback canegrub with a moderate capacity for population increase. Such species occupy an intermediate position on the r - K continuum of strategies in population dynamics (Southwood 1977). K-selected species tend to occupy stable habitats and have evolved mechanisms to limit their population densities below the carrying capacity of the environment (Southwood 1977). At the other extreme, r-selected species are extreme opportunists, occupy ephemeral habitats and have a capacity for rapid population increase, all of which make responses by natural enemies unlikely to result in regulation of the population. Kain (1979) argued that soil-dwelling agricultural pests including scarabs were generally intermediate r - K strategists, and greyback canegrub also fits that description, based on its moderate capacity for increase and ability to exceed the carrying capacity of its habitat.

8.0 EFFECTS OF CROP MANAGEMENT ON FREQUENCY OF DISEASE (Objective 6)

Following the identification of entomopathogenic diseases in greyback canegrub, the project was expanded in 1995 - 1996 to include monitoring of disease incidence under a range of cultivation and trash-management practices within districts. The impetus for this additional study was the difference in disease infection levels between the Burdekin, which is intensively cultivated and where trash is burnt each year, and other locations in far north Queensland, where ratoons were generally uncultivated and trash was retained at each harvest.

A replicated trial comparing the effects of trash blanketing, trash incorporation, and trash burning on numbers of greyback canegrub and other soil-inhabiting fauna was established in 1993 on the Tully Sugar Experiment Station (Robertson and Bakker, in prep). Greyback canegrubs collected during population sampling in 1996 and 1997 were sent to CSIRO Entomology in Canberra and held until death or pupation, as for other grub samples (above). When they died, the grubs were examined for entomopathogens. Samples were not collected from the fourth ratoon in 1998 due to the relatively poor growth of cane in several plots, which may have adversely influenced the oviposition of greyback beetles.

