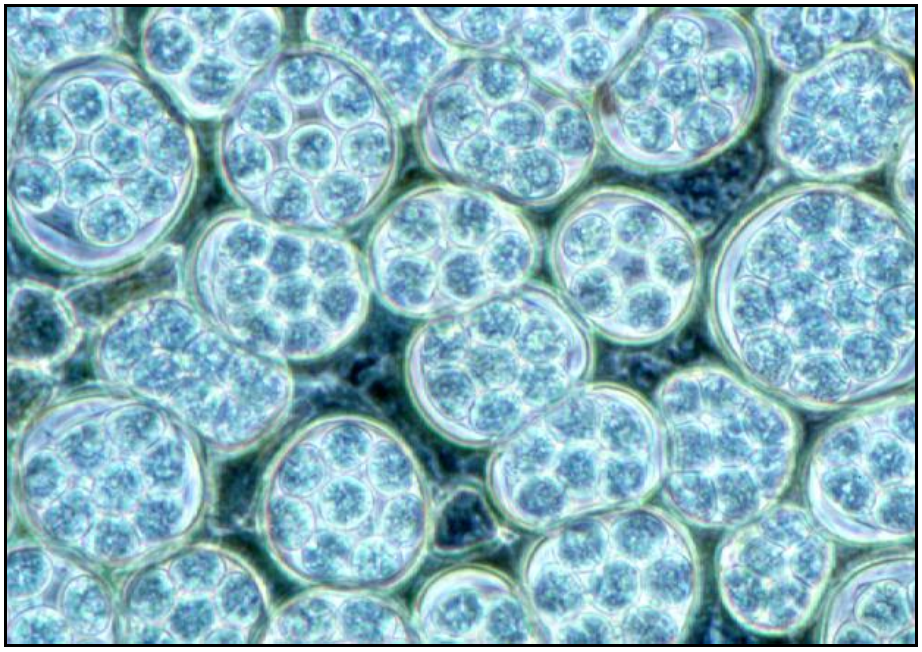
FINAL REPORT

SRDC PROJECT CS004

ENVIRONMENTAL FACTORS AFFECTING *ADELINA* IN THE BURDEKIN

by

DAVID J DALL and DAVID P LOGAN



Oocysts of Adelina*, a coccidian protozoan pathogen of greyback canegrubs*

Principal Researchers:

Dr David Dall
CSIRO Entomology
GPO Box 1700
Canberra ACT 2601
Email: david.dall@csiro.au

Dr David Logan
BSES
PO Box 117
Ayr Q 4807

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SUMMARY

The project aimed to determine why the canegrub pathogen *Adelina* does not infect a significant proportion of the canegrub population in the Burdekin Delta area.

It examined the effect of physical characteristics of soil (pH, sodicity and composition) and three other environmental parameters (temperature, relative humidity and compaction) on retention of oocyst infectivity for larvae over periods corresponding to one and two cycles of grub infestation of crops in the field. In addition, the project raced the establishment and persistence of *Adelina* infection in two experimentally inoculated field plots in the Burdekin canegrowing area.

A 'sentinel larva'-based bioassay that allows examination of the effect of various environmental factors on the retention of infectivity of *Adelina* was developed. Results of such a bioaasay showed no differences between the three types of soils tested, despite one soil coming from a region where the pathogen is very common (Bambaroo), and another from a region where it is quite rare (Burdekin Delta). The test did clearly show that drying conditions can be highly detrimental to retention of infectivity of the pathogen. Extrapolation from these results would suggest that field locations where there are prolonged periods of dry soil conditions would be at a relative disadvantage as regards maintenance of high levels of the pathogen in grub populations.

Under field conditions in the Burdekin, *Adelina* did survive at levels sufficient to cause infection in the following generation of canegrubs in some areas and in one of two years; in the other plots and year, there was no subsequent infection.

These results thus show that while oocysts of protozoan pathogens such as *Adelina* have robust external walls, they are, nevertheless, susceptible to the influence of adverse environmental conditions. Under field conditions, where exposure to any potential inoculum is likely to be more transient, the influence of an adverse environmental factor is even more acute.

