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Research Organisation: CRC for Tropical Pest Management.

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FIELD ASSESSMENT OF HELIOTHIS VIRUSES ON COTTON

R. E. Teakle, CRC for Tropical Pest Management

ABSTRACT.

A Heliothis NPV formulatiou, GemStar®, showed promise as a basis for successful selective, non-chemical insect control on cotton. Trials were conducted with the virus as a stand-alone insecticide on dryland cotton at Dalby, Q. by D. Murray (QDPI), and in conjunction with Envirofeast® on irrigated cotton at Moree by R. Mensah, (NSW Agriculture).

On dryland cotton at Dalby, Qld, yields were equivalent to those with conventional chemical control at one of the two sites. The virns failed to contain a heavy infestation of Heliothis in February 1996, but neither did conventional insecticides.

In an IPM programme on cotton with lucerne strip cropping at Moree involving 4 Envirofeast sprays followed by 3 sprays of the virns combined with Envirofeast and a clean-up chemical spray, yields were similar to those obtained with a conventional insecticidal regimen. Predator numbers were higher in the Envirofeast/GemStar plots than in other "soft" treatments, Envirofeast/endosulfan or Envirofeast/Bt endosnlfan, suggesting that any disruption to predators by GemStar was at a low level.

Bioassays indicated that GemStar® maintained its potency during on-farm, refrigerated storage. Levels of infection achieved on cotton were variable but the overall average was about 55%. This suggests that the use of the virns should be integrated with other Heliothis control measures.

Thresholds frequently exceeded 2 larvae per row metre without undue damage to the crops, suggesting that this biocontrol system provided greater flexibility in control response than with chemical insecticides. This aspect requires further investigation to determine appropriate thresholds.

INTRODUCTION.

Chemical control. Insect attack, mainly by the cotton bollworm, (*Helicoverpa armigera* and *Helicoverpa punctigera*) is a major constraint to cotton production. Chemical insecticides are the main means of Heliothis control, but their diminishing performance resulting from resistance, particularly in *Helicoverpa armigera,* has created a serious problem for the industry. Furthermore, indiscriminate destruction of beneficial species by chemical insecticides leaves the cotton crop vulnerable to insect pest attack, and prevents the adoption of effective IPM strategies. More selective means of pest control are needed.

Heliothis virus. A new formulation of a Heliothis-specific nuclear polyhedrosis virus (NPV), GemStar®, is available from Biosys Inc. in the United States. This has performed well in laboratory bioassays and preliminary field assessments on cotton in Queensland, and an application for registration for use on cotton in Australia was lodged by the manufacturers in February 1996.

In addition, *in vitro* production of a local Heliothis NPV has been achieved within the Chemical Engineering Department of the University of Queensland. Sufficient stocks for field assessment were not able to be provided but limited quantities are expected for the 1996/97 season.

Bt cotton. "Ingard" cotton is expected to provide relief against Heliothis attack, but will not be applicable in all cotton regions or situations. Since the Bt toxin is specific for caterpillars, it is most desirable that the existing natural control is conserved. Alternative, non-disruptive insecticides are also required for utilisation in Bt resistance management programmes and when "Ingard" technology will not be available, eg. for plantings in some localities (ie. Central Queensland) and after certain planting times (currently 15th November).

Assessments.
Because existing natural control is not disrupted by the Heliothis virus, the methodology used to evaluate such virus products must apportion to the virus the mortality component due to it. This is essential to the understanding of virus performance, in that the total control is the sum of that exerted by the virus plus that due to existing natural control. (This contrasts with assessments of broad-spectrum chemical insecticides.)

In addition, the potency of the virus formulation for the target species needs to be standardised by bioassay, adding further complications to assessments.

Field performance of Heliothis virus is normally estimated by the sampling of larvae from treated cotton and maintaining them in the laboratory for observation for virus infection. This is necessary but labour-intensive approach requires a suitable infrastructure including constant temperature incubation facilities and artificial diet preparation facilities.

In order to gauge the potential of the virus, assessments need to be done on cotton at a variety of locations under a range of conditions and pest densities. The present tests were done on dryland cotton near Dalby, Qld, and on irrigated cotton at Moree, NSW.

The Queensland assessments were done as part of the programme of Dr David Murray to

investigate non-chemical control strategies.