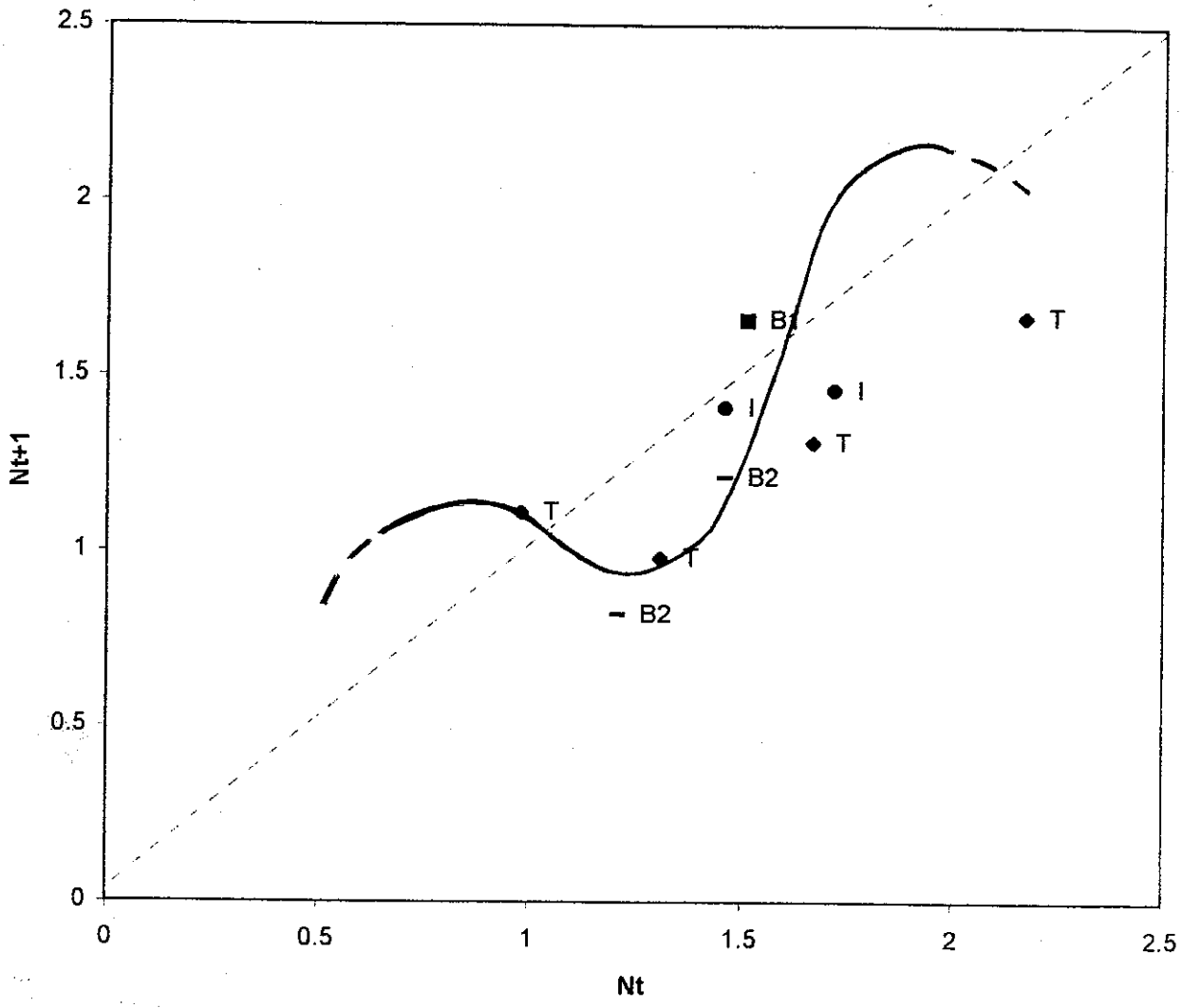

Figure 11. Plot of log density (no./m^2) of greyback canegrubs established in successive years in monitored canefields at Upriver Home Hill, Burdekin district (B1), Clare, Burdekin district (B2), Tully (T), and Mundoo, Innisfail district (I). The form of the fitted curve follows the general model of Southwood and Comins (1996) where natural enemies acting in a density-dependent way suppress population growth at intermediate densities, but populations escape natural enemy control at low and high densities. The curve (fitted by eye) is of the form used by Hassell (1978) originally for control by predators:

$$N_{t+1} = N_t e^{r(1-Nt/K)} e^{(-b\,Nt\,P)/(1+cNt+bThNt^2)}$$

(where K is the carrying capacity of the habitat, T_h is the delay in mortality caused by natural enemies, and b and c are constants from the mortality rate; see Hassell 1978).

Similar densities of greyback canegrubs were established in all three trash management treatments, both with and without insecticide treatment (suSCon^R Blue applied at planting in split-plot treatments) in the second ratoon in 1996. In the burnt-trash treatment, a mean of 38% of the grubs was infected with *Adelina* sp. 53% in the trash-incorporation treatment, and 42% in the trash-blanketed treatment. *Metarhizium* affected 7, 0, and 6% of grubs in the burnt-trash, trash-incorporated and trash blanketed treatments respectively. Numbers of grubs recovered from plots were relatively low and differences in levels of individual pathogens between treatments could not be tested statistically.

In the third ratoon in 1997, there were significantly fewer greyback canegrubs established in the trash-blanketed plots (mean 0.14 per stool) compared to burnt-trash plots (mean 1.03 per stool; $F_{(2,23)} = 6.18$, $P = 0.02$). Population density of grubs in the trash-incorporated plots was intermediate at 0.61 per stool. *Adelina* sp. was present in 33% of the total number of grubs recovered from burnt plots, 50% of grubs in the trash-incorporated plots, and 47% in samples from the trash-blanketed plots. Insufficient grubs were available to test for significant differences in disease levels between treatments. Only two greyback grubs succumbed to *Metarhizium* from all samples taken in 1997, one from burnt trash and one from incorporated trash plots.

Density-dependent infection rate by *Adelina* sp. (see section 5.2 above) may have confounded the interpretation of effects of farming practices on the disease. Higher population densities of greyback canegrub under burnt cane compared to trash blanket systems (see Robertson and Walker 1996) should result in higher levels of infection by *Adelina* sp. at least in the subsequent year, provided both systems were equally favourable for persistence and infectivity of the pathogen. Although not statistically demonstrated, lowest levels of infection by *Adelina* sp. were recorded in the Tully trial under burnt-cultivated conditions in both years, despite highest densities of grubs being recorded in those plots (Table 4). This result is unexpected from the density-dependent action of *Adelina*, and suggests that burnt and cultivated conditions may suppress the full expression of the disease.

Samples of greyback grubs were collected from farms practising burnt-cane harvesting in the Tully district, and disease levels were compared with grubs from trash blanketed canefields in the same district. A farm across Banyan Creek from Tully Sugar Experiment Station, approximately 500 to 1,000 m from the Tully monitoring site, was infested with greyback canegrub, and burnt-cane harvesting with cultivated ratoons was practiced on this farm. Infection levels of *Adelina* sp. and *M anisopliae* identified in grubs from this farm (farm 3), and from the Tully monitoring site, are compared in Table 5.

Table 5 Percent incidence of two entomopathogenic diseases in greyback canegrub collected from two adjoining farms (nos 1 & 3) practicing different trash and ratoon management at Tully, and a third with burnt trash in the northern part of Tully district (no 2, El Arish), 1994 - 1998. Farm 1 was the BSES monitoring site, field 26B. NB The burnt-trash farms (nos 2 and 3) adopted trash blanketing and no-tillage ratooning in the 1997 harvest season, after monitoring of grubs and disease in 1997.

Year	Farm	Trash management	Pathogen (%incidence)	
			Adelina sp.	*M anisopliae*
1995	1(26B)	Trash blanket	40	28
	2	Burnt trash/cultivated	10	0
1996	1	Trash blanket	13	23
	3	Burnt trash/cultivated	6	0
1997	1	Trash blanket	10	26
	2	Burnt trash/cultivated	0	0
	3	Burnt trash/cultivated	20	2
1998	1	Trash blanket	5	27
	2	Trash blanket for one year	63	0
	3	Trash blanket for one year	39	0

The incidence of *Adelina* sp. increased between 1996 and 1997 on farm 3 even with burnt cane and ratoon cultivation, while the disease declined at the Tully monitoring site nearby (farm 1, Table 5) as the density of the grub population declined (see section 4.2 above). The results of this comparison were inconclusive, but serve to illustrate the dynamic nature of interactions between greyback canegrub and incidence of its diseases within fields and within farms over time.