Improved understanding of the physical tolerances of the infective stages of *Adelina* has assisted in the design of crop-management strategies that maximise the impact of the pathogen on canegrub populations. These strategies have been incorporated into the GrubPlan program that has been successful in reducing the impact of greyback canegrub.

1. BACKGROUND

Canegrubs are estimated to cost canegrowers in the Sarina-Mossman region about $10 million each year through losses to production and purchase of insecticides. The true area-wide cost of damage to the industry, which includes losses through premature ploughout of crops, losses to contract harvesters, processing of excess quantities of soil debris at milling, etc, is certainly far higher, but is almost impossible to estimate precisely.

While some 19 species of canegrubs are recognized as infesting Australian sugarcane crops, by far the most economically important is the greyback canegrub, *Dermolepida albohirtum*.

Research by BSES and CSIRO done as part of the SRDC-supported projects BSS120 and BSS166 (Robertson *et al.* 1998; Allsopp *et al.* 2002) showed that a coccidian protozoan, putatively from the genus *Adelina*, is the most important natural factor regulating established greyback larval populations in the field.

Those studies found that up to 80% of greyback larvae were killed by *Adelina* infection at some field sites in the far north Queensland (FNQ) region, and overall, that the pathogen was estimated to cause the death of about 30% of all grubs that established in crops in FNQ. In contrast, the rate of *Adelina* infection of greyback canegrubs collected from the Burdekin region in seasons 1995-98 was never more than 1%, even thought the levels of host availability exceeded those that were sufficient to support disease outbreaks in the more northern area. Similar results were found in more recent surveys by Sallam *et al.* (2003).

It was hypothesised that this difference in incidence of infection, and hence of pest survival, was at least partially responsible for the different levels of crop damage sustained in the two areas. The reason(s) for the failure of the pathogen to thrive in the Burdekin Delta were not determined in those previous studies, but were of obvious interest. In particular, it was recognised that an understanding of the factor(s) responsible for the differential rates of pest infection by the pathogen might also allow manipulation of the incidence of disease in areas that currently host 'healthy' populations of greyback canegrubs.

Accordingly, this project aimed to investigate some of the factors hypothesised to cause or influence the observed differences between rates of grub infection in the two (FNQ and Burdekin) regions.

Our previous observations in the laboratory suggested that *Adelina* might be relatively sensitive to environmental conditions at some points in its lifecycle. This project aimed to examine the effect of several environmental variables on the retention of infectivity of the resting stages (oocysts) of the pathogen in infested host cadavers. Specifically, it aimed to examine the effect of physical characteristics of soil substrates (e.g., pH, organic content and other compositional factors) and of two other environmental parameters (temperature and relative humidity) on retention of infectivity of *Adelina* oocysts for larvae of *D. albohirtum*, over periods corresponding to one and two cycles of grub infestation of crops in the field. It also attempted to assess the carryover of infectivity in the field.

2. OBJECTIVES

The project aimed to determine why the canegrub pathogen *Adelina* does not infect a significant proportion of the canegrub population in the Burdekin Delta area. It examined the effect of several key environmental variables on the retention of infectivity of the resting stages of the pathogen.

Specifically, it:

- Examined the effect of physical characteristics of soil (pH, sodicity and composition) and three other environmental parameters (temperature, relative humidity and compaction) on retention of oocyst infectivity for larvae over periods corresponding to one and two cycles of grub infestation of crops in the field; and
- Traced the establishment and persistence of *Adelina* infection in two experimentally inoculated field plots in the Burdekin canegrowing area.

Both objectives were met.

A 'sentinel larva'-based bioassay that allows examination of the effect of various environmental factors on the retention of infectivity of *Adelina* was developed. Results of such a bioaasay showed no differences between the three types of soils tested, despite one soil coming from a region where the pathogen is very common (Bambaroo), and another from a region where it is quite rare (Burdekin Delta). The test did clearly show that drying conditions can be highly detrimental to retention of infectivity of the pathogen. Extrapolation from these results would suggest that field locations where there are prolonged periods of dry soil conditions would be at a relative disadvantage as regards maintenance of high levels of the pathogen in grub populations.