Dr Robert Mensah included the virus in his IPM investigations involving strip plantings of lucerne and Envirofeast® on commercial cotton at Moree.

OBJECTIVE

The objective was to field-test Heliothis NPV on cotton under a range of conditions.

MATERIALS AND METHODS.

Virus. GemStar® is a commercial virus formulation based on an NPV isolate from *Helicoverpa zea* and produced by Biosys Inc. in the United States. This was used at the manufacturer's recommended rate of 741 mL (1.5×10^{12} virus polyhedra)/ha. Its potency was standardised by laboratory bioassay versus *Helicoverpa armigera*. The virus is the same as in Elcar®, a product formerly registered on cotton in Australia.

Assessment sites. * Dryland cotton at Warra (2 sites) and Nandi (1 site), near Dalby, Q., (D.A.H. Murray, QDPI)
* Irrigated cotton at Moree, NSW. (1 site) (R. Mensah, NSW Agriculture).

A field assessment protocol for cotton was developed by R.E. Teakle and D. Murray (Appendix 1). Logistical difficulties prevented its being followed in a number of instances, and specific details of the trials are given in the reports of Drs Murray and Mensah.

The approaches to the use of the virus differed in Queensland and New South Wales:-

In Queensland (Warra and Nandi), Dr Murray used the virus inundatively as a stand-alone **selective bioinsecticide** complementing existing natural control. Molasses was included in the formulation, probably enhancing virus deposition on the crop through humectant activity. Treatments were unreplicated. Broad-spectrum insecticides were not employed.

In New South Wales (Moree) the virus was used by Dr Mensah as a **component of an IPM programme** involving lucerne strip cropping, in order to generate predator/parasite beneficials and to trap mirid bugs. Envirofeast® treatments were used alone initially and then in combination with the virus.
Three comparative treatments were used:

(1) Envirofeast + endosulfan,
(2) Envirofeast + Bt/endosulfan, and
(3) Envirofeast/GemStar virus mixtures.

All treatments at Moree were replicated 6 times and received a final clean-up with a Talstar/parathion mixture. The commercial nature and value of the crop unfortunately precluded the setting aside of untreated control plots.

Larval handling. Larvae were handled slightly differently at the 2 sites:-
At Dalby, the larvae were sampled directly into 28 mL cups for incubation and observation for infection or parasitism at the Toowoomba laboratory.
At Moree, the larvae were sampled into wells of 24-well tissue culture trays for despatch to Indooroopilly for observation for disease. This occasioned additional handling and maintaining the larvae under more congested conditions.

Standardisation of GemStar by bioassay.

GemStar Sampling. GemStar was stored under refrigeration (5°C) at Toowoomba (DPI) and Moree (on-farm coldroom). Samples of about 10 mL per container (9.5 L) were withdrawn using a 10 mL syringe after the containers had been agitated to mix the contents uniformly.

In addition, subsamples from Toowoomba were held under refrigeration at test sites at Warra and Nandi (Dalby), and these were tested for suitability of storage conditions by bioassay.

The pooled samples from each storage location were assessed by bioassay for potency in comparison with a GemStar standard held under refrigeration at Indooroopilly.

Test insects. These were *H. armigera* neonates (<24-h-old) from a laboratory colony maintained at Indooroopilly as per Teakle and Jensen (1985).

Bioassay. A diet-surface-contamination method was used in which *ca.* 48 test larvae per dose were exposed to 5 or 6 two-fold doses per virus formulation. They were conducted in 24-well tissue culture trays containing artificial diet (formaldehyde omitted). Doses were calculated in terms of polyhedra per mm^2 diet surface using the virus concentration supplied by the manufacturer. Thirty microlitres of dilutions of the virus suspensions were used to contaminate uniformly the diet surface in each well, as per Teakle *et al.*, (1985b). After the surface of the diet had dried, the test larvae were added and confined individually in the wells with polypropylene balls, and incubated at 25°C for 9 or more d. Mortality was recorded and probit analysis (Finney, 1972) was used to provide relative potencies.

RESULTS AND DISCUSSION.

Potency assessments on GemStar.

The dose-mortality data on consignments of GemStar sent to Toowoomba and Moree and held on farms at Warra and Nandi indicated that they were not significantly different in potency from the GemStar standard ($P > 0.05$) (Tables 1 and 2).