Current work is aimed at determining whether the intensity of cultivation prior to planting of cane influences the level of pathogens subsequently recovered in greyback canegrub.

9.0 PATHOGENICITY OF SELECTED MICRO-ORGANISMS (Objective 7)

9.1 Infections established in the field

Two microorganisms (*Adelina* sp. and *Metarhizium anisopliae*) were collected as field-established infections in numbers sufficient to allow systematic analysis of patterns of infectivity and pathogenicity.

Field collection of larvae from sites in far north Queensland throughout the 1994 season suggested that the incidence of infection, particularly for *Metahizium*, declined as the season progressed (Table 6). Several factors, operating alone or in combination, were postulated as potentially responsible for this phenomenon, namely an increased resistance of older larvae to establishment of microbial infection, a reduced rate of

development or severity of infections that established in older larvae, and the possibility that late sampling simply resulted in collection of 'survivors' that had escaped infection earlier in their development.

Table 6 Rates of infection by pathogens in greyback canegrubs collected throughout the 1994 season in far north Queensland.

		Rate of infection %	
Collection date	**Total collected**	***Adelina* sp.**	***M anisopliae***
February	148	10.1	12.8
March	190	11.1	5.8
April	214	22.0	1.4
May	112	3.6	0.0
June	176	10.8	0.6

The possibility that the progression of disease was prolonged or ameliorated in larvae infected later in the season (and thus at a later developmental stage) was assessed by dissection of individuals that died as pupae, and of some individuals that successfully emerged as adults. None of these pupae or adults were observed to carry microbial infections, showing that low-level infections were not being overlooked in the experimental method employed, and that larvae infected with microbial agents invariably died before pupation. This latter point in particular has important ramifications for the transmission patterns of these diseases. The diseases are unlikely to be carried as sublethal infections by adults, and hence are likely to spread only slowly from field to field.

Given that limited information on infections was obtained from larvae collected late in the 1994 season, efforts were subsequently made to collect all field samples relatively early in the season. Therefore, in years 1995 - 1998, all field samples were collected by mid-May.

For the purposes of analysis, larvae collected in the 1995 - 1998 seasons were considered to originate from two distinct geographic areas, *viz* the Burdekin district (see Section 4.1), and a broad 'far north Queensland' region comprising collection sites from Bambaroo northwards, and including the Herbert Valley, Tully and Innisfail districts (Sections 4.2 - 4.4). Mortality data were analysed on the basis of larval age at death ('days post-hatching'), assuming standard emergence dates of 15 November and 15 December for the Burdekin and far north Queensland regions respectively (Figure 13). In this analysis, the proportion of the extant larval collection that died in each 30 day period was recorded as '% mortality', and cause of death within that group was then ascribed as shown. Total sample size thus varied at each recording point as larvae were added by collection, and eliminated by mortality.

As discussed in Section 4.1 and shown in Figure 12, the incidence of disease in the Burdekin region was extremely low in all sampling seasons, and this effectively prevented the detection of patterns of microbial epizootiology in those samples. In contrast, similar and repeated patterns of microbial pathogenicity and larval mortality were apparent in larvae collected from the far north Queensland region through seasons 1995 - 1998. This analysis, together with observation of field-collected larvae in the laboratory, revealed that

Adelina and *Metarhizium* pathogens display very different patterns of infection, morbidity, mortality and post-mortem consequences in their larval hosts.

Infection with *Adelina* has long been known to be a consequence of ingestion of infectious propagules (sporocysts within oocysts), whereas infection with *M anisopliae* results from the penetration of the larval cuticle by a hyphal body from a germinating spore (eg see Tanada and Kaya 1993). We observed that infection of larvae with *Adelina* was frequently accompanied by a lengthy period (weeks to months) of morbidity before death, and that during this time animals became progressively more lethargic and showed little or no feeding activity. Symptoms of the infection were otherwise extremely subtle; infected grubs were sometimes recognisable by development of a slightly fawn-brown coloration, which contrasted with the creamy-white appearance of healthy specimens. In contrast, infection with *Metarhizium* was not discernible by any external symptoms before death of the host; infected larvae apparently behaved and fed normally until suddenly succumbing to the infection.

Occurrence of mortality caused by these pathogens also appeared to show very different temporal patterns. As shown in Figure 12 (and by implication in Table 6), deaths associated with *M anisopliae* were most commonly observed in younger larvae, while deaths caused by *Adelina* tended to occur both over a longer period, and predominate in older animals. It was recognised, however, that the sampling strategy used in the 1995 - 1998 seasons (ie early collection, followed by a lengthy period of observation in sterile medium in the laboratory) might impose a bias against estimation of the incidence of a microbial agent that was capable of establishing infection at any time throughout larval life. Accordingly, data relating to larvae collected in the far north Queensland region during 1995 - 1998, and subsequently diagnosed with *Adelina* or *Metarhizium* infection, were analysed in further detail (Table 7). Specifically, larvae were categorised by their stage of development at collection (ie as first, second or third instar), and the incidence and times of mortality then analysed separately for each group.

Table 7 Parameters of mortality due to *Adelina* and *Metarhizium anisopliae* in greyback canegrub larvae collected at different developmental stages in the 1995 - 1998 seasons.