Under field conditions in the Burdekin, *Adelina* did survive at levels sufficient to cause infection in the following generation of canegrubs in some areas and in one of two years; in the other plots and year, there was no subsequent infection.

These results thus show that while oocysts of protozoan pathogens such as *Adelina* have robust external walls, they are, nevertheless, susceptible to the influence of adverse environmental conditions. Under field conditions, where exposure to any potential inoculum is likely to be more transient, the influence of an adverse environmental factor is even more acute.

3. METHODOLOGY AND RESULTS

3.1 Laboratory bioassay

3.1.1 Methodology

3.1.1.1 Soil samples

Soil samples were obtained from three field locations in sugar growing regions:

- an established sugar-growing site from Bambaroo, at the southern edge of the FNQ growing region, and known to have greyback grub populations with very high levels of *Adelina* infection;
- an established sugar-growing site from the Burdekin River Delta, where grub populations generally show very low levels of *Adelina* infection ('old Burdekin'); and
- a prospective sugar industry 'expansion' site from the Burdekin River Delta, where grub populations have not been studied ('new' Burdekin).

Soil samples were adjusted to approximate homogeneity of texture by breaking lumps in a mortar and pestle, and sieving through a fine mesh sieve. The soils were then dried at 35°C until no further loss of weight could be observed.

Samples of each of the three soils were then analysed for their key physical and physico-chemical parameters (CSIRO Land & Water, Canberra) (Table 1).

Table 1. Physico-chemical properties of field-collected soils used in experiments

Soil property	Soil origin		
	Bambaroo	**'Old' Burdekin**	**'New' Burdekin**
Field capacity[1]	20.0	30.0	26.0
Wilting point[2]	6.7	11.2	8.3
pH[3]	5.2	7.0	6.9
pH[4]	4.5	6.2	6.0
Total carbon (%)	1.2	1.6	1.2
Organic carbon (%)	1.2	1.6	1.2
Total nitrogen (%)	0.07	0.06	0.07

[1]at 100 cm water column; quoted in g water per 100 g soil; [2]at 15 bar; g water per 100 g soil; [3]in aqueous solution; [4]in 10 mM $CaCl_2$

3.1.1.2 Experimental treatment of oocysts

Greyback canegrubs were collected at field locations in north Queensland and transported to Canberra, where they were individually maintained at 28°C in a light loam/peat moss mixture, and fed *ad libitum* with diced carrot. Tissue smears were prepared from cadavers of grubs that died, and were examined by phase contrast light microscopy for the presence of the resting stage (oocyst) of the pathogen.

Cadavers containing large numbers of oocysts were stored individually in 15 mL sealed plastic tubes at room temperature, under moist conditions, until their subsequent use in experiments. Duration of storage ranged from a few days to a maximum of about 3 months.

Pathogen-infested cadavers were randomly assigned to nine different experimental treatments, each comprising incubation in one of the three soil types, adjusted

appropriately (where necessary) to one of three soil moisture levels (dry, wilting point, field capacity), and subsequently maintained at either 20, 25 or 28°C.

Cadavers were placed individually in treatment 'arenas' comprising 150 mL plastic tubs, each containing about 25 g of soil prepared as described above. The total weight of each arena (comprising the prepared soil matrix, cadaver and container) was recorded at the commencement of treatment. Arenas were thereafter weighed at monthly intervals, and extra water was added if/ as necessary to return the contents to the appropriate (initial) weight, and thus, to the corresponding intended moisture level.

Exposure to experimental treatment regimes was maintained for approximately 8 months or 20 months (May-December), these being durations that approximately correspond to the period in the field between death of infected grubs and incidence of the next one or two generations of canegrubs.

Immediately prior to introduction of sentinel eggs/larvae into the treatment arenas (see below), the contents of all arenas were adjusted to field capacity moisture level by addition of the required amount of water, and a small quantity of loam/ potting mixture containing germinating rye grass seeds was added.

