



PROJECT REPORT No. 72

**THE ETHIRIMOL CONTENT OF
COMMERCIALY-TREATED
CEREAL SEEDS**

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THE ETHIRIMOL CONTENT OF COMMERCIALY-TREATED CEREAL SEEDS

by

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Final report of a six month project commencing in April 1992 by Vanessa Fox, an undergraduate student from Brunel University, whilst on an industrial placement at Horticulture Research International. The work was initiated at the request of Mr Trevor Martin, chairman of the BCPC Seed Treatment Working Party, and formed the second part of a larger study entitled "Sampling protocol for treated seeds". The experimental work presented in this report was funded by a grant of £8,550 from the Home-Grown Cereals Authority (Project No. 0071/1/91).

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1.0 INTRODUCTION

The aim of this study was to establish the accuracy, uniformity and physical stability of a commercially-used fungicide treatment applied to cereal seeds. It was part of a larger project, undertaken by the Seed Treatment Working Party of the British Crop Protection Council, to develop a procedure to enable a single sample of treated seed to be taken to represent the whole treated batch. Standard seed sampling procedures are unsuitable for treated seed samples because they involve much mixing and reduction in sample size. Seed treatments often produce dust and therefore loss of treatment when handled.

A single, widely-used fungicide treatment was selected for the study. This treatment comprised a mixture of three fungicides - ethirimol, flutriafol and thiabendazole - formulated and sold by ICI Agrochemicals under the brand name "Ferrax". The analytical procedure was based on the determination, by high performance liquid chromatography (hplc), of ethirimol, the major component in the formulation.

This work was a continuation of the project undertaken in 1990 at HRI-W by Mr Morgan of Nottingham Polytechnic, when the hplc method was developed.

1.1 FERRAX

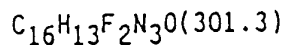
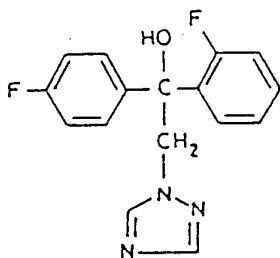
"Ferrax" is a systemic fungicide formulated by ICI Plant Protection for use on winter and spring barley. It is a pink coloured liquid which, when applied as a seed treatment, dries to leave a pink powdery coating to the seed.

It contains 400 g/l ethirimol
37.5 g/l flutriafol
10.0 g/l thiabendazole

1.1.1 ETHIRIMOL

5-butyl-2-ethylamino-6-methylpyrimidin-4-ol

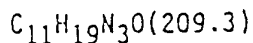
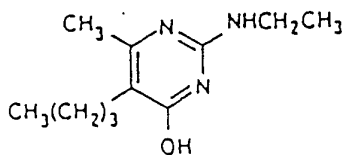
A hydroxypyrimidine, this was the basis of an earlier ICI fungicide, Milstem, and gives protection against powdery mildew.



1.1.2 FLUTRIAFOL

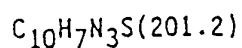
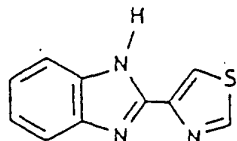
(RS)-1-(2-fluorophenyl)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl)ethanol

A triazole compound giving protection against a wide range of air-borne foliar disease and seed and soil borne diseases.



1.1.3 THIABENDAZOLE
2-(thiazol-4-yl)benzimidazole

From the benzimidazole group, it helps protect against Fusarium seedling blights.



Ethirimol was the first systemic cereal seed treatment used for control of foliar disease. Its use in conjunction with conventional mercury products gave an effective treatment against many seed-borne and soil-borne diseases of barley. Widespread use of the product led to changes in the sensitivity of UK barley powdery mildew populations (Shephard 1975). Restrictions were placed on its use in order to preserve the effectiveness of the compound. New triazole fungicides then took over much of the cereal fungicide market leading to downward shifts in its sensitivity, whilst the sensitivity to ethirimol rose from levels recorded when the restrictions were introduced. It was then decided to explore the possibility of combining the use of ethirimol and the triazole fungicides (Skidmore 1983).