	Instar at collection		
	I	**II**	**III**
Total number collected	213	1152	1534
Assumed mean age at collection (days)	25	45	75
Killed by *Adelina* alone			
Number	41	346	426
Percentage of total	19.2	30.0	27.8
Days to death post-collection (±s.e.)	67±4	53.1±1	40±1
Killed by *Metarhizium* alone			
Number	16	78	47
Percentage of total	7.5	6.8	3.1
Days to death post-collection (±s.e.)	14±2	13±1	17±3
Co-infected with *Adelina* and *Metarhizium*			
Number	1	4	2
Percentage of total	0.5	0.3	0.1
Days to death post-collection	(0)	(0, 0, 16, 23)	(12,40)

As shown in Table 7, occurrence of *M anisopliae* infection showed significant variation between instars; while there was no significant difference between larvae collected as first and second instars with respect to their likelihood to develop infection, those collected as third instars were significantly less likely to develop infection than either of the earlier instars. This 'maturation immunity' (Tanada and Kaya 1993) shown by third instar larvae may be due to a variety of factors, including changes to cuticular structure or thickness. The mean time elapsed between collection and death in the laboratory did not vary substantially between the three instars, suggesting that there was little difference in the speed of action of the agent once infection of a host was established.

In the case of *Adelina,* larvae collected at different developmental stages also showed significant variation in their likelihood to die from infection (Table 7). In this case it was observed that larvae collected as first instars were significantly less likely to have acquired an infection than those collected as second or third instars. This observation is presumed to indicate the existence of a window of susceptibility to infection that extends throughout the first instar, and perhaps into the early second instar. Clear difference was also observed in the elapsed time to death by *Adelina* for different instars, with that period being longest in first instar larvae, and shortest in third instars (Table 7). This observation is consistent with the interpretation that infected larvae collected from the field harboured a slow-acting disease contracted at an early stage of development, whose associated mortality was apparent only long after infection. Results of our laboratory trials (see below) strongly support this supposition. On the basis of the calculations shown in Table 7, mean age at death for field collected larvae was estimated to be between 92 and 115 days post-hatching, a figure that agrees closely with results from the laboratory experiments (Section 9.2, below).

After consideration of the analyses of data presented in Tables 6 and 7 we were satisfied that any bias potentially associated with temporal aspects of our sampling strategy had not resulted in undue distortion of our estimates of incidence of disease.

Following death, individuals killed by the *Metarhizium* fungus were readily identifiable by the covering of white or green hyphae and spores that enveloped the cadaver. The cadavers themselves assumed a 'mummified' form, and although subject to progressive degradation over time, retained an identifiable form for weeks to months *post-mortem*; cadavers in this form were also commonly observed in the field. In contrast, cadavers of individuals that were killed by *Adelina* quickly became liquified, and rapidly and completely decayed and disappeared within hours or a few days of death, even when held individually in the protected laboratory environment. We believe that this post-mortem response adequately explains the fact that the disease had not previously been recorded from the host in the field, despite being the most common cause of larval mortality.