3.1.1.3 Sentinel larvae and assessment of retention of oocyst infectivity

Adult greyback beetles were collected from feeding sites in the field in northern Queensland in November and December, and sent to the laboratory in Canberra where they were held in groups of about 50 in plastic 'hobby crates' containing a small amount of damp peat moss. Eggs laid by those adults were individually collected, transferred to a moist sterile loam/potting soil substrate and allowed to develop to near maturity. Six mature eggs were placed into each of the treatment arenas, and 14 days were allowed for the eggs to hatch and for young larvae to burrow and feed in the arena contents. These 'sentinel larvae' were then recovered and reared individually as described above for field-collected grubs. A second group of six eggs was then replaced into each of the same arenas, and these larvae were treated as described for the first sentinel cohort. Sentinel larvae were maintained with twice-weekly observation until death, then assessed for presence of infection by examination of tissue smears as above.

3.1.2 Results

Rates of emergence of sentinel larvae from mature eggs were routinely near 100%, but rates of survival to a point where status of infection could be reasonably determined (see below) were highly variable, both within and between different replicates of transmission trials.

Accordingly, despite the fact that trials had been established with the intention of systematically assessing transmission rates on the basis of the rate of acquisition of infection by sentinel larvae, this intent had to be adapted to the realities of the situation.

As a consequence, each exposure of individual cadavers to sentinels was considered to represent a single acquisition trial, and was scored as being either

- a positive transmission event (where transmission to at least one sentinel larva was recorded);
- a negative transmission event (where no transmission could be demonstrated); or
- a null result (where no sentinel larva in the trial survived long enough for a positive or negative result to be satisfactorily assigned, i.e., survived until to the date of the first positive transmission event recorded for that experiment).

Results of all experiments, scored in this manner, are shown in Table 2. Overall, a total of 115 valid (ie either positive or negative) trials was assessed, comprising 74 for the 8-month treatment duration, and 41 for the 20-month duration. Of these, 56 and 22 trials, respectively, gave a positive result. Given the existence of many missing data cells due to the null results recorded, the data shown in Table 2 were pooled on the basis of the three treatment factors under examination, with results as shown in Tables 3.1-3.3.

Examination of the data shown in Tables 3.1-3.3 suggested that the type of soil to which the cadaver was exposed had no effect on persistence of infectivity of oocysts for sentinel larvae (Table 3.1). Likewise exposure of the infested cadavers to different temperatures did not produce any discernable trend as regards persistence of oocyst infectivity (Table 3.2).

In contrast, exposure to different levels of relative humidity had a marked effect on persistence of oocyst infectivity for sentinel larvae. After 8 months of treatment there were indications of a trend towards reduction of infectivity under drying conditions, with > 95% of trials of cadavers and oocysts maintained at field capacity being positive, but < 65% of those maintained under dry conditions being positive (χ^2 = 1.65, 2 df, P = 0.44). After exposure to 20 months of treatment the trend was even more pronounced, with > 85% of trials of cadavers and oocysts maintained at field capacity remaining positive, but < 22% of those maintained under dry conditions being positive (χ^2 = 5.4, 2 df, P = 0.067).

Table 2. Outcomes of acquisition trials using infested greyback cadavers and sentinel larvae. See text and notes for interpretation.

