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Monitoring of insecticide resistance in field sampled buffalo flies

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SUMMARY

During March to May 2000, a survey of insecticide resistance was conducted on populations of buffalo flies *Haematobia irritans exigua* sampled from 26 cattle properties throughout major buffalo fly infested areas of northern New South Wales and Queensland, Australia. Properties were sampled from the following regions:

Region	Town/Station	Total
Northern New South Wales	Lennox Head Kyogle (2) Ewingsdale Brushgrove Woodenbong Urbanville	7^{T}
South-eastern Queensland	Gympie Broweena* Wonga* Maryborough (2)*	5
Central Queensland	Bajool (3)* Rockhampton* Stanwell* Belmont	6
Northern Queensland	Kuttabul Mirani* Bloomsbury* Proserpine* Bowen* Charters Towers*	6
Far Northern Queensland	Chillagoe* Mena Creek	2

*Same farm sampled in 1995 survey; T = only 3 farms were assayed at correct temperature.

Buffalo flies were collected from yarded cattle with a sweep net and transferred into disposable plastic Petrie dishes containing filter papers impregnated with discriminating concentrations of various insecticides. Mortality was counted after a 2-hour interval and the percentage survival calculated and used as an indicative or equivalent measure of percentage resistance. The discriminating concentrations used in the 2000 survey were the same as those used in surveys conducted for the Meat Research Corporation in 1994 and 1995 by Agrisearch Services Pty Ltd. The following insecticides and discriminating concentrations were assayed in the 2000 survey:

Insecticide	2000 Discriminating Concentrations *ug* ai per cm^2 filter paper
Organophosphate Insecticides (OP)	
1. Diazinon	2.4 (DC95), 3.4 (DC94)
2. Chlorfenvinphos	6.8 (DC95), 10.8 (DC94)
Synthetic Pyrethroid Insecticides (SP)	
1. Cypermethrin	3.4 (DC95)
2. Flumethrin	3.3 (DC95)
3. Deltamethrin	2.0 (DC95)
4. Cyhalothrin	6.4 (DC95)

DC94 = discriminating concentration used in 1994

DC95 = discriminating concentration used in 1995

Two DCs were used for diazinon and for chlorfenvinphos, because of the greater interest in any change in resistance to these organophosphate insecticides.

Information was also gathered about current buffalo fly management practices being used on the cattle properties surveyed.

2000 Survey Results Summary

A total of 26 farms were sampled for buffalo fly bioassays throughout the whole of New South Wales and Queensland in the 2000 survey. Also, due to reasons of practicality all the assays conducted during the 2000 survey were carried out over a limited time (4-5 days for each region). Given the limited sample size and time frame of this 2000 survey, some caution should be exercised when interpreting the results.

The 2000 survey indicated the possible appearance of diazinon resistance in buffalo flies in the northern New South Wales' region for the first time as measured using the filter paper bioassay technique. On the northern New South Wales' properties sampled in 2000 (3), the average percentages of buffalo fly survival (ie 26.4% and 67.7%) at the respective 1994 and 1995 diazinon DCs were considerably higher than the corresponding levels of survival in assays in 1994 (0.5%) and 1995 (9.5%). These degrees of increased diazinon tolerance were not seen in any other sampled regions in 2000, particularly in south eastern Queensland - where a similar result may have been expected. Nor had similar levels of diazinon tolerance ever been seen in the previous surveys (ie 1994 and 1995).

A concerningly high level of resistance to the other organophosphate insecticide: chlorfenvinphos was also found in assays in the northern New South Wales region. In 2000, the average percentages of buffalo fly survival to respective chlorfenvinphos DCs (ie 43.7% and 80.9%), were significantly higher than the corresponding levels of survival in the 1994 (2.5%) and 1995 (21.5%) assays. In other regions: there was a slight rise in the level of chlorfenvinphos tolerance in buffalo flies sampled in the north Queensland region, however there were unchanged and/or low to moderate levels of tolerance to chlorfenvinphos assayed in the south eastern and far north Queensland regions.

Continued high levels of resistance to the synthetic pyrethroid insecticides were generally indicated in buffalo flies in all regions of New South Wales and Queensland. Slightly reduced levels of tolerance to deltamethrin and cyhalothrin may indicate that resistance genes in the population have become diluted.

The questionnaire of current management practices towards buffalo fly carried out in 2000 was taken from a relatively small number of properties and is therefore limited as far as drawing too many conclusions about the wider grazing community. The 2000 survey did indicate an interesting link between fly control management practices and the development of insecticide resistance. That is, that in the regions where organophosphate resistance percentages was suggested, the recent use of these same insecticides had been commonplace. As well, application efficiencies of these insecticides - particularly chlorfenvinphos through back spray and back rubber – were generally not good. Lack of thorough spraying of animals with diluted insecticides was identified as a problem in the 1994 and 1995 survey. The relative cheapness of the organophosphate insecticides (containing chlorfenvinphos and diazinon) that are intended for use through back spraying may lead to less care being exercised with their use, eventually contributing to under-dosing and the selection for resistance. By way of contrast, in the south eastern region of Queensland where chemical abstinence and keen interest in dung beetles welfare were reported, levels of organophosphate resistance indicated increased only slightly since 1995. It also appeared encouraging that the general reduced usage of deltamethrin and cyhalothrin insecticides may have contributed towards an overall decrease in the levels of resistance to these chemicals.

The main message to come from the 2000 survey is that continued care needs to be taken with the use of insecticide products to help prevent (or delay) the onset of insecticide resistance. To help achieve this aim the author feels that there needs to be a co-ordinated industry-wide approach, with regular monitoring of buffalo fly resistance via current and/or newer technologies, to evaluate agreed resistance management strategies.

INTRODUCTION

The buffalo fly *Haematobia irritans exigua* is a dung breeding, blood feeding ectoparasite of cattle, and it remains a very serious economic pest of cattle production in subtropical and tropical regions of Australia.

In 1994 and 1995, the Meat Research Corporation sponsored Agrisearch Services Pty Ltd to investigate insecticide resistance in buffalo fly populations on over 100 cattle properties throughout major cattle producing regions of New South Wales and Queensland. A field bioassay method using treated filter papers containing discriminating concentrations of different insecticides was used. The assay was calibrated on a known susceptible fly population. The percentage of assayed flies that survive one insecticide's discriminating concentration is used as an indicative measure of the percentage of that sampled population that are resistant to that one insecticide.

High levels of resistance to the synthetic pyrethroid group of insecticides were detected in buffalo fly populations in all eastern regions in 1995: 79.1% resistance to cypermethrin, 84.8% resistance to flumethrin, 81.3% resistance to deltamethrin and 90.2% resistance to cyhalothrin. Also in 1995, levels of resistance to the organophosphate group of insecticides were lower and incidences were more sporadic. The main areas in which organophosphate resistance was recorded in 1995 were northern New South Wales (9.5% resistance to diazinon and 21.5% resistance to chlorfenvinphos), south eastern Queensland (49.7% resistance to chlorfenvinphos) and far north Queensland (14.4% resistance to chlorfenvinphos).

Since the last survey (ie 1995), some changes in buffalo fly management practices have occurred, such as an increased use of certain organophosphate insecticides - in particular the diazinon impregnated ear tags - and a decrease in use of certain pyrethroid insecticide sprays for buffalo fly control. The main aims of this project were to monitor any changes that may have occurred since 1994 and 1995 in a) the resistance status of buffalo fly populations, and b) the management tactics used by cattle producers.

This report contains the experimental methods used and presents the results obtained.

The study was conducted under project MLA/00/01 (AHW.003).

EXPERIMENTAL DETAILS

1.1 Bioassay Method

A discriminating concentration (DC) bioassay technique was used. The technique and discriminating concentrations that were used in 1994 and 1995 (Farnsworth, Collett and Ridley 1997) were used again in the 2000 survey. DCs were determined from standardising dose-mortality assays on buffalo flies bred at CSIRO, DTAP, Longpocket Laboratory and maintained insecticide free for the previous 8 years. On each property, buffalo flies were captured using a sweep net from the backs and sides of yarded cattle, anaesthetised with CO_2 and immediately exposed to insecticide impregnated filter papers inside disposable plastic Petrie dishes. On each property, 2-4 replicate Petrie dishes were used and a total of approximately 100 flies were exposed to each DC. Petrie dishes were maintained at around 28- 31 degrees C and mortality was counted at the end of 2 hours and the degree of resistance determined (the percentage survival in each bioassay is equivalent to the percentage resistance of the sampled population).

Before calculating percentage survival Abbott's formula was used to correct for any control mortality:

$$Pmab = [(Po - Pc)/(100 - Pc)] \times 100$$

Where: Pmab = corrected mortality (%)

Po = observed mortality (%)

Pc = control mortality (%)

The following insecticides and discriminating concentrations were used:

Insecticide	2000 Discriminating Concentrations – ug ai per cm^2 filter paper (code)
Organophosphate Insecticides (OP)	
1. Diazinon	2.4 (DC95), 3.4 (DC94)
2. Chlorfenvinphos	6.8 (DC95), 10.8 (DC94)
Synthetic Pyrethroid Insecticides (SP)	
1. Cypermethrin	3.4 (DC95)
2. Flumethrin	3.3 (DC95)
3. Deltamethrin	2.0 (DC95)
4. Cyhalothrin	6.4 (DC95)

DC94 = discriminating concentration used in 1994

DC95 = discriminating concentration used in 1995

Two DCs were used for diazinon and for chlorfenvinphos, to increase the sensitivity of detection of any change in resistance to these organophosphate insecticides.

Results are expressed as the percent mortality of assayed flies at respective DCs (**Tables 1-5**). The percentages of flies that survive in the DC bioassays (ie 100% - percent mortality) are also expressed as the percentage resistance (**Figures 1 – 12**).

1.2 Farms and Regions Surveyed

Buffalo flies were sampled and assayed from a total of 26 cattle properties. Properties that were surveyed in 1994 and 1995 were targeted in 2000, however factors such as weather and timing of recent insecticide treatments limited this objective. Properties were avoided if they had recently used buffalo fly insecticides (i.e. within 2 weeks).

Properties were sampled from the following regions and towns:

Region	Town/Station	Total
Northern New South Wales	Lennox Head Kyogle (2) Ewingsdale Brushgrove Woodenbong Urbanville	7[T]
South-eastern Queensland	Gympie Broweena* Wonga* Maryborough (2)*	5
Central Queensland	Bajool (3)* Rockhampton* Stanwell* Belmont	6
Northern Queensland	Kuttabul Mirani* Bloomsbury* Proserpine* Bowen* Charters Towers*	6
Far northern Queensland	Chillagoe* Mena Creek	2

* same farm sampled in 1995 survey; T = only 3 farms were assayed at the correct temperature.

1.3 Questionnaire

Each cattle owner/manager was interviewed about details of his or her management of buffalo flies, and their interest in dung beetles. Copy of the questionnaire is shown in the appendices.

1.4 Photographs

Several photographs were taken to demonstrate typical infestations of buffalo flies on cattle and the fly collection and bioassay methodology. These photos are included in the Appendices.

RESULTS

The results are summarised in **Tables 1-5** (percentage bioassay mortality) and presented graphically in **Figures 1-12** (percentage bioassay mortality and percentage resistance) and are presented in full in the appendices.

1.5 Organophosphate Insecticide Resistance

1.5.1 Northern New South Wales (Table 1 and Figure 1)

Results for consideration of resistance are based on DC assays of flies collected from 3 northern New South Wales' farms. Assays of flies that were sampled from the first 4 farms, were maintained at ambient temperatures (experimentor error), instead of regulated temperatures of 28-31 degrees C. The toxicities of the organophosphate insecticides are known to be sensitive to changes in temperature (Personal communication, Bill Doherty QDPI, Oonoonba Veterinary Laboratory). The limited number of farms sampled in northern New South Wales (as well as other regions) for the resistance assays in the 2000 survey needs to be considered when inferring general trends.