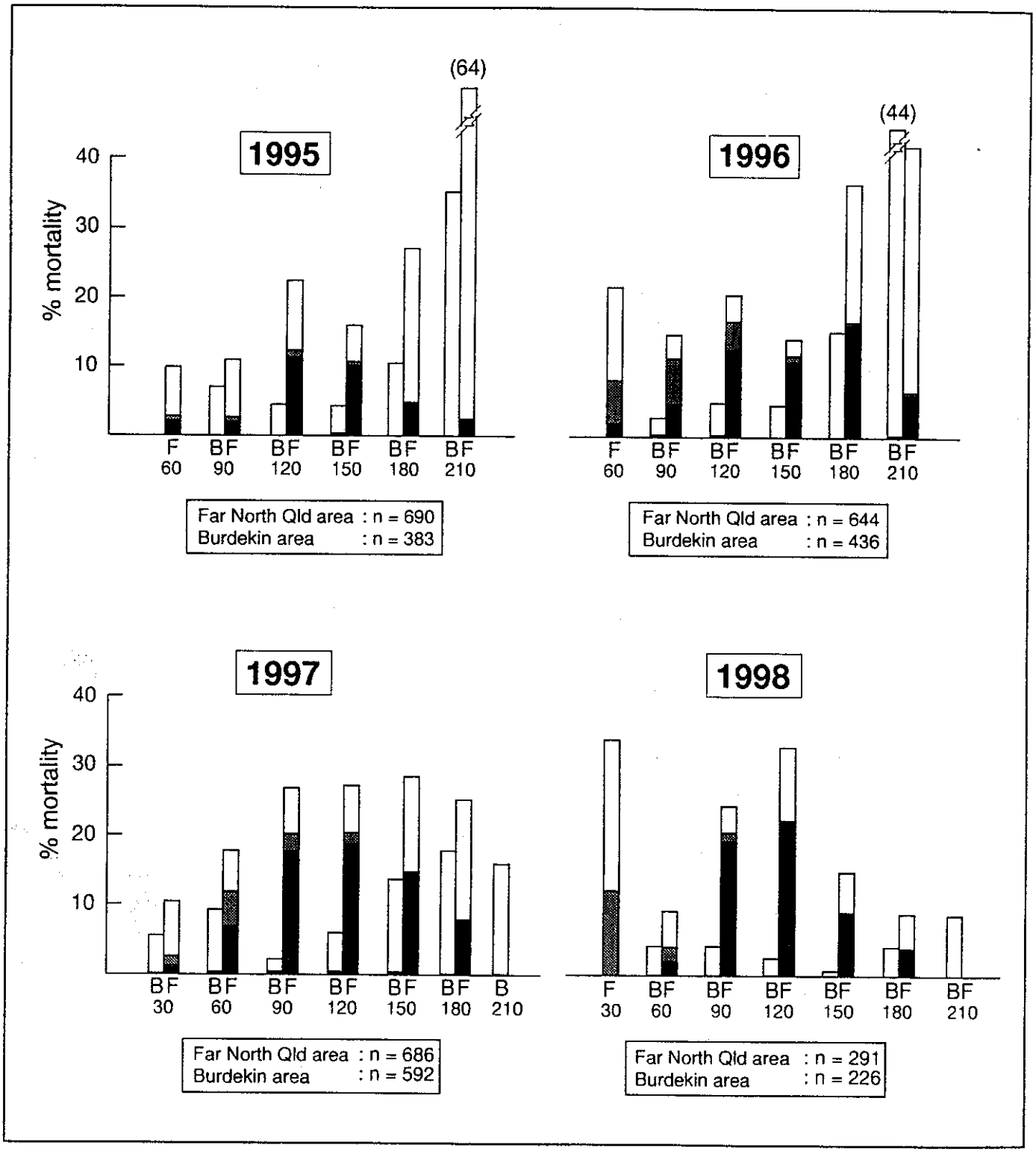

Figure 12. Monthly mortality profiles by cause of death from Burdekin (B) and Far North Queensland (F) for seasons 1995-98. Solid portions show levels of infection by *Adelina*, hatched portion shows infection by *Metarhizium*, and remaining portions show deaths by other micro-organisms or where no cause of death could be ascertained. Days post-hatching are shown below samples.

Data contained in Figure 12 were also analysed as a cumulative mortality curve (Figure 13). Not surprisingly, this analysis showed that within-season survival of larval populations differed markedly between the two geographical regions, largely as a result of the impact of *Adelina* and *Metarhizium* in the far north Queensland region. Thus, as shown in Figure 13, some 40% of larvae from the far north Queensland samples were observed to die within 120 days of hatching, whereas only 10% of larvae from the Burdekin district died in the same period. As discussed in Section 9.3 (below), this observation suggests that the disease organisms play an important role in reduction of crop damage by greyback canegrub in the far north Queensland region.

9.2 Infections established in the laboratory

As noted above, *Metarhizium anisopliae* was a commonly observed pathogen in field-collected larvae. An extensive program of research involving BSES, CSIRO and industrial partners is already investigating this pathogen and host combination; accordingly, no laboratory studies were undertaken with this pathogen as part of the current project.

Laboratory feeding trials with other pathogens were designed to determine the maximum possible impact of the pathogens on larvae, in all cases assuming that this would be most marked when very young larvae were exposed to the disease (see above). All trials reported here thus used first instar larvae collected 2 - 3 days after hatching from eggs laid in the laboratory by field-collected adults. Larvae were then immediately exposed to propagules of the micro-organisms. In some experiments larvae were presented with artificial diet contaminated with propagules, while in others the larvae were allowed to develop for a pre-determined period in peat moss potting soil medium contaminated in the same way. After exposure to infection, larvae were individually maintained at 28^{o}, and monitored twice weekly. In all instances larval growth was assessed by weight gain, and infection status was ultimately determined by examination of fat body smears using light microscopy.

9.2.1 *Adelina* feeding trials

A series of experiments was done with the coccidian protozoan *Adelina*. In the first experiments, larvae were allowed to consume a small piece of diet contaminated with 10, 10^2 or 10^3 oocysts before transfer and rearing under standard conditions. Other experiments either used the same method and parameters, or exposed larvae to purified oocysts in artificial substrate.

In the first series of experiments, 31, 35 and 65% of larvae that survived beyond two weeks post-feeding (n=13, 17 and 23 respectively) were found to be infected by the three respective doses. The development of infected larvae was reduced in comparison to that of uninfected controls, with the former dying 8 - 22 weeks after initial exposure. Time to death was observed to vary with dose; mean times (± s.e.) to death were 115±25, 91±16, 81±4 days for the lowest to highest doses respectively. As observed for field-infected animals, larvae infected with *Adelina* in the laboratory became lethargic and ceased feeding many weeks before death.