Virus infection levels on cotton. Comparative levels of virus infection achieved at Moree and Narrabri were consistently between 50% and 62%. At Dalby, however, the levels of infection varied between <20% and 90% (mean 55%). The reasons for such differences

Table 1 Potency ratings of GemStar at different locations

Location	LC_{50} (polyhedra/mm^2 diet surface) (95% CL)	Dose/mortality response (Y= bx + c)	Relative potency (95% CL)
Indooroopilly (Standard)	0.18 (0.13 - 0.27)	Y= 1.5x + 6.0	1.0
Moree	0.13 (0.03 - 1.74)	Y= 1.8x + 6.6	1.3 (0.7 - 2.5)
Toowoomba	0.14 (0.11 - 0.18)	Y= 1.9x + 6.6	1.2 (0.6 - 2.4)
Indooroopilly (Standard)	0.28 (0.21 - 0.40)	Y= 1.3x + 5.7	1.0
Nandi (Dalby) (W Arthur property)	0.30 (0.17 - 0.70)	Y= 1.6x + 5.8	0.9 (0.6 - 1.3)
Warra (Dalby) (G Bidstrup property)	0.24 (0.19 - 0.33)	Y= 1.5x + 5.9	1.1 (0.8 - 1.4)

Table 3 Percent virus infection in small and medium size larvae on cotton sprayed with GemStar® at Dalby, Qld.

Location	Virus infection incidence (%)	
	Larval size	
	VS & S	M
Nandi	46	40
Warra		
1)	58	47
2)	30	41

Table 2 Mortality data for GemStar bioassays

GEMSTAR storage location	Dose (PIB/mm^2)	Cumulative no. larvae dead at days (25°C) post-exposure							Total no. larvae
		4	5	6	7	8	9	10	
Control	0	-	-	-	-	1	1	2	46
Indooroopilly (standard)	0.025	-	1	1	2	4	5	6	46
	0.05	-	2	6	8	10	11	13	41
	0.1	-	-	4	8	11	15	18	48
	0.2	-	-	6	8	15	21	23	46
	0.4	-	6	17	19	27	34	34	47
Moree	0.025	-	2	4	4	7	11	11	46
	0.05	-	-	3	3	6	6	6	46
	0.1	-	2	5	8	13	13	20	45
	0.2	-	2	12	18	22	23	25	43
	0.4	-	6	23	26	31	36	36	41
Toowoomba	0.025	-	-	1	1	2	4	5	45
	0.05	-	1	3	3	8	9	13	44
	0.1	1	1	2	5	12	15	16	43
	0.2	-	2	9	13	17	24	28	45
	0.4	-	4	14	16	18	23	28	45
(Control)	0	-	-	-	-	-	-	-	40
Indooroopilly (Standard)	0.025	-	-	3	3	3	3	3	46
	0.05	-	1	3	4	7	7	7	43
	0.1	1	1	3	3	6	9	11	46
	0.2	-	-	3	5	16	21	23	44
	0.4	-	2	9	12	22	24	26	46
	0.8	1	4	20	22	29	31	32	46
W Arthur (on-farm) Dalby	0.025	-	-	-	-	3	4	4	48
	0.05	-	-	-	-	-	1	2	44
	0.1	-	1	3	3	8	10	11	46
	0.2	-	1	8	8	14	19	20	46
	0.4	-	4	12	13	19	20	21	47
	0.8	-	7	18	24	36	38	38	45
G Bidstrup (on-farm) Dalby	0.025	-	-	2	2	3	3	5	47
	0.05	-	1	3	3	4	7	7	44
	0.1	-	1	3	5	6	7	8	46
	0.2	1	1	6	9	16	20	21	44
	0.4	-	2	13	17	26	29	31	46
	0.8	2	4	17	22	31	33	33	45