In northern New South Wales, the percentages of buffalo flies surviving in DC assays with both organophosphate insecticides (diazinon and chlorfenvinphos) in 2000 were much greater than in similar assays in 1994 and 1995. In diazinon assays in 1994 the DC94 killed an average of 99.5% of flies, and in diazinon assays in 1995 the DC95 killed an average of 90.5% of flies. In 2000 diazinon assays however, the DC94 killed an average of only 73.6% and the DC95 killed only an average of 32.3% of flies, suggesting large increases in resistance levels since the surveys of 1994 and 1995.

In 1994 assays with chlorfenvinphos the DC94 killed an average of 97.5% of flies, and in 1995 assays with chlorfenvinphos the DC95 killed an average of 78.5.5% of flies. In 2000 assays with chlorfenvinphos however, the DC94 killed an average of only 56.3% and the DC95 killed an average of only 19.1% of flies, also implicating large increases in resistance levels since the surveys of 1994 and 1995.

1.5.2 South Eastern Queensland (Table 2 and Figure 3)

In south eastern Queensland there appeared to be only a minor and probably insignificant change in tolerance or resistance to diazinon. In assays in the 2000 survey, an average of 8.6% of buffalo flies survived the DC95 whereas an average of 2.7% survived the same DC in 1995. There was even less change in tolerance of flies assayed in 1994 and 2000 using the same DC94.

In 1994, tolerance to chlorfenvinphos was low with an average of 8.4% survival in assays, however in 2000, tolerance had increased with an average of 21.4% survival using the same DC94. In 1995, tolerance to chlorfenvinphos was quite high with an average of 49.7% survival in assays, but in 2000, tolerance had decreased with an average of 39.7% survival in assays. These contrasting results suggest that chlorfenvinphos resistance levels were essentially unchanged.

1.5.3 Central Queensland (Table 3 and Figure 5)

In Central Queensland, the assayed percentages of buffalo fly resistant to diazinon and chlorfenvinphos (ie surviving respective DCs) in 2000 were the same or lower than levels detected in 1994 and 1995.

1.5.4 North Queensland (Table 4 and Figure 7)

In north Queensland, the assayed percentages of buffalo flies resistant to diazinon (ie surviving the DCs) in 2000 were the same or only slightly higher than the percentages detected in assays in 1994 and 1995 (still <10%).

There was however, an apparent change in buffalo fly sensitivity to chlorfenvinphos. In 1994, chlorfenvinphos resistance was low with 3.3% survival at the DC94, and in 2000, resistance had increased only slightly with 7.6% survival at the same DC94. Based on the DC95 however, the chlorfenvinphos resistance level increased sharply with 7.9% survival of flies in the 1995 assay, and 42.4% survival of flies in the 2000 assay.

1.5.5 Far North Queensland (Table 5 and Figure 9)

In far north Queensland, levels of buffalo fly sensitivity to diazinon and chlorfenvinphos in 2000 were essentially unchanged from the levels detected in 1994 and 1995 assays. A low level of chlorfenvinphos tolerance (14.4% average survival in assay) in 1995 was not basically different from the resistance level (15.0% average survival in assay) detected in 2000 using the DC95.

1.6 Synthetic Pyrethroid (SP) Insecticide Resistance

1.6.1 Northern New South Wales (Table 1 and Figure 2)

In northern New South Wales, the mortalities of buffalo flies in assays with the SPs in 2000 were generally similar to those in similar assays in 1995, indicating the continuation of strong degrees of resistance to all four SP insecticides assayed. The levels of sensitivity to cypermethrin and flumethrin were lower in 2000 than in 1995, indicating increases in resistance levels. The levels of resistance to deltamethrin and cyhalothrin however, were lower in 2000 than in 1995, indicating encouraging dilutions of resistance to these SP insecticides over the past 5 years.

1.6.2 South Eastern Queensland (Table 2 and Figure 4)

In south eastern Queensland, results of 2000 assays indicated a similar situation with regard to SP resistance that was seen in northern New South Wales. That is, that assays indicated the continuation of strong degrees of resistance to all four SP insecticides. The levels of resistance to cypermethrin and flumethrin were greater in 2000 than in 1995. The levels of resistance to deltamethrin and cyhalothrin however, were lower in 2000 than in 1995, indicating encouraging dilutions of resistance in the buffalo fly population over the past 5 years.

1.6.3 Central Queensland (Table 3 and Figure 6)

In central Queensland, results of the 2000 assays indicated a continuation of strong degrees of resistance to all four SP insecticides. Level of resistance to flumethrin was higher in 2000 than in 1995. Levels of resistance to cypermethrin, deltamethrin and cyhalothrin however, were all lower in 2000 than in 1995, indicating the dilution of resistance in the buffalo fly population over the past 5 years.

1.6.4 North Queensland (Table 4 and Figure 8)

In north Queensland, the results of the 2000 assays indicated a similar situation with regard to SP resistance that was seen in northern New South Wales and south eastern Queensland. That is, that strong degrees of resistance to all four SP insecticides are still present in the buffalo fly population. The levels of resistance to cypermethrin and flumethrin were higher in 2000 than in 1995. The levels of resistance to deltamethrin and cyhalothrin however, were lower in 2000 than in 1995, indicating dilution of resistance levels in the buffalo fly population over the past 5 years.

1.6.5 Far North Queensland (Table 5 and Figure 10)

In far north Queensland, 2000 assay results indicated that SP insecticide resistance was still present in buffalo flies at significant levels. There were signs of worsening resistance to cypermethrin, however there was also an encouraging dilution of the level of resistance to deltamethrin between 1995 and 2000.

1.7 Questionnaire

1.7.1 Northern New South Wales (7 Properties)

In northern New South Wales, the average number of cattle per property was 205, and these were mainly European breeds. The most common insecticide used on sampled properties, either by slow release ear tag (Spike), backrubber or over-spray was diazinon (Di-Jet or Nucidol). Other insecticides used were chlorfenvinphos (Supona), flumethrin (Bayticol). Amitraz and ivermectin (and flumethrin) were also used for tick control. One property had not used any insecticides for buffalo fly control in the previous 12 months. There was no monitoring or controlled release of dung beetles.

1.7.2 South Eastern Queensland (5 Properties)

In south eastern Queensland, the average number of cattle per property was 542, and these were mainly Brahman cross breeds. Two (out of 5) properties had used no insecticide in the past 12 months and one of these was practising selective culling of buffalo fly sensitive animals. The most commonly used insecticide by either backrubber or over-spray was chlorfenvinphos (Supona), application of which was less than ideal with respect to adequate coverage of animals. No slow release diazinon ear tags (Spike) were used on any of the sampled properties. Amitraz was used on most of the sampled properties for tick control. There was a general interest in dung beetle with 2 (out the 5) properties actively monitoring their dung beetles species.

1.7.3 Central Queensland (6 Properties)

In central Queensland, the average number of cattle per property was 1167, and these were mainly Brahman cross breeds. All but one of the 6 sampled properties were using no insecticides for buffalo fly control. One property (with milking cows) was using chlorfenvinphos (Supona) or diazinon (Di-Jet) via over-spray. No slow release diazinon ear tags were used on any of the sampled properties. Two properties were treating for cattle ticks with amitraz or ivermectin. There was only a very casual interest in dung beetles.

1.7.4 North Queensland (6 Properties)

In north Queensland, the average number of cattle per property was 1883, all Brahman cross breeds. Four of the six sampled properties were using chlorfenvinphos (one via Barricade S, the remainder using Supona), all as over-sprays onto animals loosely packed into holding yards (i.e.. giving less than ideal spray coverage). No slow release diazinon ear tags were used on any of the sampled properties, although one property had used zeta-cypermethrin+PBO (Python) tags. Amitraz and fllumethrin (Bayticol) were used on two of the sampled properties for tick control. There was little interest in dung beetle.

1.7.5 Far North Queensland (2 Properties)

The two far north Queensland properties were distinctly different. One (Mena Creek) was a high rainfall, intensive fattening property and the other (Chillagoe) was a low rainfall, extensive breeding operation. There was minimal insecticide usage of both properties with the former employing slow release diazinon ear tags (Spike) and removing them after 16 weeks while on the latter property each animal was treated on average once per year using chlorfenvinphos (Supona) over-spray. Dung beetles were of minor interest to either property manager.

Table 1: Percent Buffalo Fly Mortalities at Discriminating Concentrations - Northern New South Wales, March 2000.

Insecticide (code/year)	DC *ug/cm2*	Lennox *Howe*	Ewingsdale *Pearson*	Brushgrove *Clark*	Woodenbong *Smith**	Urbenville *Clements**	Kyogle *Harrison**	Kyogle *Carter**
Diazinon(DC94)	3.4	57.1	93.8	69.9	64.9	20.7	28.3	39.7
Diazinon (DC95)	2.4	5.8	46.6	44.5	20.7	6.8	7.7	5.6
Chlorfenvinfos (DC94)	10.8	7.6	98.6	62.8	98.2	29.0	88.9	47.7
Chlorfenvinfos (DC95)	6.8	0.0	22.5	34.8	3.8	0.9	20.1	13.3
Cypermethrin (DC95)	3.4	0.0	2.6	30.1	3.2	4.0	5.3	0.0
Flumethrin (DC95)	3.3	0.0	1.4	22.5	0.0	0.1	0.0	0.0
Deltamethrin (DC95)	2.0	6.5	12.6	24.8	8.7	12.8	25.4	15.2
Cyhalothrin (DC95)	6.4	2.6	4.6	34.7	4.9	0.2	2.8	0.0
Acetone Control		11.9	1.5	15.0	2.5	0.6	2.8	3.6

Insecticide (code/year)	DC *ug/cm2*	Mean NNSW Mortality 2000 IT	AT*	TOT.AV	Mean Mortality (NNSW) 1994#	1995#
Diazinon(DC94)	3.4	73.6	38.4	53.5	99.5	
Diazinon (DC95)	2.4	32.3	10.2	19.7		90.5
Chlorfenvinfos (DC94)	10.8	56.3	66.0	61.8	97.5	
Chlorfenvinfos (DC95)	6.8	19.1	9.5	13.6		78.5
Cypermethrin (DC95)	3.4	10.9	3.1	6.5		26.7
Flumethrin (DC95)	3.3	8.0	0.0	3.4		20.7
Deltamethrin (DC95)	2.0	14.6	15.5	15.1		7.7
Cyhalothrin (DC95)	6.4	14.0	2.0	7.1		9.5

* AT = Ambient temperature IT = ideal temperature (ie 28-34 deg.C) # = results of 1994/1995 resistance survey for MRC

Table 2: Percent Buffalo Fly Mortalities at Discriminating Concentrations - South Eastern Queensland, April 2000.

Insecticide	**DC**	**Gympie**	**Broweena**	**Wonga**	**Maryborough**	**Maryborough**	**Mean Mortality (SEQ)**		
(code/year)	***ug/cm2***	*Bishop*	*Rockemer*	*Crossley*	*DBargenquast*	*RBargenquast*	**2000**	**1994#**	**1995#**
Diazinon(DC94)	3.4	98.6	100.0	99.2	100.0	100.0	99.6	98.0	
Diazinon (DC95)	2.4	69.8	100.0	92.2	99.4	95.5	91.4		97.3
Chlorfenvinfos (DC94)	10.8	32.2	97.7	81.6	89.3	92.1	78.6	91.6	
Chlorfenvinfos (DC95)	6.8	17.3	78.1	33.7	92.0	80.1	60.3		50.3
Cypermethrin (DC95)	3.4	1.0	6.1	0.1	1.8	1.4	2.1		11.8
Flumethrin (DC95)	3.3	0.5	0.0	0.0	0.4	4.2	1.0		11.0
Deltamethrin (DC95)	2.0	30.1	14.9	21.5	25.7	27.7	24.0		12.7
Cyhalothrin (DC95)	6.4	9.9	5.9	12.2	14.8	14.9	11.5		10.1
Acetone Control		1.1	2.5	3.0	2.8	2.1	2.3		-

= results of 1994/1995 resistance survey for MRC

Table 3: Percent Buffalo Fly Mortalities at Discriminating Concentrations - Central Queensland, May 2000.