Ethirimol, flutriafol and thiabendazole are well established agrochemicals with no particular hazards to the user or consumer and have low environmental impact (Godwin, Shephard & Noon 1988).

2.0 MATERIALS AND METHODS

2.1 Seed Sampling

Most of the samples were collected by Mr Peter Hewett, a member of the BCPC Seed Treatment Working Party who had initiated the project. Samples were taken during continuous treatment procedures (run) and also from sacks on pallets comparable approximately to those from the runs. These were all taken at different times from different treatment plants around the country.

Further samples were obtained by Mr David Suett and myself from a local treatment plant. They were from one-tonne and half-tonne sacks on pallets. From each sack four samples, of approximately 10 g, were taken from each level; top, middle and bottom. The sack was then opened and two samples were taken from the top and two more from deeper inside. Samples were taken from the different levels using a sampling spear, which comprised a pointed tube with an oval hole near the pointed end. The spear was inserted into the sack to a predetermined distance, with the hole facing downwards: the spear was then turned through 180° (until the hole faced upwards) withdrawn slowly and the seed transferred to a sample tube. To sample the top and inside the sack a stick sampler was used, this consisted of two metal tubes with one fitting loosely inside the other. Both tubes had slots that were super-imposed by turning the inner tube. The sampler was put into the top of the sack with the slots open and seed fell into them, the slots were then closed and the sampler withdrawn (Bould 1986).

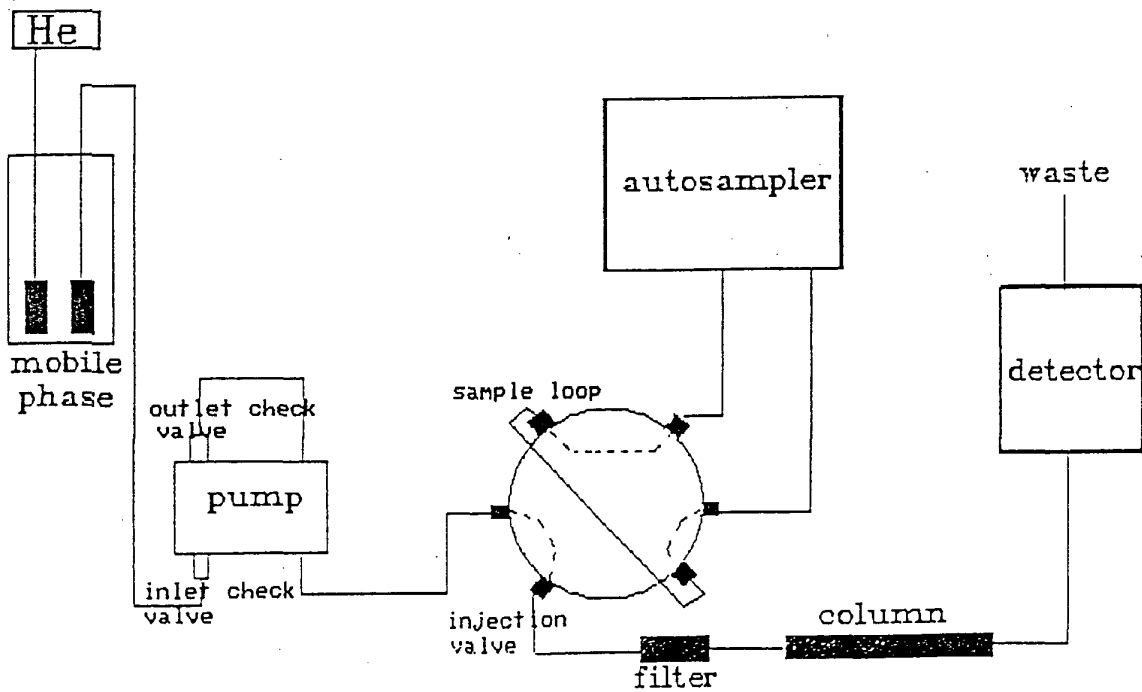
In order to study the impact of the drilling procedure on seed loadings a bulk sample was obtained. Three samples were taken initially from the top, middle and bottom of the sack. The contents were then spread evenly within the hopper of a

Ransome Nordsten seed drill and five further samples were taken at intervals along the hopper. The drill, which had twenty-five outlets, was then activated and the seed was collected in bags attached to each outlet. Samples were taken from one in five bags and analysed. From two bags twenty samples were taken, ten were analysed and the other ten were mixed together. Three samples from this mixture were taken and used to compare against the ten unmixed.