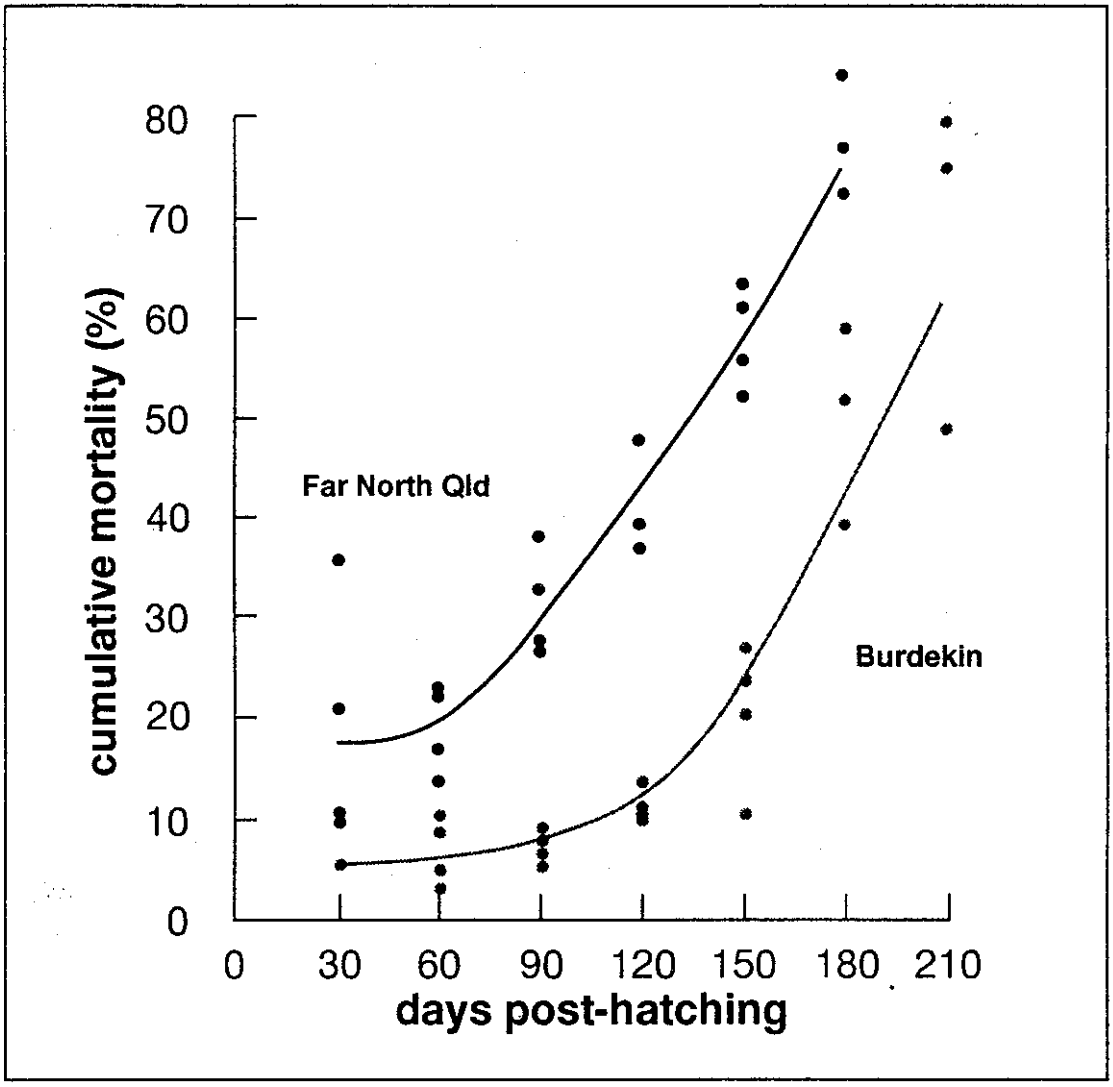

Figure 13. Cumulative mortality curves for greyback larvae collected from Far North Queensland and Burdekin districts for seasons 1995-98.

Other experiments used highly purified preparations of oocycts from larvae that had been stored at 5°C for > six months after death; in these experiments there was complete failure to achieve infection. It remains unclear whether this result was due to an inability of the microbial agent to retain infectivity after prolonged storage at low temperature, mechanical damage of oocycts during the purification process, or a combination of these or other factors. Other work as part of BSS166 has since shown that long-term cold storage also affects the ability of *Adelina* to establish infection under field conditions, suggesting that the first of these explanations may be of primary importance.

9.2.2 '*Nosema*' feeding trials

Two series of experiments were done with the microsporidian protozoan referred to as '*Nosema*'. In the first, young larvae were allowed to consume a small piece of diet contaminated with either $5x10^4$ or $1x10^6$ spores, before transfer to standard rearing conditions. In the second, larvae were allowed to develop for seven days under normal rearing conditions in substrate contaminated with either $2x10^5$ or $2x10^6$ spores per gram.

In the first series of experiments, 9 of 22 (41%), and 9 of 9 (100%) exposed larvae (that survived beyond two weeks post-feeding) were ultimately found to be infected at the lower and higher dose respectively. In the second series, none of 13, and 21 of 29 larvae (72%) exposed to the lower and higher doses respectively were found to be infected. Examination of a subsample (n=6) of infected larvae from the latter trial showed an average production of $5x10^9$ spores per host.

Consequences of infection with '*Nosema*' appeared very similar in all experiments. As shown in Figure 14, which describes development of 19 infected larvae exposed to a dose of $2x10^6$ spores per gram of substrate, infected animals grew at close to the normal rate for about eight weeks post-infection, but then invariably failed to undergo a moult from second to third instar. From this point, infected larvae showed little feeding activity and their weights steadily declined until death at an average of 16 weeks post-infection.

It is possible that the observed uniformity of response in both growth and final spore yield is a consequence of the establishment of infection in larvae of a uniform age. This hypothesis will be further investigated in another SRDC-supported project (CE003) through establishment of infection in larvae of different ages and sizes. Nevertheless, the experiments reported here demonstrated that infection with '*Nosema*' can dramatically affect the development and survival of greyback larvae.