Insecticide	**DC**	**Bajool**	**Bajool**	**Bajool**	**Rockhampton**	**Stanwell**	**Belmont**	**Mean Mortality (CQ)**		
(code/year)	***ug/cm2***	*Stunzer*	*Leahy*	*Jenkinson*	*Coombs*	*Coombs*	*Agforce*	**2000**	**1994#**	**1995#**
Diazinon(DC94)	3.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.9	
Diazinon (DC95)	2.4	100.0	100.0	95.8	100.0	100.0	97.8	98.9		97.2
Chlorfenvinfos (DC94)	10.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.7	
Chlorfenvinfos (DC95)	6.8	98.0	100.0	100.0	100.0	86.2	98.5	97.1		92.1
Cypermethrin (DC95)	3.4	26.7	2.3	14.8	72.7	10.7	3.5	21.8		14.5
Flumethrin (DC95)	3.3	3.1	2.3	0.0	1.5	0.0	0.0	1.1		14.2
Deltamethrin (DC95)	2.0	70.7	78.6	33.2	82.8	19.6	24.3	51.5		23.9
Cyhalothrin (DC95)	6.4	73.1	9.7	22.0	76.8	19.8	16.8	36.4		9.9
Acetone Control		0.0	0.0	0.5	0.0	0.0	0	0.1		-

= results of 1994/1995 resistance survey for MRC

Table 4: Percent Buffalo Fly Mortalities at Discriminating Concentrations - North Queensland, April 2000.

Insecticide	**DC**	**Kuttabul**	**Mirani**	**Bloomsbury**	**Proserpine**	**Bowen**	**Ct. Towers**	**Mean Mortality (NQ)**		
(code/year)	*ug/cm2*	*Wenck*	*Pullen*	*Jefferson*	*Faust*	*Potts*	*Nielsen*	**2000**	**1994#**	**1995#**
Diazinon(DC94)	3.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.9	
Diazinon (DC95)	2.4	-	82.7	100.0	100.0	-	96.2	94.7		97.2
Chlorfenvinfos (DC94)	10.8	72.2	90.6	100.0	94.6	100.0	97.1	92.4	96.7	
Chlorfenvinfos (DC95)	6.8	33.6	27.0	99.0	84.2	95.8	6.2	57.6		92.1
Cypermethrin (DC95)	3.4	8.1	6.2	4.4	7.3	18.2	7.4	8.6		14.5
Flumethrin (DC95)	3.3	4.3	0.0	0.0	2.0	1.1	-	1.5		14.2
Deltamethrin (DC95)	2.0	8.8	76.4	10.1	29.6	84.9	24.3	39.0		23.9
Cyhalothrin (DC95)	6.4	10.2	11.3	6.4	25.3	30.5	17.9	16.9		9.9
Acetone Control		0.0	0.0	0.5	2.2	0.0	0	0.5		-

= results of 1994/1995 resistance survey for MRC

Table 5: Percent Buffalo Fly Mortalities at Discriminating Concentrations - Far North Queensland, April 2000.

Insecticide	**DC**	**Mena Ck**	**Chillagoe**	**Mean Mortality (NQ)**		
(code/year)	*ug/cm2*	*Locke*	*Pantovic*	**2000**	**1994#**	**1995#**
Diazinon(DC94)	3.4	100.0	100.0	100.0	99.9	
Diazinon (DC95)	2.4	98.5	100.0	99.3		97.7
Chlorfenvinfos (DC94)	10.8	100.0	100.0	100.0	85.2	
Chlorfenvinfos (DC95)	6.8	84.2	85.9	85.0		85.6
Cypermethrin (DC95)	3.4	4.5	20.3	12.4		36.8
Flumethrin (DC95)	3.3	-	6.1	6.1		-
Deltamethrin (DC95)	2.0	0.0	77.3	38.6		25.3
Cyhalothrin (DC95)	6.4	-	57.7	57.7		-
Acetone Control		0.0	0.0	0.0		-

= results of 1994/1995 resistance survey for MRC

Figure 1: Buffalo Fly Mortality/Resistance (%), Organophosphates, 1994,1995 & 2000 - Northern NSW

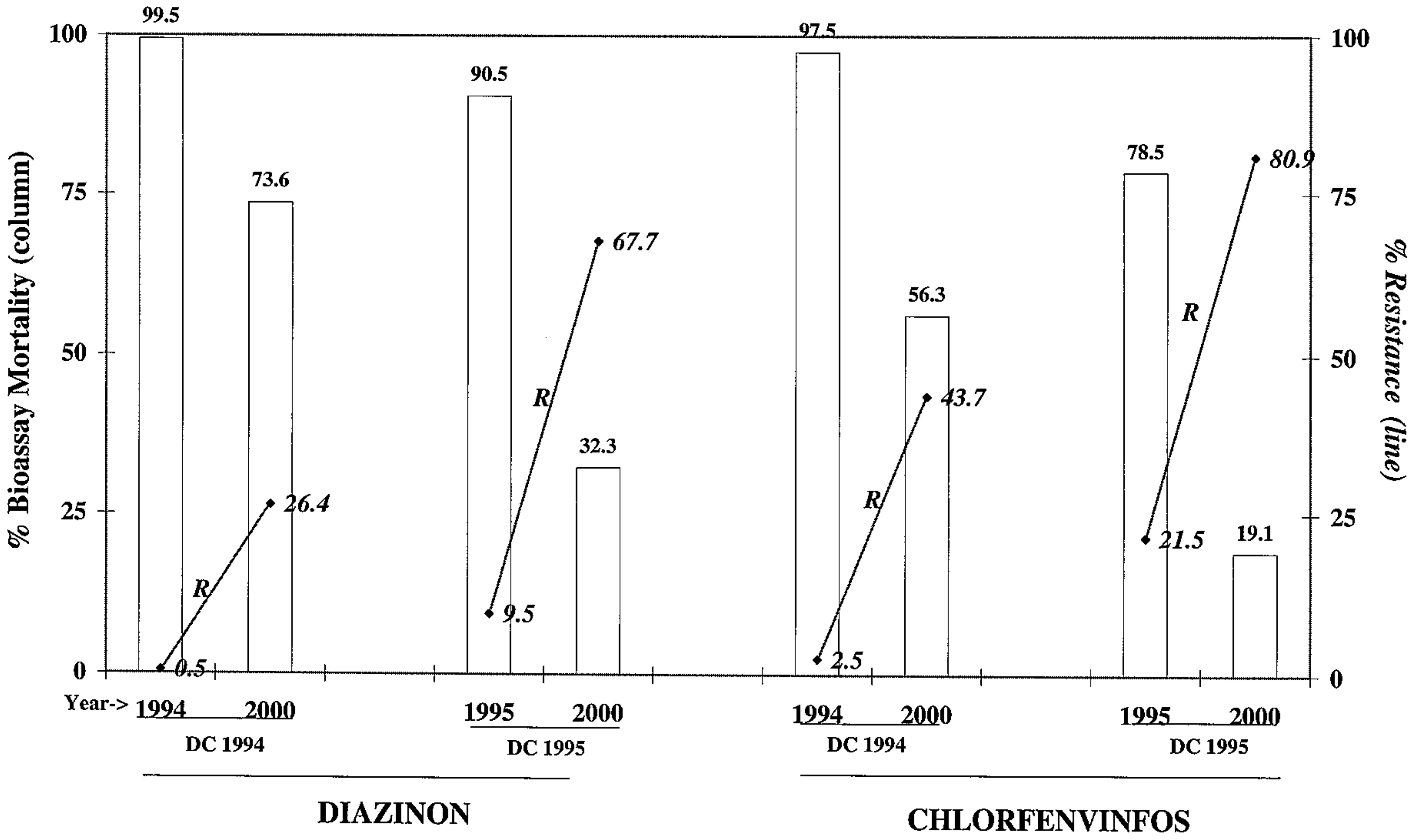


Figure 2: Buffalo Fly Mortality/Resistance (%), Synthetic Pyrethroids, 1995 & 2000 - Northern NSW

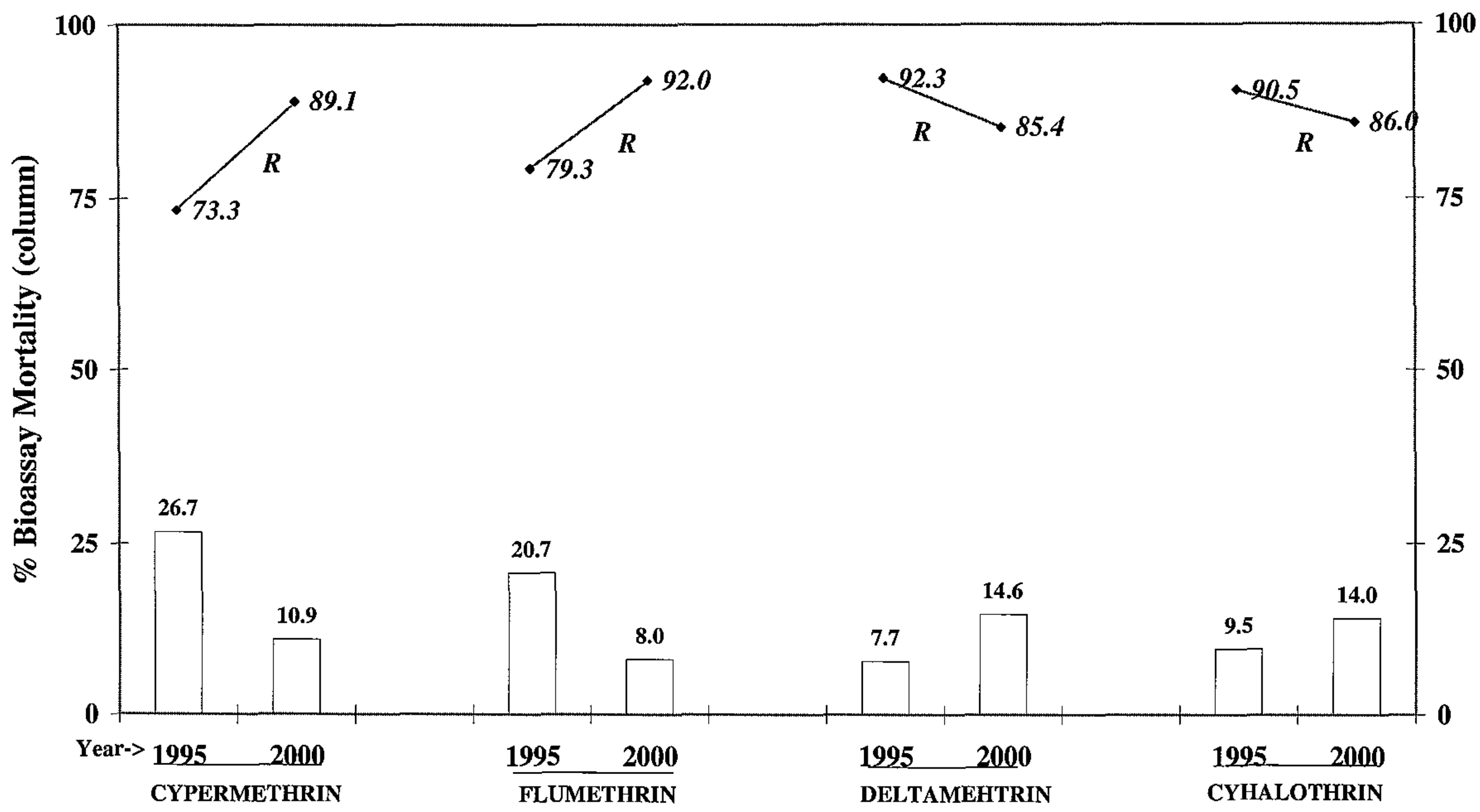


Figure 3: Buffalo Fly Mortality/Resistance (%), Organophosphates, 1994,1995 & 2000 - Southeast QLD