2.2 Seed Analysis

The ethirimol content on the seed was determined by reverse-phase hplc.

FIGURE 1: Flow diagram of the hplc system used



The stationary phase was hydrophobic (organic) and the mobile phase was hydrophilic (polar). The column used, a 25 cm Hypersil ODSII (Shandon Scientific Ltd), contained silica with the C18 (octadecylsilane) chain bonded to the surface.

Reverse-phase chromatography is the most common form of hplc because it is convenient, versatile and results are generally reproducible. Convenience comes from the fact that the column can equilibrate when eluent compositions are changed over a short time period. Reproducibility is achieved from the quick equilibration of the system and the stability of the column.

Many different stationary phases can be used in the system giving a broad range of compounds that can be separated efficiently.

2.2.1 Preparation of extracting solvent

2.1 g of citric acid was dissolved in 100 ml of water giving a 0.1M solution. 1.33 g of di-potassium hydrogen orthophosphate trihydrate was dissolved in 500 ml of water. 0.1M citric acid was then added dropwise to give a pH 8.0 buffer solution, using a Whatman pH meter.

The extractant, buffer:methanol:tetrahydrofuran (15:19:16), was then prepared.

2.2.2 Preparation of analytical standard

About 100 mg of ethirimol was accurately weighed into a 100 ml volumetric flask and made up to the mark with the extractant, giving an approximate 1 mg/ml ethirimol solution. This was then diluted exactly to give

100 ug/ml ethirimol standard solution, to be used for all the analyses.

2.2.3 Procedure

The samples were collected in stoppered plastic sample tubes. Each tube was weighed and the contents transferred into a 100 ml Duran bottle, the tube was re-weighed and the difference taken as the mass of seed. Any dressing left behind was considered to be of negligible mass. The tube was then rinsed with extractant and all washings transferred to the duran bottle. For samples of approximately 10 g, 50 ml of extractant was used. The samples were tumbled end over end for half an hour, then filtered through No. 1 Whatman filter paper. The extracts were diluted with extractant by a factor of five to bring them into the working range of 100 ug/ml. Each sample was then placed into a 2 ml vial with a screw top and rubber septum coated with PTFE to ensure a solvent resistant seal.

The Spectra-Physics SP8100 Liquid Chromatograph system used had built-in autosampler facilities. The mobile phase was; pH 8 phosphate buffer: methanol:acetonitrile (4:3:3) and the flow rate was 1.45 ml/min. The detector was a Cecil CE2112 Variable Wavelength UV Monitor set at a wavelength of 220 nm.

Before every sample run, two injections of standards were made to check the retention times and to ensure that the column and operating conditions had equilibrated. The samples were analysed using "bracketed" external standards, an arrangement in which a standard was followed by five samples then another standard. From this the concentrations of the samples

were determined from the standards that bracketed them. It was also a useful method for observing any changes in the chromatography, as the peak area of the standards should not vary by more than 5% throughout the run. For each run a record was kept as shown in Fig. 2.