9.2.3 Entomopoxvirus (DaEPV *sensu stricto*, and $DaEPV_{SR}$) feeding trials

Feeding trials were done with a previously described entomopoxvirus (EPV) from greyback canegrub (DaEPV *sensu* Goodwin and Filshie 1975; Goodwin and Roberts 1975; henceforth referred to as DaEPV) and a newly identified EPV from the same host, which was first isolated from a larva collected at Stone River in the Herbert Valley (henceforth referred to as $DaEPV_{SR}$).

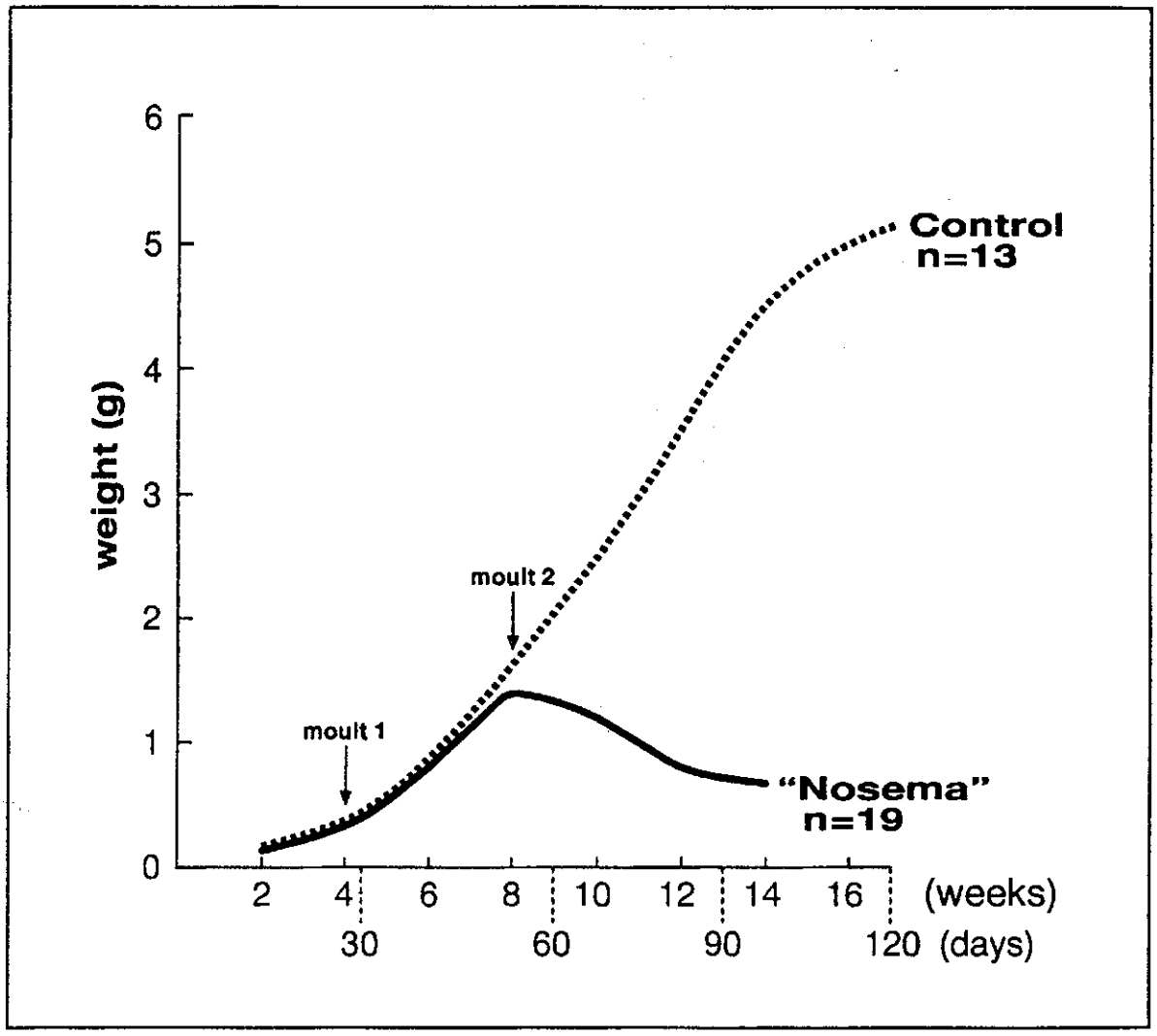

Figure 14. Growth curve for greyback larvae infected with '*Nosema*' microsporidian disease compared with growth curve for healthy larvae.

Infection with both of the viruses caused reduction in weight gain as compared to controls, and in a manner reminiscent of the effects of '*Nosema*' infection, larvae ceased to feed some considerable time before death. Infected larvae died some 12 - 15 weeks post-infection.

Investigation of the ultrastructure of infection by DaEPV revealed the existence of a previously unknown replicative strategy in which mature spheroids are apparently actively expelled from infected cells. In contrast, replication of $DaEPV_{SR}$ apparently involves morphogenesis of both spheroids and spindle bodies within a virally-derived membrane-bound vesicle inside the infected cell.