Table 4 Infectivity of GemStar for *Helicoverpa* spp. on cotton, R Mensah trials

Trial location	Date sprayed	Date assessed	Treatment	Number of larvae sampled (proportions of sizes represented)	Percent larval (infection with virus)	Other mortality factors (%)
"Norwood" Moree	16.12.95	19.12.95	Control (untreated)	28 (0.36 0.6 0.04) VS & S M L	0	-
			GemStar virus	56 (0.2 0.54 0.27) VS & S M L	50 (0.36 0.6 0.4) VS & S M L	
"Norwood" Moree	12.1.96	15.1.96	Control (untreated)	20 (0.35 0.45 0.2.) VS & S M L	10 (0.0 0.22 0.0) VS & S M L	-
			GemStar virus	10 (0.8 0.2 0.0) VS & S M L	60 (0.62 0.5 0.0.) VS & S M L	
Auscott, Narrabri	24.1.96	29.1.96	Control (untreated)	29 (0.38 0.48 0.14) VS & S M L	0	Ascovirus 21 *Microplitis* 3
			GemStar[a] virus	58 (0.52 0.48 0.0) VS & S M L	62 (0.62 0.60 0.0) VS & S M L	
"Norwood" Moree	30.1.96	2.2.96	Control (untreated)	35	0	-
			GemStar virus	37	53[b]	Ascovirus 8 *Microplitis* 5

[a] Applied aerially

[b] In calculating GemStar virus disease incidence, larvae infected with Ascovirus or parasitised with *Microplitis* sp. were excluded from the total

are not clear. It was suggested (R. Mensah, pers. comm., 1996) that the inclusion of Envirofeast® in the formulation could be responsible for the greater consistency of the resulting infection levels. This would suggest that the dampening effect of Envirofeast® caused it to both inhibit high infection levels and enhance low levels of infection. Other explanations are more likely.

The greater variability recorded at Dalby compared with that at Moree could possibly have been caused by other factors, such as larva sampling and handling, larval uniformity and size, and contamination efficiency (eg. spray equipment and plant surface moisture), and timing of application in relation to larval hatching or sampling. In addition, only VS/S (<7 mm) and M (7-15 mm) larvae were sampled at Dalby, whereas L (>15 mm) larvae were sampled, as well, at Moree. The reason for excluding L larvae at Dalby was to avoid bias due to their being more easily located than the other sizes. The between-plot variation at Moree was not recorded. These may have indicated variability in infection levels similar to those at Dalby.

Larger larvae need greater virus doses to infect owing to developmental resistance (Teakle et al., 1985a), and some of the L larvae could have been virtually immune to the virus. However, levels of infection were not always inversely proportional to larval size at Dalby (Table 3). This suggests that greater intake of virus by larger larvae may largely compensate for developmental resistance.

Loss of activity on cotton.

A comparison of larval mortality responses in laboratory bioassays and on cotton in the field suggests that there is considerable loss of activity in the field. Laboratory data (Table 1) indicated that 50% mortality could be achieved with doses as small as 0.13 to 0.3 polyhedron per mm^2 diet surface, whereas the recommended rate for GemStar® on cotton is equivalent to 150 polyhedra per mm^2 ground surface, and this resulted in a mean of 55% larval mortality.

Reasons for the apparent mortality disparity are:-
* Non-uniform deposition of virus on cotton plants in the field, with lower plant parts and under-surfaces receiving relatively low levels of contamination, and some of the virus ending up on the ground.

* Concealed feeding of larvae on cotton vs. exposed feeding on contaminated surfaces in the bioassay. Larvae were largely associated with reproductive structures on cotton and those already established in them would have acquired virus only during entry and exit. Any delay in acquisition would have resulted in virus inactivation on the surface (mainly due to sunlight) (Teakle *et al.*, 1996) and an increase in developmental resistance in larvae, as well as growth dilution of virus on the plant surface.

* Non-uniform sizes of larvae in the field with some young (eg. VS or S) larvae fortuitously avoiding virus prior to sampling/virus inactivation, and some older larvae receiving sub-lethal doses.

Trial design, Moree. Commercial considerations dictated a small size and unreplicated nature of the untreated control plot at Moree. This is unfortunate in that all treatments

received Envirofeast®. Consequently, the methodology used did not indicate whether Envirofeast®-enhanced predation of diseased/disabled larvae occurred at Moree and other sites in NSW. If so, such selective removal of infected larvae could have resulted in the more uniform residual disease levels recorded, and the true infection levels would then have been higher than the 50-62% levels noted.

Parasitism. Negligible parasitism was recorded at Moree except late-season, when Ascovirus-infected larvae were also noted (Table 4). At Dalby, parasitism and Ascovirus infection were present through December, January and February. These may mutually inhibit virus infection (Murray *et al.*, 1995; Teakle *et al.*, 1985b), but, since they are all ultimately lethal, the cause of death may not be regarded as important to the grower.