Treatment	8-month treatment					20-month treatment		
	Exp 1	Exp 2	Exp 12	Exp 13	Exp 14	Exp 3	Exp 4	Exp 5
BM D 20	1	1	x	0	x	0	x	0
BM W 20	1	0	1	x	x	x	x	x
BM F 20	x	1	1	1	x	x	x	x
BM D 25	1	1	0	0	0	0	x	x
BM W 25	x	x	0	x	x	1	1	x
BM F 25	1	x	x	1	x	1	x	1
BM D 28	1	1	1	x	x	1	0	0
BM W 28	1	x	0	1	x	0	0	1
BM F 28	x	x	1	1	x	1	x	1
OB D 20	1	1	0	x	x	0	1	0
OB W 20	1	1	x	0	x	1	x	x
OB F 20	1	1	x	1	x	x	x	x
OB D 25	1	x	0	x	x	0	x	x
OB W 25	1	1	0	0	1	x	1	1
OB F 25	x	1	1	x	x	1	0	x
OB D 28	1	1	x	0	x	0	x	x
OB W 28	1	0	1	1	x	x	0	0
OB F 28	1	1	0	1	x	1	x	1
NB D 20	1	1	0	0	x	x	1	x
NB W 20	1	1	1	0	x	x	x	x
NB F 20	x	1	1	1	x	x	1	x
NB D 25	1	x	x	x	x	0	x	0
NB W 25	1	0	x	x	x	1	x	x
NB F 25	x	0	x	1	x	1	x	1
NB D 28	1	x	x	x	x	x	x	x
NB W 28	1	1	0	1	x	0	x	0
NB F 28	x	1	x	1	x	1	0	1

1. Soil type used in trial is denoted as BM (Bambaroo), OB ('old' Burdekin) or NB ('new' Burdekin); moisture level is denoted as D (dry), W (wilting point) or F (field capacity); exposure temperature is indicated (20, 25 and 28°C).
2. A positive transmission event is indicated as '1', a negative event as '0', and a null result as 'x'.

Table 3.1. Sentinel acquisition data combined by soil type to which cadaver was exposed

Soil origin	Acquisitions			
	8-month duration		20-month duration	
	outcome[3]	%	outcome	%
BM[1]	18/25	72.0	8/15	53.3
OB	21/27	77.7	7/14	50.0
NB	17/22	77.3	7/12	58.3
Total[2]	56/74	-	22/41	-

[1] designations as for Table 2

[2] for cross-checking purposes only

[3] denotes number of positive trials/total number of valid trials (combined positive and negative trials)

Table 3.2. Sentinel acquisition data combined by temperature to which cadaver was exposed

Temperature °C	Acquisitions			
	8-month duration		20-month duration	
	outcome[1]	%	outcome	%
20	22/29	75.9	4/8	50.0
25	13/22	59.1	10/15	66.7
28	21/23	91.3	8/18	44.4
Total	56/74	-	22/41	-

Table 3.3. Sentinel acquisition data combined by relative humidity to which cadaver was exposed

Relative humidity	Acquisitions			
	8-month duration		20-month duration	
	outcome	%	outcome	%
D[1]	16/25	64.0	3/14	21.4
W	19/27	70.4	7/13	53.9
F	21/22	95.5	12/14	85.7
Total	56/74	-	22/41	-

[1] D = dry; W= wilting point; F = field capacity

3.1.3 Conclusions

During the course of this project we have developed a 'sentinel larva'-based bioassay that allows examination of the effect of various environmental factors on the retention of infectivity of a coccidian protozoan pathogen of the greyback canegrub *D. albohirtum*. Although this is a relatively difficult assay, mostly because of the fragility of the neonate beetle larvae, it has allowed us to examine the effect of three factors that had been hypothesised as potentially likely to influence pathogen persistence in the field.

Our results did not show any differences between the three types of soils tested, despite the fact that one soil was taken from a region where the pathogen is very common (Bambaroo), and another from a region where it is quite rare (Burdekin Delta).

In contrast, our results clearly showed that drying conditions can be highly detrimental to retention of infectivity of the pathogen. Extrapolation from these results would suggest that field locations where there are prolonged periods of dry soil conditions would be at a relative disadvantage as regards maintenance of high levels of the pathogen in grub populations.

These results thus show that while oocysts of protozoan pathogens such as *Adelina* have robust external walls, they are, nevertheless, susceptible to the influence of adverse environmental conditions.

We note also that these assays – where relatively large numbers of young larvae are placed in close confinement with inoculum for a lengthy period – are likely to maximise the potential for acquisition of infection. Under field conditions, where exposure to any potential inoculum is likely to be more transient, it is possible that the influence of an adverse environmental factor will be even more acute.