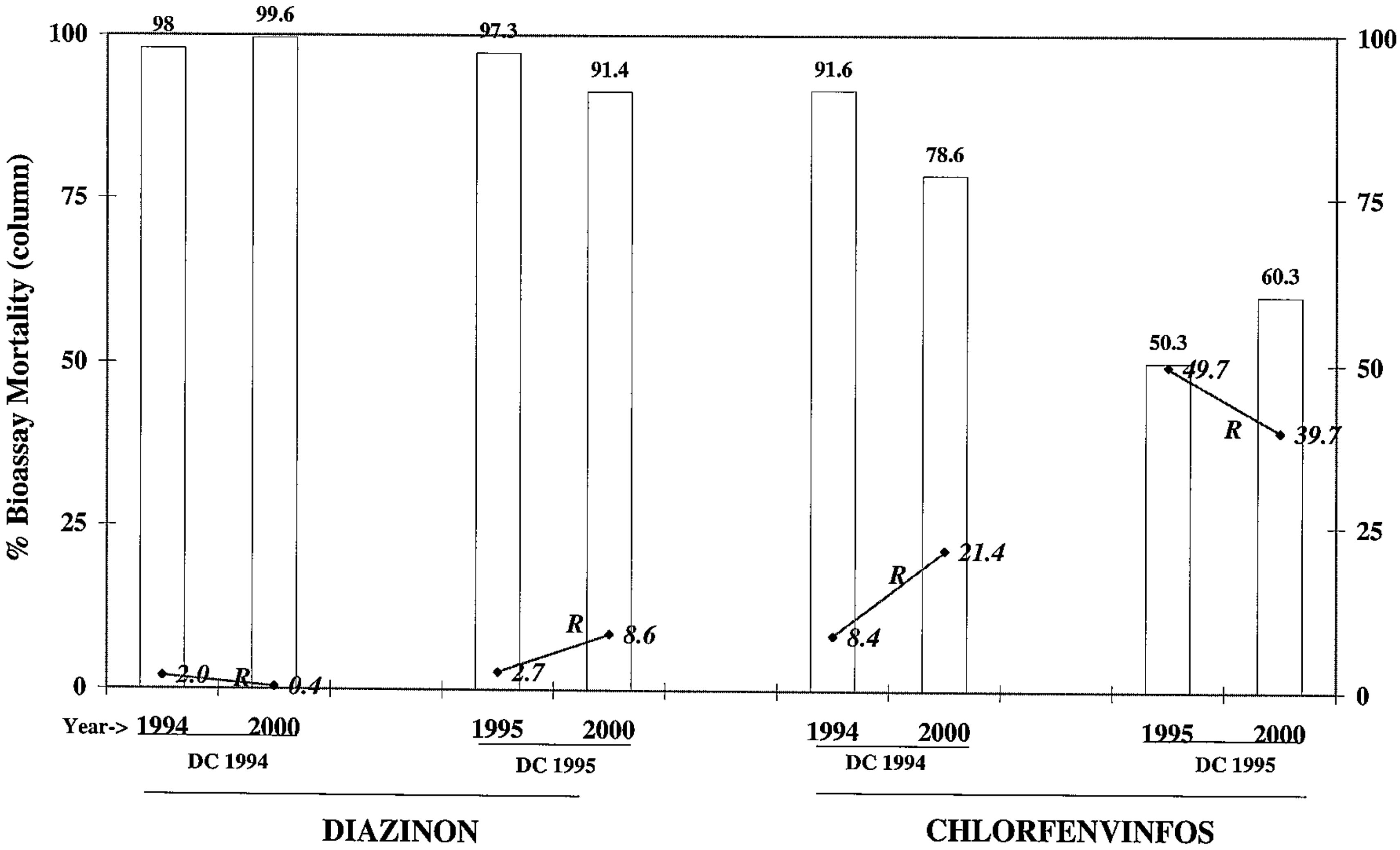


Figure 4: Buffalo Fly Mortality/Resistance (%), Synthetic Pyrethroids, 1995 & 2000 - Southeast QLD

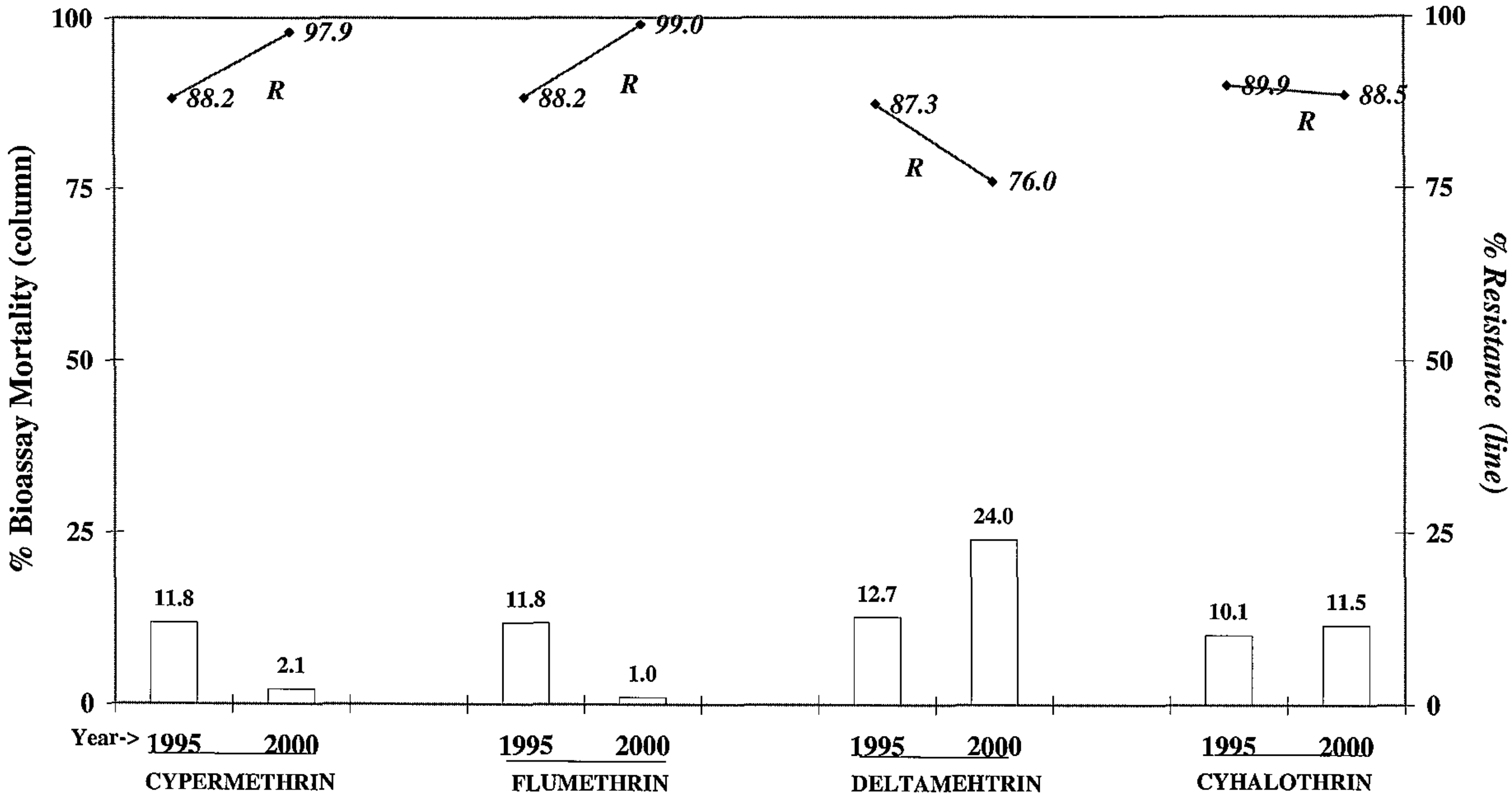


Figure 5: Buffalo Fly Mortality/Resistance (%), Organophosphates, 1994,1995 & 2000 - Central QLD

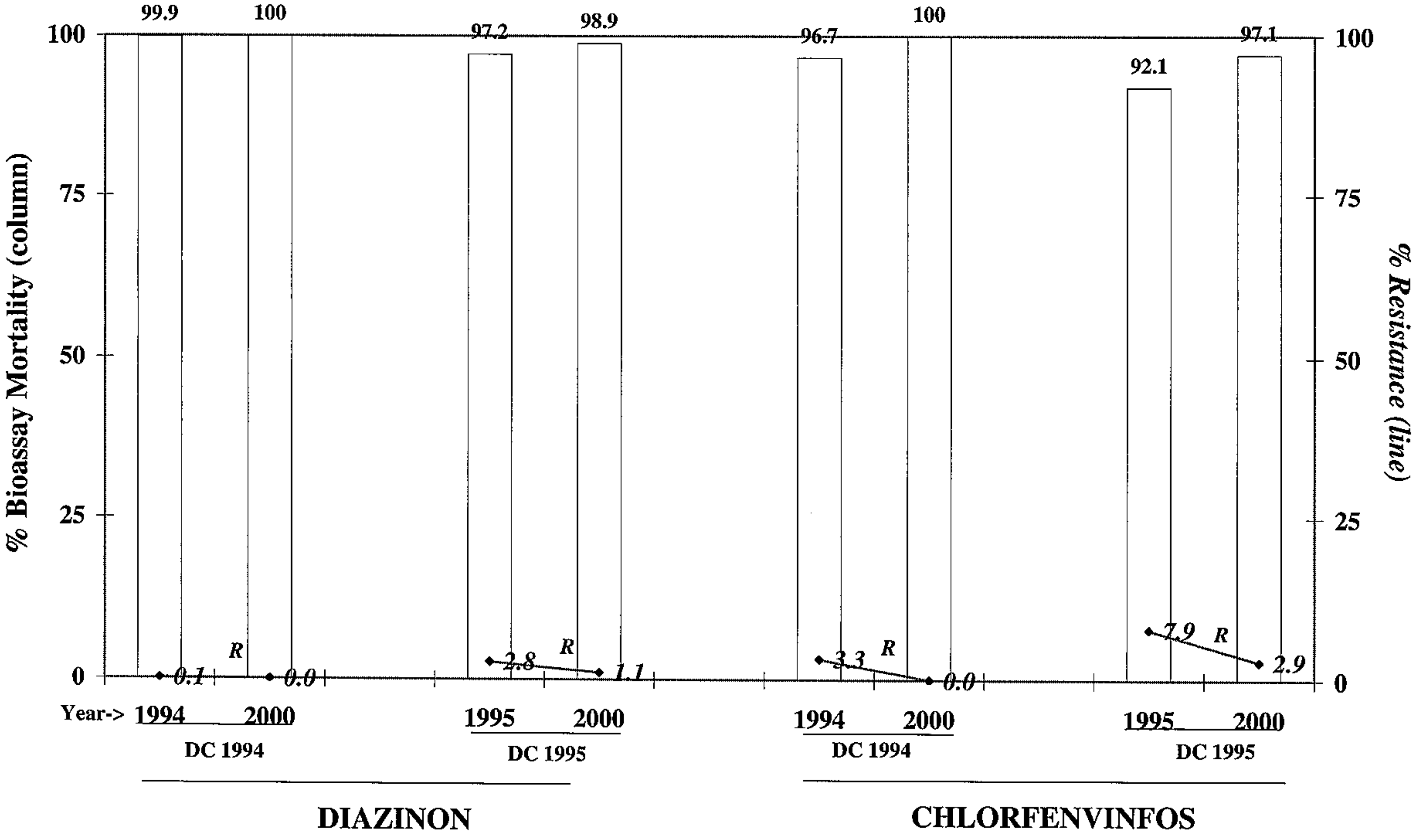


Figure 6: Buffalo Fly Mortality/Resistance (%), Synthetic Pyrethroids, 1995 & 2000 - Central QLD