IPM roles. The relative contributions of Envirofeast® and GemStar® to Heliothis control in the IPM system developed by Dr Mensah are not known. Direct Heliothis mortality between 50 and 62% could be attributed to the GemStar treatments, but the impact of the Envirofeast was not quantified. The mechanisms (oviposition repellancy/predator enhancement) were also not assessed, but quantitative data on oviposition repellancy for *H. armigera* and *H. punctigera* have recently been published (Mensah, R.K. Aust. J. Ent. 35: 323-329, 1996). As a predator enhancer, Envirofeast operates as a general insect suppressant with a positive contribution to Heliothis control. The possibility of synergism (or antagonism) between Envirofeast and GemStar should not be discounted.

Action thresholds. At both Dalby and Moree sites, thresholds of 2 larvae per row metre (normally observed in EntomoLOGIC) were regularly exceeded without undue damage resulting. This indicates that IPM thresholds need to be moderated in the light of existing natural control. Initially, simple predator:prey ratios could be used and then refined in the light of further experience. The use of these ratios to determine thresholds would require an education programme for pest managers.

CONCLUSIONS.

The Heliothis virus can serve as an important component of insect pest management on both raingrown and irrigated cotton without reliance on chemical insecticides in situations where small and medium-sized Heliothis larvae are present at near-threshold pest densities.

RECOMMENDATIONS AND APPLICATION TO INDUSTRY.

Sustainability in cotton production will require a multi-faceted aproach, not relying exclusively on broad-spectrum pesticides or transgenic plants. Biopesticides such as Heliothis NPV products can provide the basis for ecologically sound insect control within an IPM framework. This form of technology requires a flexible approach based on scientific understanding in order to succeed and achieve consistent results. Experience with Elcar® almost 20 years ago indicates that short cuts are likely to lead to failure.

Heliothis NPV will probably find greatest application on raingrown cotton initially. This can provide a base for new programmes employing biopesticides such as recombinant NPV to evolve.

FURTHER STUDIES.

Envirofeast® efficacy:

* This needs to be quantified in relation to predator composition and numbers on cotton. A correlation between predator presence and control of Heliothis and other pests needs to be determined.

* The mechanism for oviposition repellancy needs to be established.

Virus efficacy:

* The role of the virus on "Ingard" cotton has not been investigated. In view of the potential for anti-feedant action of Bt toxin, comparative studies on conventional and "Ingard" cotton are required.

* The relative performance of GemStar alone versus Envirofeast/GemStar on cotton and any bias in insect sampling for virus disease incidence need to be assessed.

* The relationship of virus efficacy to timing of application and larval hatch needs to be better understood.

* The capacity for dissemination of the virus and secondary spread of virus disease via predators and parasitoids needs to be investigated.

* Appropriate Heliothis thresholds, which take account of co-existing biocontrol, need to be determined.

* *In vitro* virus assessments: limited efficacy testing is planned for 1996/97 to be followed by extensive testing in 1997/98.

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APPENDIX 1.

ASSESSMENT OF "GEMSTAR" ON COTTON

R.E. Teakle, CRC for Tropical Pest Management
D.A.H. Murray, QDPI, Toowoomba.

Requirements (sprays and mortality assessments):

* GemStar at 741 mL ha^{-1}.
* Molasses, 1% at 80 L ha^{-1} (predissolved to 50% the day before spraying, if necessary).
* Wetting agent (non-ionic detergent), 0.01% for high volume treatment, eg. 80 L ha^{-1}.
* 28 mL cups with artificial diet, >40 per treatment at days 0, 1, 4 and 7. Also high-sided trays to hold cups with larvae during transportation.
* incubator or room at 25°C, phase contrast microscope, slides, coverslips.

Rate: 741 ml (approx. 1.5 x 10^{12} virus polyhedra) in high volome, eg. ca. 80 L ha^{1}.

Diluent (no Envirofeast): 1% molasses plus 0.01% non-ionic detergent (eg. Triton 100).

Note:
* It may be necessary to predissolve molasses in water to 50% before use, eg. the day before. Otherwise there could be difficulty in getting the molasses into solution.
* The "control" plot receives diluent only. (A further untreated control may be used to exclude any effect of the diluent, eg. attraction of predators or pests by molasses.)
* Molasses is not needed if Envirofeast is included.

Timing of application: To coincide with egg hatching and in the late afternoon.