3.2 Exposure under field conditions

3.2.1 Methodology

Field trial 1 was established in late 1999 in a commercial canefield at BSES Burdekin using all available *Adelina*-infected cadavers transported from Canberra to Ayr. Eggs and first-instar larvae of *D. albohirtum* were introduced by hand into eight 5-m sections of a late-planted plant crop (Q165) on 15 December at a rate of 15 instar equivalents per metre of row. 6 days later *Adelina*-infected cadavers were placed into six of these areas at a rate of 5 cadavers per metre row; two areas were left 'untreated'. All larvae collected on 6 March 2000 were transported to the laboratory in Canberra for maintenance and post-mortem diagnosis to determine infection rates.

The trial was cut early in the 2000 harvest season in an attempt to promote grub infestation in the first-ratoon crop; it was not trash blanketed. Larvae were recovered from the plots in March 2001 and sent to Canberra as above.

Field trial 2 was similar, but established in 2000. It was sampled only in March 2001.

3.2.2 Results and conclusions

Of the grubs collected from Trial 1in March 2000, the overall infection rate with *Adelina* was 25% in the plots inoculated with cadavers, whilst no larvae were infested in the 'untreated' plots (Table 4). However, all of the infected larvae came from the one inoculated plot – why this was so is not clear, but may reflect the low number of larvae collected from the plots.

All of the *Adelina*-infected grubs recovered died in the laboratory during 28 March-12 April, giving minimum and maximum periods between infection and death of 97 and 113 days, respectively. This result is consistent with 'time-to-death' estimates for larvae previously infected and held under laboratory conditions and for naturally infected larvae collected from the field during BSS120.

Table 4. Infection levels in larvae collected from Field Trial 1 in March 2000

Plot	Number collected	Number dead	Number infected with *Adelina*	Other infections
Controls (combined)	4	4	0	1 - nematodes
Adelina 1	2	1	0	1 - *Metarhizium*
Adelina 2	2	1	0	1 - nematode
Adelina 3	6	4	0	1 - nematode
Adelina 4	0	-	-	
Adelina 5	1	1	0	
Adelina 6	13	9	6	
Total – *Adelina* plots	24	16	6	

None of the larvae collected from either of the two trials in March 2001 (79 in total) were infected with *Adelina*.

4. OUTPUTS

The experiments have shown that soil type *per se* is not an important factor in regulating the persistence of *Adelina* from one generation of canegrubs to the next. Soil moisture, however, can have a significant effect, with low soil moistures significantly decreasing potential survival and carry over of the pathogen. Under field conditions in the Burdekin, *Adelina* did survive at levels sufficient to cause infection in the following generation of canegrubs in some areas and in one of two years; in the other plots and year, there was no subsequent infection.

These results are consistent with the absence of infection in field populations of greyback canegrubs in the Burdekin. The main factor causing this is probably the significant wet-dry cycle under irrigation in the Burdekin area, exacerbated by the absence of trash blanketing. This has significant implications for management of greyback canegrubs in the Burdekin.

The results are also consistent with studies by Sallam *et al.* (2003) that showed that *Adelina* survived better under weed cover in a fallow than under bare soil in the fallow.

5. EXPECTED OUTCOMES

Improved understanding of the physical tolerances of the infective stages of *Adelina* has assisted in the design of crop-management strategies that maximise the impact of the pathogen on canegrub populations. These strategies have been incorporated into the GrubPlan program (Hunt *et al.* 2002) that has been successful in reducing the impact of greyback canegrub (Hunt *et al.* 2003).

6. FUTURE RESEARCH NEEDS AND RECOMMENDATIONS

While we have tested three different factors that might be of relevance in the sugar-growing environment, others, such as mechanical disturbance, remain to be tested; this was not tested by Sallam *et al.* (2003). This could have significant effect on the carry-over of infective stages.

7. PUBLICATIONS ARISING FROM THE PROJECT

None as yet – one planned.

8. ACKNOWLEDGEMENTS

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