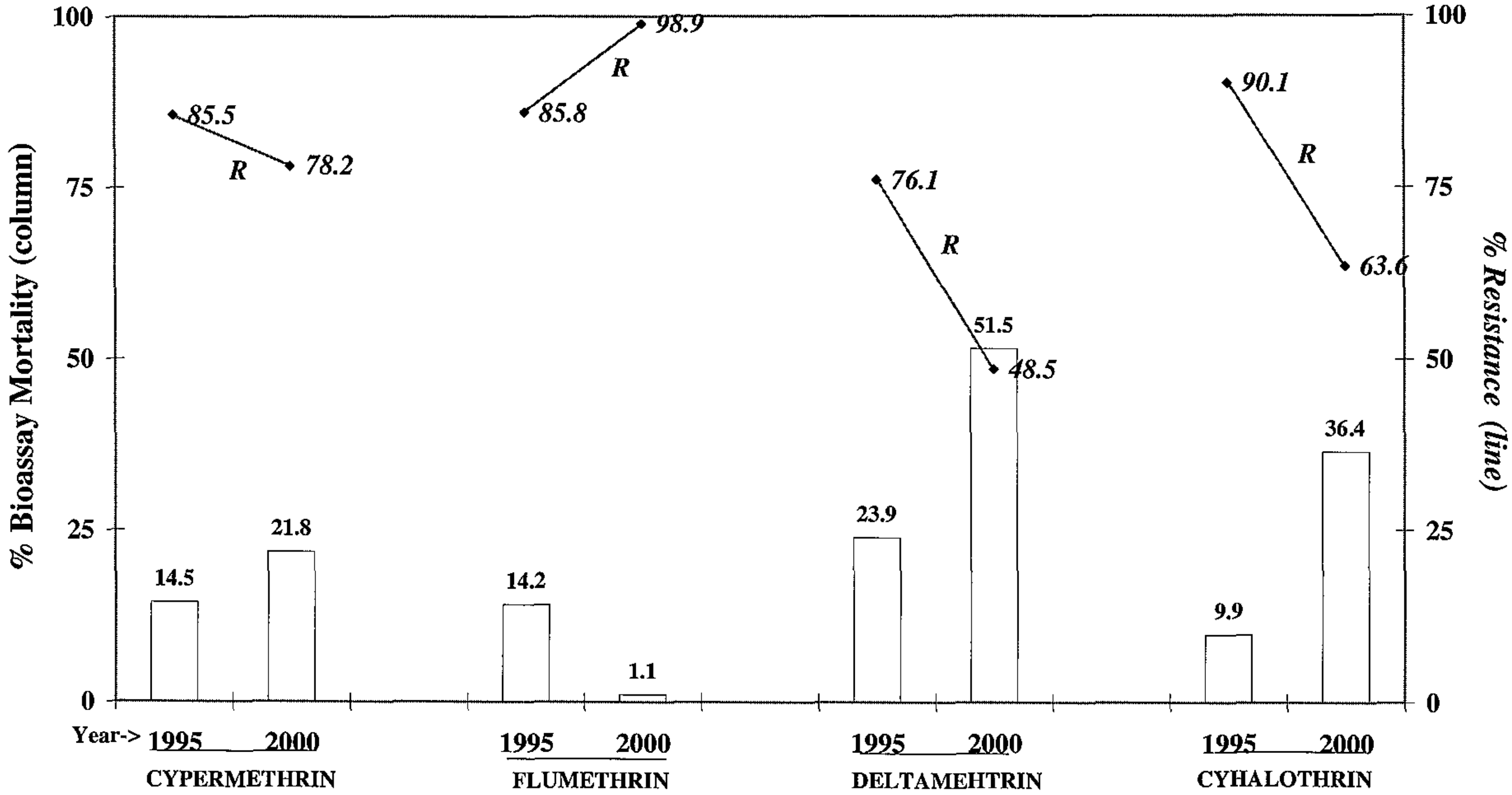


Figure 7: Buffalo Fly Mortality/Resistance (%), Organophosphates, 1994,1995 & 2000 - North QLD

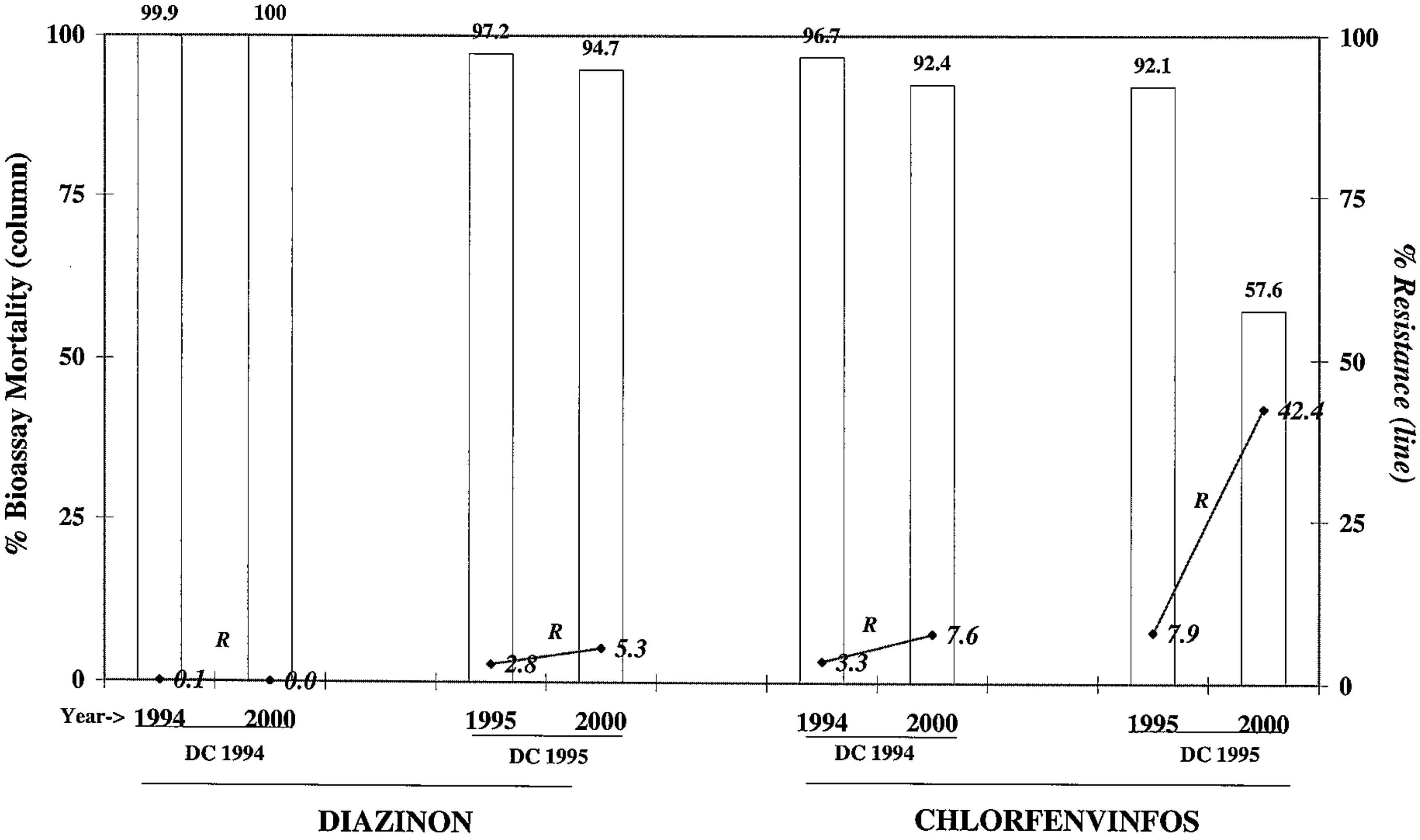


Figure 8: Buffalo Fly Mortality/Resistance (%), Synthetic Pyrethroids, 1995 & 2000 - North QLD

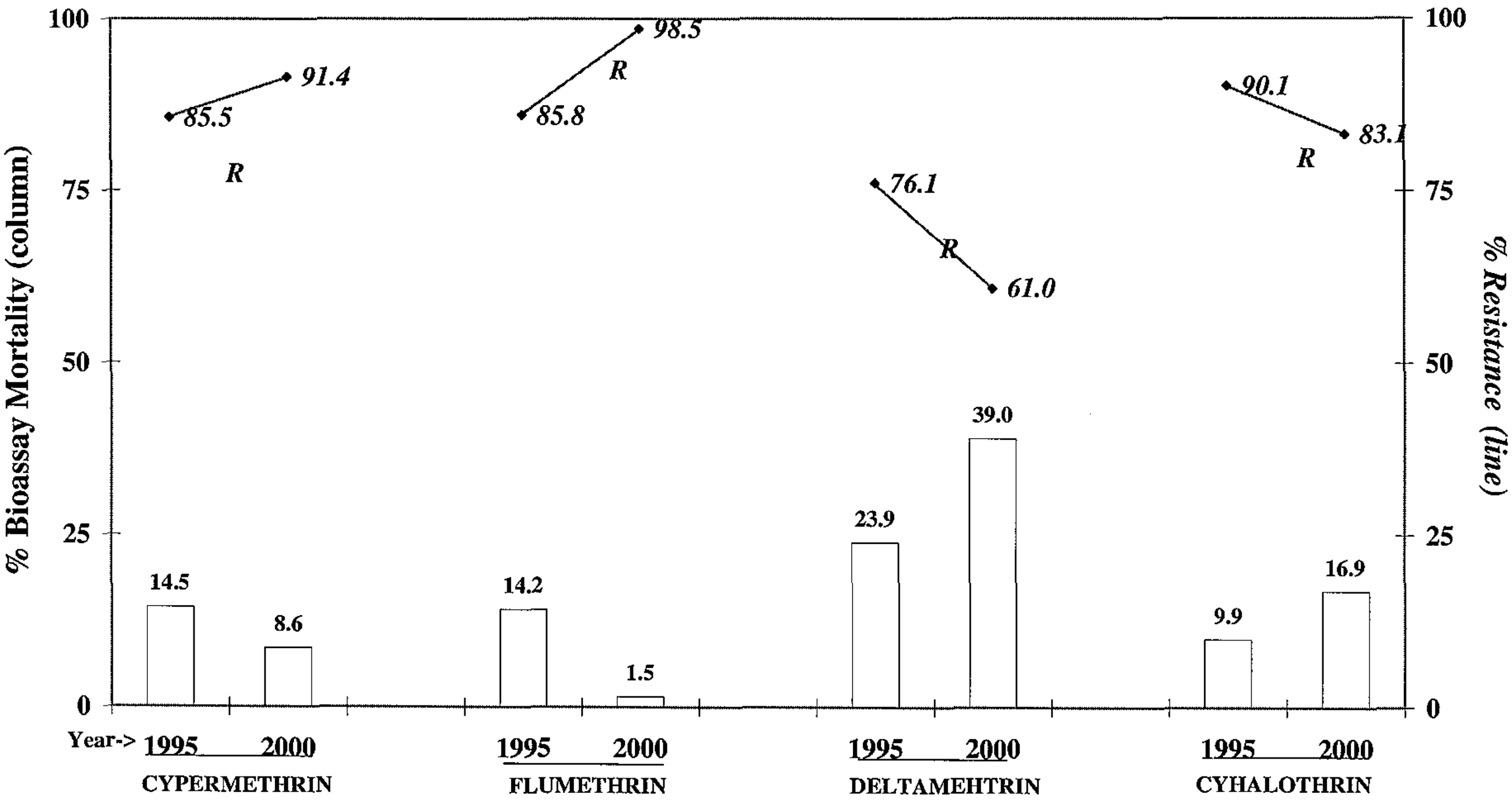


Figure 9: Buffalo Fly Mortality/Resistance (%), Organophosphates, 1994,1995 & 2000 - Far north QLD