Note:
* The virus is readily inactivated by sunlight. Application late in the day allows at least 18 h of high-potency virus to remain on the crop. In addition, it is possible that high daytime temperatures may retard feeding on the contaminated crop, resulting in a low infection rate of larvae.

* The control plot should be sited to avoid drift, and sampled first to avoid cross-contamination (see below).

* If weather conditions prevent ground-rig spraying, aerial application may be needed to avoid treating large larvae.

Efficacy assessment: **Virus infection levels are recorded in at least 40 larvae sampled from untreated (control) and treated plots. Samples are collected on day 0 (pre-spray), aud ou Days 1, 4, and 7.**

Note:

* The sampling at Days 1 and 4 is desirable in that it gives an indication of the rate of virus acquision by the larvae, but sampling on Days 0 and 1 can be omitted if logistic difficulties occur. A rate response can be derived from the infection levels at Days 1 or 4, but not in samples after this period. This is because secondary spread of the disease can occur following early deaths, particularly if virus spread is aided by moisture on the plants.

* Samples are made by hand rather than by suction to avoid cross-contamination.

* Record egg (White, Brown) and larval development (**VS** <3 mm; **S** 3-7 mm; **M** 7-15 mm or **L** > 15 mm) and densities.

* At least 40 larvae per treatment should be taken and incubated at 25°C (mean summer temperature).

Small larvae can be initially sampled into 24-well tissue culture trays with artificial diet and polypropylene balls. However, to avoid double-handling all sizes should be sampled directly into 28 mL cups containing diet. These should be provided with 8 pin holes in the lid to allow ventillation: for VS larvae mark the lid to indicate that the provision of holes should be delayed for about 3 days, to avoid larvae escaping through them.

* Mortality is recorded daily to get an idea of the time/mortality response.

* Associated diseases (eg. Ascovirus) and parasitoids are also recorded.

* The causes of death are confirmed microscopically, if necessary.

Associated observations:

(a) Squaring rates are recorded on 4 x 1 metre row lengths per treatment. Fruit retention is estimated from 20 (4 groups of 5) non-tipped plants. Final positions of harvestable bolls and yield are also estimated.

(b) Beneficial insect population densities are recorded.

(c) Rainfall registrations during application and sampling are recorded.

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COMMUNICATION OF RESULTS.

Articles.

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TEAKLE, R.E., MURRAY, D.A.H., MENSAH, R. and MONSOUR, C.J. (1996). Heliothis virus: Can it rise from the ashes of "Elcar"? Proc. Eighth Australian Cotton Conference, Broadbeach, Queensland, August 1996, pp. 347-354.

Seminars.

TEAKLE, R.E. "Evaluating baculoviruses", CRCTPM., U. of Qld, 27 June, 1996.

TEAKLE, R.E. "Heliothis NPV as a biopesticide for cotton". Biopesticide Seminar, CRCTPM., U. of Queensland, 24 September 1996.

Industry Meeting (Rhone-Poulenc).

Discussing GemStar® and Envirofeast®, Moree, NSW, 19 September 1996.

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TEAKLE, R.E., MURRAY, D.A.H., MENSAH, R. and MONSOUR, C.J. (1996). Heliothis virus: Can it rise from the ashes of "Elcar"? Proc. Eighth Australian Cotton Conference, Broadbeach, Queensland, August 1996, pp. 347-354.

CRC FOR TROPICAL PEST MANAGEMENT

ACCOUNT : **24: Bt Formulations CRDC (Bob Teakle)**

1996/97 FINANCIAL STATEMENT

AS AT : November 1996

	1994/95	1995/96	1996/97	TOTAL
INCOME:				
OTHER	31,810	13,840	0	**45,650**
TOTAL INCOME	31,810	13,840	0	**45,650**
OPERATING:				
PERSONNEL	22,317	299	0	**22,616**
CONS/MTCE	1,682	19,000	2,198	**22,880**
TRAVEL	154	0	0	**154**
TOTAL OPERATING	24,153	19,299	2,198	**45,650**
CAPITAL:				
COMPTR EQUIP	0	0	0	**0**
OTHER EQUIP	0	0	0	**0**
TOTAL CAPITAL	0	0	0	**0**
TOTAL EXPENSES	24,153	19,299	2,198	**45,650**
SURPLUS/(DEFICIT)	7,657	(5,459)	(2,198)	**0**

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