9.3 Conclusions from observations on field-collected and laboratory-infected larvae

Integration of work with field-collected and laboratory-infected larvae allows us to make a good estimate of the influence and characteristics of various diseases on activity and survival of greyback canegrub larvae. The previously unrecognised pathogen Adelina sp. *nov* (which we are in the process of describing with a proposed name of *Adelina dermolepidae*) was responsible for the deaths of about 28% of all larvae collected in the field in far north Queensland. The previously known disease *Metarhizium anisopliae* was also found to have some natural effect, being responsible for the death of about 5% of the larvae. Four other pathogens were also found as described in Section 5.2; overall, disease was responsible for mortality of about 33% of all larvae. Work in this project suggests that crop management is important to disease occurrence (Section 5.3), and this is being further investigated in a related project (BSS166).

In addition to the *Metarhizium* isolate (FI 1045) collected as part of this project and since developed for commercial use, we have identified a second pathogen that we believe also has promise as a control agent. The microsporidian '*Nosema*' is rare in the field (incidence of < 0.5%), but it is highly pathogenic and possesses other characteristics that would appear to suit this purpose (eg tolerance to handling, good storage capacity). A further project aimed at investigating the practicability of its use has been initiated (CE003).

10.0 *IN VITRO* AND HETEROLOGOUS CULTURE OF SELECTED MICRO-ORGANISMS (Objective 8)

Over the course of the project we made several attempts to establish replication of the microsporidian '*Nosema*' and the EPVs in cells in culture (both of beetle and caterpillar origin), and in the case of '*Nosema'*, in caterpillars. In no case were we able to establish replication either *in vitro* or in a heterologous host. These attempts were empirical in their nature, the aim being to meet the undefined requirements of establishing pathogen replication. At this stage, production of any of these agents (excluding *M anisopliae*) will be most effectively achieved *in vivo*.

11.0 CONCLUSIONS

This project and allied studies identified three important factors in the determination of population density of greyback canegrub within areas where it is a major pest of sugarcane. The first is the propensity of greyback beetles to select tall crops of sugarcane in which to lay their eggs, quantified by Ward and Cook (1996). This behaviour affects the distribution of grubs at the local farm level, but does not affect the area-wide population dynamics of the pest. Variation in recruitment rate between late stage grubs in one generation, and established young grubs in the following generation was identified as the key factor of population change even within tall cane. This change was determined by variations in beetle migration, and in natality and mortality rates between late-stage grubs and subsequently established young grubs in the next generation, all of which are poorly understood.

The second factor is the relatively constant population density of newly established grubs under cane stools, commonly 2 - 4 per stool, irrespective of the relative population density of adults caught in light traps at the start of the season. This observation, and other research in the Burdekin by Ward (1998) which showed high mortality when a high density of neonate grubs was introduced to enclosures, suggests that intraspecific competition limits the upper density of grub populations.

The third important factor is the density-dependent mortality of grubs caused by entomopathogens, and by *Adelina* sp. in particular. Grub populations are maintained near the establishment population density throughout their development period in the absence of disease, leading to severe damage to sugarcane. This is the situation in the Burdekin district. Where disease incidence is relatively high as in parts of the Herbert, Tully and Innisfail districts, grub population density declines through the season. *Adelina* sp. appears to act in a delayed density-dependent way, with peak incidence following high population density of the grub hosts, and then decline in incidence as the population of grubs declines at low levels. *Adelina* sp. may cause oscillatory cycles in greyback canegrub population densities, which explains the cycles of alternating severe and low levels of damage seen in far north Queensland before the introduction of BHC.

The results of this study suggest that control of grubs, rather than control of beetles, is the only rational method of control of greyback canegrub. The significant impact of entomopathogens should be used in management of greyback canegrub, and farming practices should be developed to foster and augment biological control of greyback canegrub. Incidence of entomopathogens appears to be related to the degree of disturbance of canegrowing soils; minimum tillage planting, zero-tillage ratooning and trash blanketing should be developed and extended to all districts suffering damage by greyback canegrub. Development of *Metarhizium* as an augmentative biological control is proceeding and will be a useful component of management for greyback canegrub. Further research on *Adelina* and '*Nosema*' species may also result in augmentative biological control agents able to be reintroduced when greyback populations increase in the virtual absence of natural control.

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APPENDIX 1

Summary of the incidence of two entomopathogenic diseases recorded from greyback canegrub during extensive survey of grub populations. Grubs were held at CSIRO Entomology, Canberra, until death or pupation each year between 1994 and 1998.

Location	**Year**	**Pathogen (% incidence)**	
		***Adelina* sp.**	***M Anisopliae***
Tully			
Tully 25A[1]	1996	14	43
	1997	8	28
	1998	11	44
Tully (1/1)[2]	1995	63	32
(1/2)	1996	4	0
(1/3)	1997	20	2
(1/4)	1998	39	0
Mulgrave	1994	0	2
Mackay	1995	0	0
Proserpine	1998	0	0
Mareeba	1998	0	3
El Arish			
El Arish (1/1)	1994	9	3
El Arish (2/1)	1995	10	23
El Arish (2/2)	1997	0	0
El Arish (2/3)	1998	63	0

[1] Field 25A, planted in 1995, adjoining intensively monitored site (field 26B).
[2] Farm 1, field 1 (Musumeci).