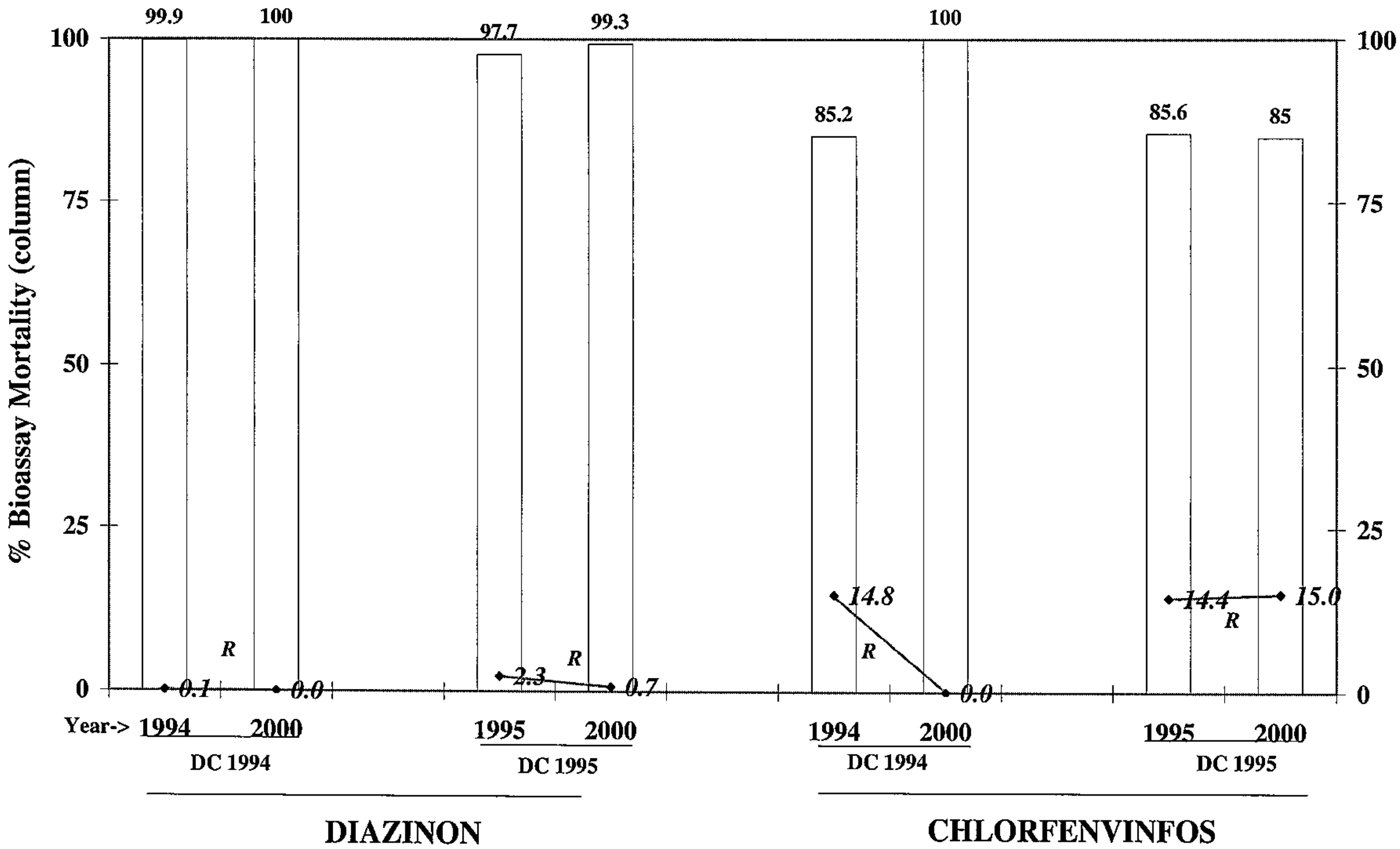


Figure 10: Buffalo Fly Mortality/Resistance (%), Synthetic Pyrethroids, 1995 & 2000 - Far north QLD

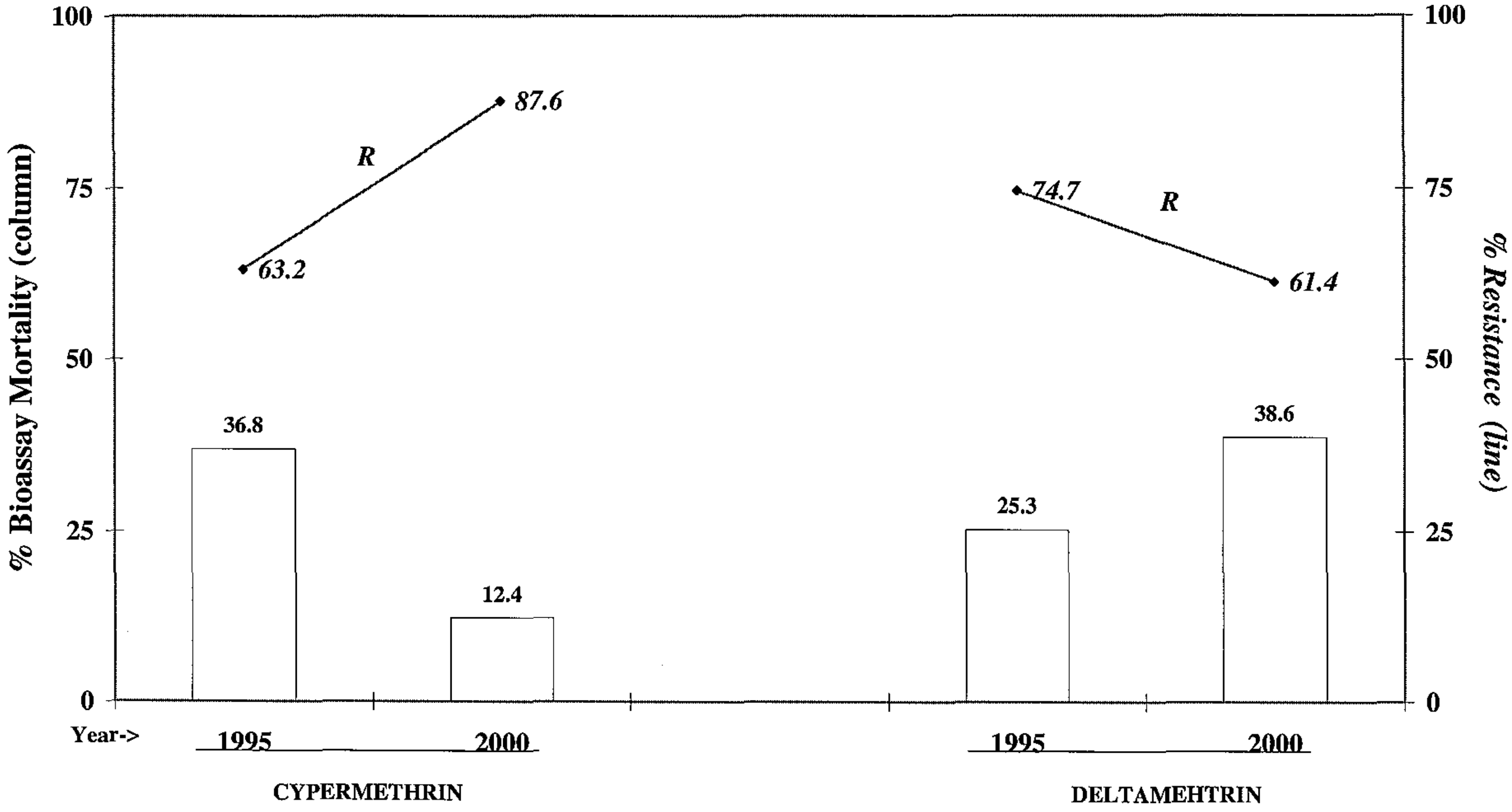
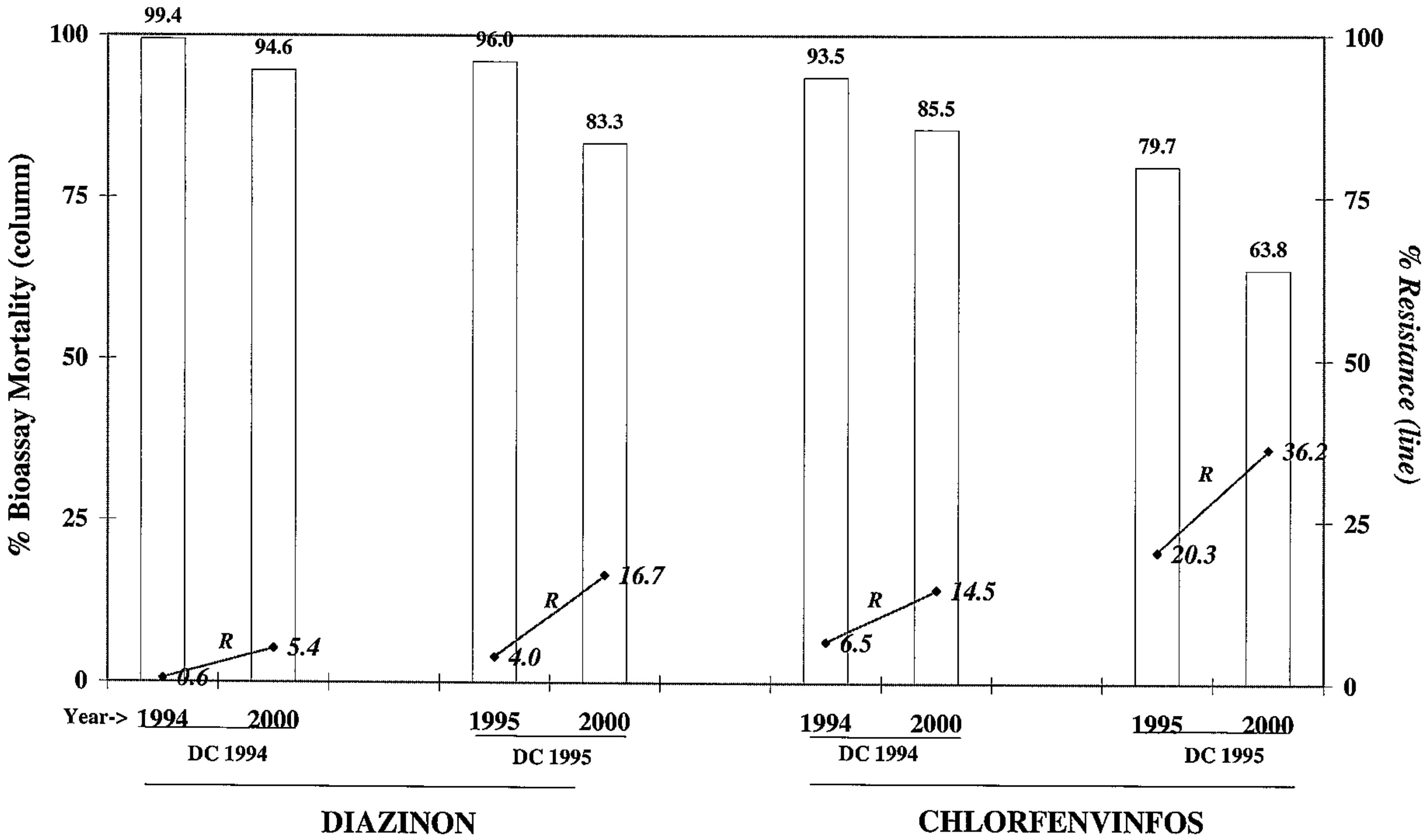
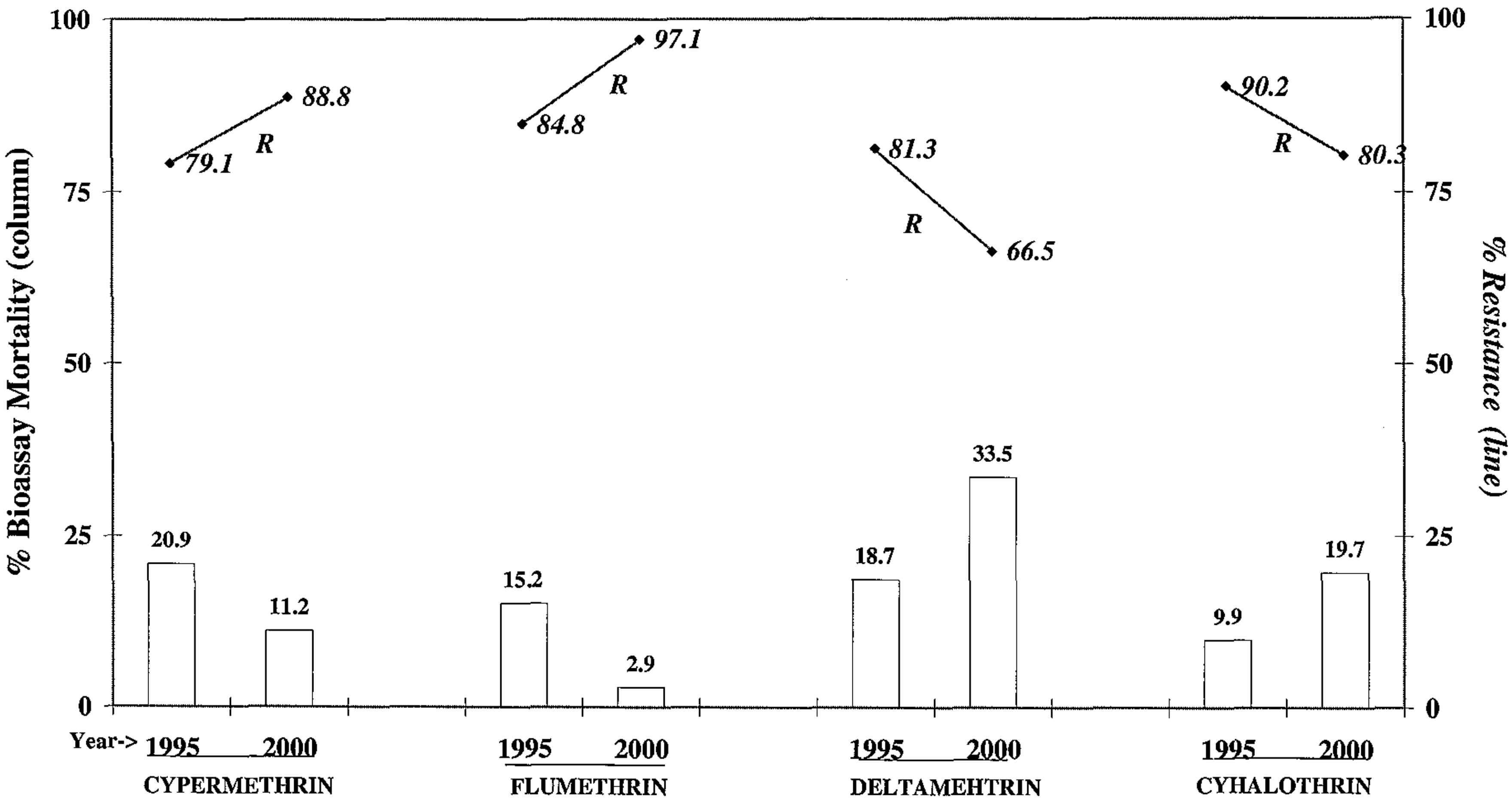


Figure 11: Buffalo Fly Mortality/Resistance (%), Mean Organophosphates, 1994,1995 & 2000 - NSW & QLD



<u>Figure 12</u>: Buffalo Fly Mortality/Resistance (%), Mean Synthetic Pyrethroids, 1995 & 2000 - NSW & QLD



DISCUSSION AND CONCLUSIONS

A total of 26 farms were sampled for flies throughout the whole of New South Wales and Queensland in the 2000 survey. Due to reasons of practicality all the assays conducted during the 2000 survey were carried out over a limited time (4-5 days for each region). Given the limited sample size and time frame of this 2000 survey therefore, some caution should be exercised when interpreting the results.

The 2000 survey indicated the possible appearance of diazinon resistance in buffalo flies in the northern New South Wales' region for the first time as measured using the filter paper bioassay technique. On the northern New South Wales' properties sampled in 2000 (3), the average percentages of buffalo fly survival (ie 26.4% and 67.7%) at the respective 1994 and 1995 diazinon DCs were considerably higher than the corresponding levels of survival in assays in 1994 (0.5%) and 1995 (9.5%). These degrees of increased diazinon tolerance were not seen in any other sampled regions in 2000, particularly in south eastern Queensland - where a similar result may have been expected. Nor had similar levels of diazinon tolerance ever been seen in the previous surveys (ie 1994 and 1995).

A concerningly high level of resistance to the other organophosphate insecticide: chlorfenvinphos was also found in assays in the northern New South Wales region. In 2000, the average percentages of buffalo fly survival to respective chlorfenvinphos DCs (ie 43.7% and 80.9%), were significantly higher than the corresponding levels of survival in the 1994 (2.5%) and 1995 (21.5%) assays. In other regions: there was a slight rise in the level of chlorfenvinphos tolerance in buffalo flies sampled in the north Queensland region, however there were unchanged and/or low to moderate levels of tolerance to chlorfenvinphos assayed in the south eastern and far north Queensland regions.

Continued high levels of resistance to the synthetic pyrethroid insecticides were generally indicated in buffalo flies in all regions of New South Wales and Queensland. Slightly reduced levels of tolerance to deltamethrin and cyhalothrin may indicate that resistance genes have become diluted.

The questionnaire of current management practices towards buffalo fly carried out in 2000 was taken from a relatively small number of properties and is therefore limited as far as drawing too many conclusions about the wider grazing community. The 2000 survey did indicate an interesting link between fly control management practices and the development of insecticide resistance. That is, that in the regions where organophosphate resistance percentages was suggested, the recent use of these same insecticides had been commonplace. As well, application efficiencies of these insecticides - particularly chlorfenvinphos through back spray and back rubber – were generally not good. Lack of thorough spraying of animals with diluted insecticides was identified as a problem in the 1994 and 1995 survey. The relative cheapness of the organophosphate insecticides (containing chlorfenvinphos and diazinon) that are intended for use through back spraying may lead to less care being exercised with their use, eventually contributing to under-dosing and the selection for resistance. By way of contrast, in the south eastern region of Queensland where chemical abstinence and keen interest in dung beetles welfare were reported, levels of organophosphate resistance indicated increased only slightly since 1995. It also appeared encouraging that the general reduced usage of deltamethrin and cyhalothrin insecticides may have contributed towards an overall decrease in the levels of resistance to these chemicals.

The main message to come from the 2000 survey is that continued care needs to be taken with the use of insecticide products to help prevent (or delay) the onset of insecticide resistance. To help achieve this aim the author feels that there needs to be a co-ordinated industry-wide approach, with regular monitoring of buffalo fly resistance via current and/or newer technologies, to evaluate agreed resistance management strategies.

6. APPENDICES

6.1 Buffalo Fly Mortality in Resistance Bioassays - Northern New South Wales (March, 2000)

		Margaret Howe, Lennox Head				Graham Pearson, Bwingdale				Tony Clarke, Brushgrove				John Smith, Woodenbong				Les Clements, Urbenville				Roly Harrison, Kyogle				M&M Carter, Kyogle			
INSECTICIDE	DC ug/cm2	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB
Diazinon(D4)	3.4	69	111	62.2	57.1	154	164	93.9	93.8	96	129	74.4	69.9	94	143	65.7	64.9	21	99	21.2	20.7	53	175	30.3	28.3	41	98	41.8	39.7
Diazinon (D5)	2.4	23	135	17.0	5.8	73	154	47.4	46.6	94	178	52.8	44.5	41	181	22.7	20.7	9	122	7.4	6.8	12	117	10.3	7.7	9	100	9.0	5.6
Chlorfenvinfos (C4)	10.8	24	129	18.6	7.6	139	141	98.6	98.6	91	133	68.4	62.8	115	117	98.3	98.2	38	129	29.5	29.0	157	176	89.2	88.9	54	109	49.5	47.7
Chlorfenvinfos (C5)	6.8	6	109	5.5	0.0	35	148	23.6	22.5	66	148	44.6	34.8	8	128	6.3	3.8	2	136	1.5	0.9	40	179	22.3	20.1	20	122	16.4	13.3
Cypermethrin (Y)	3.4	8	111	7.2	0.0	5	124	4.0	2.6	65	160	40.6	30.1	8	142	5.6	3.2	5	110	4.5	4.0	10	126	7.9	5.3	2	81	2.5	0.0
Flumethrin (F)	3.3	5	110	4.5	0.0	4	141	2.8	1.4	43	126	34.1	22.5	1	98	1.0	0.0	1	145	0.7	0.1	0	119	0.0	0.0	0	91	0.0	0.0
Deltamethrin (L)	2.0	22	125	17.6	6.5	16	115	13.9	12.6	57	158	36.1	24.8	17	155	11.0	8.7	19	143	13.3	12.8	42	153	27.5	25.4	19	104	18.3	15.2
Cyhalothrin (H)	6.4	16	113	14.2	2.6	7	117	6.0	4.6	57	128	44.5	34.7	9	124	7.3	4.9	1	129	0.8	0.2	8	144	5.6	2.8	2	96	2.1	0.0
Acetone Control		14	118	11.9		2	137	1.5		25	167	15.0		3	119	2.5		1	154	0.6		3	106	2.8		4	111	3.6	

%MAB = Mortality after correction for control mortality using Abbott's formula

6.2 Buffalo Fly Mortality in Resistance Bioassays - South Eastern Queensland (April, 2000)

		Shane Bishop, Gympie				L. Rockemer, Broweena				J. Crossley, Wonga				D. Bargenquast, Maryborough				R. Bargenquast, Maryborough			
INSECTICIDE	DC ug/cm2	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB
Diazinon(D4)	3.4	71	72	98.6	98.6	93	93	100.0	100.0	129	130	99.2	99.2	171	171	100.0	100.0	76	76	100.0	100.0
Diazinon (D5)	2.4	54	77	70.1	69.8	114	114	100.0	100.0	159	172	92.4	92.2	176	177	99.4	99.4	87	91	95.6	95.5
Chlorfenvinfos (C4)	10.8	29	88	33.0	32.2	131	134	97.8	97.7	92	112	82.1	81.6	164	183	89.6	89.3	84	91	92.3	92.1
Chlorfenvinfos (C5)	6.8	12	66	18.2	17.3	96	122	78.7	78.1	45	126	35.7	33.7	190	206	92.2	92.0	87	108	80.6	80.1
Cypermethrin (Y)	3.4	1	47	2.1	1.0	8	95	8.4	6.1	4	130	3.1	0.1	6	131	4.6	1.8	3	86	3.5	1.4
Flumethrin (F)	3.3	1	63	1.6	0.5	3	126	2.4	-0.1	0	136	0.0	-3.1	6	187	3.2	0.4	4	64	6.3	4.2
Deltamethrin (L)	2.0	25	81	30.9	30.1	23	135	17.0	14.9	25	105	23.8	21.5	50	180	27.8	25.7	26	89	29.2	27.7
Cyhalothrin (H)	6.4	6	55	10.9	9.9	11	133	8.3	5.9	19	128	14.8	12.2	26	151	17.2	14.8	17	102	16.7	14.9
Acetone Control		1	94	1.1		3	122	2.5		4	135	3.0		4	143	2.8		2	96	2.1	

%MAB = Mortality after correction for control mortality using Abbott's formula

6.3 Buffalo Fly Mortality in Resistance Bioassays - Central Queensland (May, 2000)

		O&G Stunzer, Ulam				G. Leahy, Ulam				D. Jenkinson, Ulam				T. Coombs, Meura Plains				T. Coombs, Stanwell				G. Halford, Belmont			
INSECTICIDE	DC ug/cm2	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB
Diazinon(D4)	3.4	115	115	100.0	100.0	92	92	100.0	100.0	218	218	100.0	100.0	140	140	100.0	100.0	105	105	100.0	100.0	105	105	100.0	100.0
Diazinon (D5)	2.4	95	95	100.0	100.0	125	125	100.0	100.0	166	173	96.0	95.8	125	125	100.0	100.0	121	121	100.0	100.0	89	91	97.8	97.8
Chlorfenvinfos (C4)	10.8	90	90	100.0	100.0	110	110	100.0	100.0	178	178	100.0	100.0	126	126	100.0	100.0	98	98	100.0	100.0	94	94	100.0	100.0
Chlorfenvinfos (C5)	6.8	99	101	98.0	98.0	128	128	100.0	100.0	202	202	100.0	100.0	115	115	100.0	100.0	94	109	86.2	86.2	132	134	98.5	98.5
Cypermethrin (Y)	3.4	76	285	26.7	26.7	2	87	2.3	2.3	45	260	17.3	14.8	120	165	72.7	72.7	8	75	10.7	10.7	3	85	3.5	3.5
Flumethrin (F)	3.3	5	161	3.1	3.1	3	132	2.3	2.3	2	249	0.8	-2.3	2	132	1.5	1.5	0	68	0.0	0.0	0	78	0.0	0.0
Deltamethrin (L)	2.0	193	273	70.7	70.7	110	140	78.6	78.6	102	290	35.2	33.2	120	145	82.8	82.8	20	102	19.6	19.6	25	103	24.3	24.3
Cyhalothrin (H)	6.4	95	130	73.1	73.1	12	124	9.7	9.7	49	201	24.4	22.0	106	138	76.8	76.8	22	111	19.8	19.8	21	125	16.8	16.8
Acetone Control		0	112	0.0		0	130	0.0		1	192	0.5		0	120	0.0		0	90	0.0		0	130	0.0	

%MAB = Mortality after correction for control mortality using Abbott's formula

6.4 Buffalo Fly Mortality in Resistance Bioassays - Northern Queensland (April, 2000)

		N. Wenck, Kuttabul				I. Pullen, Mirani				K. Jefferson, Bloomsbury				P. Faust, Proserpine				G. Potts, Bowen				D. Nielsen, Charters Towers			
INSECTICIDE	DC ug/cm2	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB
Diazinon(D4)	3.4	87	87	100.0	100.0	115	115	100.0	100.0	126	126	100.0	100.0	78	78	100.0	100.0	185	185	100.0	100.0	90	90	100.0	100.0
Diazinon (D5)	2.4	0	0	-	-	105	127	82.7	82.7	168	168	100.0	100.0	162	162	100.0	100.0	0	0	-	-	75	78	96.2	96.2
Chlorfenvinfos (C4)	10.8	52	72	72.2	72.2	116	128	90.6	90.6	206	206	100.0	100.0	90	95	94.7	94.6	96	96	100.0	100.0	102	105	97.1	97.1
Chlorfenvinfos (C5)	6.8	37	110	33.6	33.6	31	115	27.0	27.0	104	105	99.0	99.0	93	110	84.5	84.2	206	215	95.8	95.8	4	65	6.2	6.2
Cypermethrin (Y)	3.4	7	86	8.1	8.1	5	81	6.2	6.2	5	88	5.7	4.4	12	129	9.3	7.3	35	192	18.2	18.2	7	95	7.4	7.4
Flumethrin (F)	3.3	4	94	4.3	4.3	0	82	0.0	0.0	8	180	4.4	3.2	3	72	4.2	2.0	1	88	1.1	1.1	0	0	-	-
Deltamethrin (L)	2.0	5	57	8.8	8.8	42	55	76.4	76.4	22	195	11.3	10.1	33	106	31.1	29.6	158	186	84.9	84.9	28	115	24.3	24.3
Cyhalothrin (H)	6.4	9	88	10.2	10.2	7	62	11.3	11.3	10	132	7.6	6.4	35	130	26.9	25.3	40	131	30.5	30.5	15	84	17.9	17.9
Acetone Control		0	65	0.0		0	71	0.0		2	149	1.3		2	89	2.2		0	119	0.0		0	180	0.0	

%MAB = Mortality after correction for control mortality using Abbott's formula

6.5 Buffalo Fly Mortality in Resistance Bioassays - Far Northern Queensland (April, 2000)

		P. Pantovic Chillagoe				B. Locke Mena Creek			
INSECTICIDE	DC ug/cm2	Total Dead	Total	%Mort	%MAB	Total Dead	Total	%Mort	%MAB
Diazinon(D4)	3.4	78	78	100.0	100.0	100	100	100.0	100.0
Diazinon (D5)	2.4	92	92	100.0	100.0	66	67	98.5	98.5
Chlorfenvinfos (C4)	10.8	80	80	100.0	100.0	95	95	100.0	100.0
Chlorfenvinfos (C5)	6.8	85	99	85.9	85.9	80	95	84.2	84.2
Cypermethrin (Y)	3.4	12	59	20.3	20.3	4	88	4.5	4.5
Flumethrin (F)	3.3	2	33	6.1	6.1				
Deltamethrin (L)	2.0	85	110	77.3	77.3	0	62	0.0	0.0
Cyhalothrin (H)	6.4	41	71	57.7	57.7				
Acetone Control		0	45	0.0		0	180	0.0	

%MAB = Mortality after correction for control mortality using Abbott's formula

6.6 Questionnaire Details - 2000 Insecticide Resistance Survey

6.6.1 Northern New South Wales

Region	Number	Name & Address	Cattle	Buffalo Fly	Days Control	Ticks	Dung Beetle
Northern New South Wales	1	R. Clark Elmore', Brushgrove	250 Friesian/ Jersey	Di-Jet Backrubber	4 or 5	Nil	No monitor
Average number of cattle = 205	2	G. Pearson Ewingdale	50 Hereford	Spike Tags 1 pair/year	16 weeks	Bayticol Ivomec	No monitor
	3	M. Howe Lennox Head	86 BrahmanX	Spike Tags 1 pair/year	16 weeks	Nil	No monitor
	4	M&M Carter Kyogle	440 Friesian	Di-Jet Spray 800 mL/cow (3/yr) Nucidol Backrubber	2 weeks Spike tags on beef cows	Nil	Causual monitor
	5	R. Harrison Kyogle	300 Hereford	Supona spray Di-Jet spray (10/yr) 200 mL/cow	2/3 weeks poor control	Tactic every 3 wks Spring/Autm	No monitor
	6	J. Smith Woodenbong	380 milk 700 beef	None	-	Tactic every 3 wks Spring/Autm	No monitor
	7	L. Clements Urbenville	150 Hereford Anugus	Spike Tags Di-Jet spray	16 weeks	Amitraz dip every 3 wks Spring/Autm	No monitor

6.6.2 South Eastern Queensland

Region	Number	Name & Address	Cattle	Buffalo Fly	Days Control	Ticks	Dung Beetle
South Eastern Queensland	1	D. Bargenquast Broweena Maryborough	250 BrahmanX Charolais	Supona back spray 20 mL/L, water stripe up back 6/7 per year	4 weeks (neighbours use Supona)	Amitrazspray wet all over	No monitor
Average number of cattle = 542	2	J. Crossley Wonga Gympie	300 BrahmanX	Nil in last 12 months diazinon/supona back-rubbers before that	- (neighbours use Supona)	Cull very sus-ceptible ones	No but Feehan IDd 5 sppecies on farm
	3	L. Rickemer Broweena	2000 BrahmanX	Supona 5/6 times per year. Strip down back (runs down sides)	3-4 weeks	Cows dipped amitraz twice 12 weeks	Casual monitor
	4	S. Bishop Gympie	100 Brahman Bulls	Nil in last 12 months cull susceptibles cows and use dung beetles	- No MCLs for dung bs	Nil	Monitor and re-lease. 5 spp. present
	5	R. Bargenquast Broweena	60 mixed breeds	Supona backrubber less effective on flies	4 weeks	Tactic pl. dip 3/4 times per year	Casual monitor

6.6.3 Central Queensland

Region	Number	Name & Address	Cattle	Buffalo Fly	Days Control	Ticks	Dung Beetle
Central Queensland	1	O Stunzer Prior Park Bajool	2000 BrahmanX	Nil	-	Nil	Casual monitor
Average number of cattle = 1167	2	G Leahy Willbrook Bajool	2000 BrahmanX	Nil	- (Original Tixafly trials)	Ivomec 1per year	No monitor
	3	R. Jenkinson Gelndale Bajool	320 BrahmanX 160 milk	Nil	-	Amitaz once per year	No monitor
	4	T. Coombs Meura Plains Rockhampton	300 BrahmanX	Nil	-	Nil	Causual monitor
	5	T. Coombs Stanwell Park	180 Friesian	Di-jet or Supona backspray	-		No monitor
	6	G. Halford Belmont Rockhampton	2200 Belmont red Hereford	-			No monitor

6.6.4 North Queensland

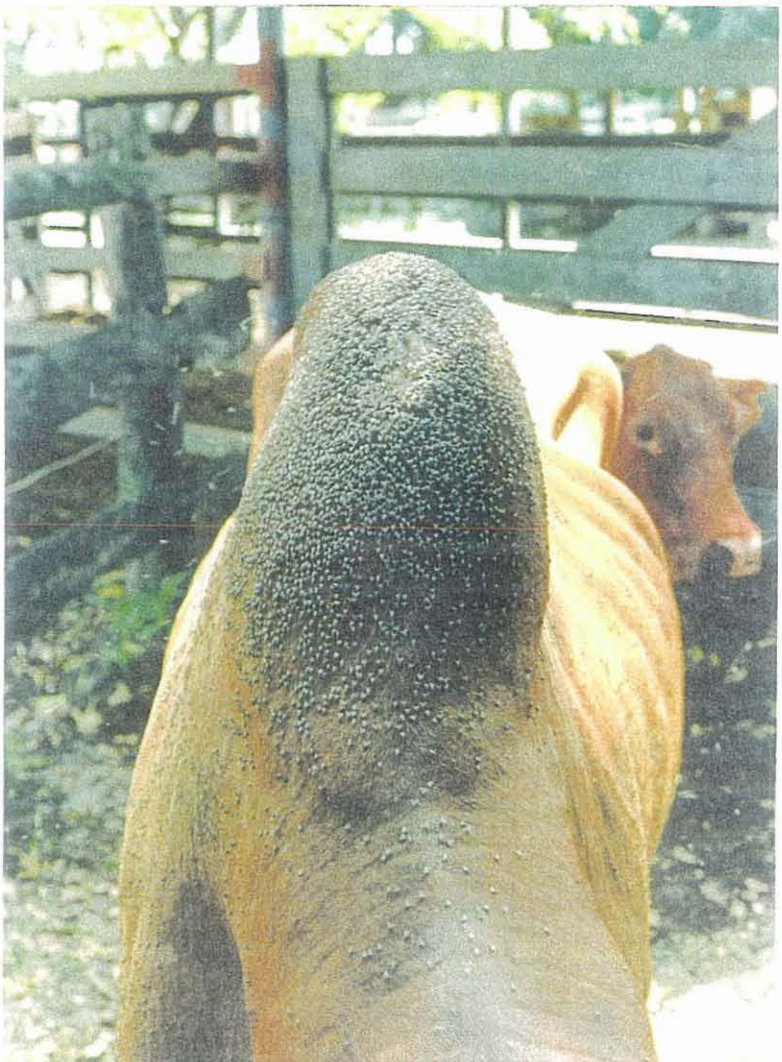
Region	Number	Name & Address	Cattle	Buffalo Fly	Days Control	Ticks	Dung Beetle
Northern Queensland Average number of cattle = 1883	1	N. Wenck Kuttabul	300 BrahmanX Longhorns	BarricadeS 4 times per year. Spray till wet all over	10-14 days	BarricadeS	Casual monitor
	2	I. Pullen Adamac Brahman Stud, Mirani	200 Brahman	Di-Jet spray Spring & Autumn. Python tags over summer	- tags removed after 16 wks	-	No monitor
	3	K. Jefferson Taranga Bloomsbury	6000 BrahmanX	Supona spray 3 times per year. Spray over top with mist	3 weeks		No monitor
	4	P. Faust Breadalbane Stn. Proserpine	2500 BrahmanX	Supona 3 per year	-	Nil	Causual monitor
	5	G.&G. Potts Millwarra Pastoral Bowen	2000 Brahman	Supona overspray 2 per year	-	Tactic dip	No monitor
	6	D.Nielsen P.O. Woodstock	300 BrahmanX	Supona or Sumifly 6 total per year	2-3 weeks (neighbours use Supona)	Bayticol 1 per year	Casual monitor

6.6.5 Far North Queensland

Region	Number	Name & Address	Cattle	Buffalo Fly	Days Control	Ticks	Dung Beetle
Far Northern Queensland	1	P. Pantovic Bullwarra Stn Chillagoe	8000 BrahmanX	Overspray with Supona 1 or 2 per year	-	-	No monitor
	2	B. Locke Utchee Creek Mena Creek	300 BrahmanX	Spike tags	16 weeks. Tags removed after 16 wks	Tactic plunge dip. 1 or 2 per year	No monitor

6.7 Photographs

6.1.1 Buffalo Fly Infested Cattle



6.1.2 Experimenter Catching Flies in a Sweep Net

6.1.3 Buffalo Flies in Petrie Dishes Containing Impregnated Filter Papers