FIGURE 2: Hplc analysis record form

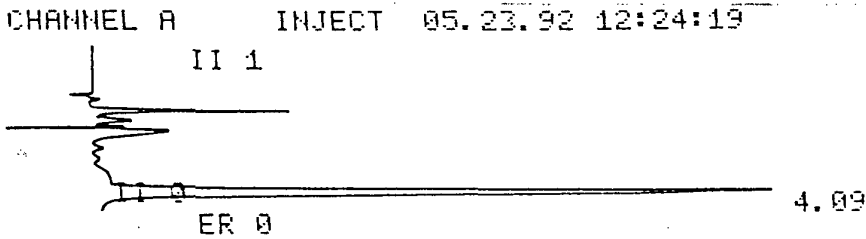
HPLC CHROMATOGRAPHIC CONDITIONS RECORD FORM

NATURE OF ASSAY : Samples BB
COMPOUND : Gonimol
DATE : 13-7-92 OPERATOR : V. Fox
SYSTEM : SP5510 pump
MOBILE PHASE : 40 % PHS phosphate buffer FLOW RATE : 1.45 ml min⁻¹
30 % methanol
30 % Acetonitrile
COLUMN TYPE : hypersil ods II LENGTH : 25 cm
PRESSURE: 2400 psi @ start; 2800 psi @ finish
DETECTOR: WAVELENGTH: 220 nm ABSORBANCE RANGE: 2
STANDARD CONCENTRATIONS: 100 ug ml⁻¹
RETENTION TIME: 4.09 mins
DATA CAPTURE: SYSTEM: SP4270 Integrator
RUN TIME: 5 mins START INTEGRATION @ 3.5 mins
COMMENTS :

Small changes to these conditions were sometimes necessary particularly to the flow rate, due to minor fluctuations of mobile phase composition.

A typical chromatogram;

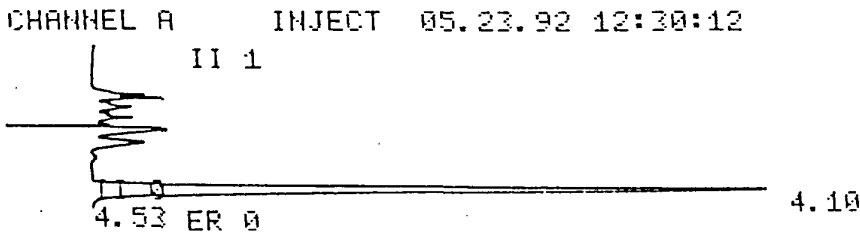
Standard;



ETHIRIMOL 05.23.92 12:24:19 CH= "A" PS= 1.

FILE	METHOD	RT	AREA BC
1.	0.	4.09	79502 01
TOTAL			79502

Sample;



ETHIRIMOL 05.23.92 12:30:12 CH= "A" PS= 1.

FILE	METHOD	RT	AREA BC
1.	0.	4.1	85911 02
2		4.53	40 03
TOTAL			85951

For each injection the integrator, a Spectra-Physics SP4270, reported the retention time (the time between injection and elution) of each peak and its relative area. From this it was possible to calculate the concentration of ethirimol in each sample;

$$\text{Conc sample} = \frac{\text{Conc standard} \times \text{Area sample}}{\text{Area standard}}$$

This gives the concentration in the diluted sample as ug/ml.

This was then converted into mg/kg;

$$\text{Conc mg/kg} = \text{conc sample} \times k \times \frac{V}{M}$$

where k = dilution factor

V = Volume of extracting solvent used

M = Mass of seed

The commercially recommended application rate of "Ferrax" is 500 ml/tonne of seed giving a label application rate for ethirimol of 2000 mg/kg.

Fresh analytical working standard solutions were prepared at four-week intervals and, before use, were compared with the standard solutions in current use. No significant differences were found between consecutive sets of standard solutions, which were always stored at <5°C.

2.3 Seed to Seed Variability

Two sets of twenty five single seeds from 1 kg quality control samples were analysed individually for their mass and loading. Each seed was weighed and placed in a vial. 1 ml of extracting solvent was added and then left in the dark at room temperature overnight.

2.4 Retention Test

A 220 g sample of seeds from a 1 kg quality control sample was used for this test, which followed the procedure of Jeffs (1986). Two initial 10 g samples were taken. The seed was then dropped down a 40 cm long perspex tube, fitted with a funnel on the top to prevent seed hitting the sides. The tube was fixed above a metal sieve so that when the seed was dropped the dressing being lost passed through it. The seed was dropped one hundred times in total with a 10 g sample taken every ten drops. The samples were then analysed as before.

3.0 RESULTS AND DISCUSSION

The resulting data have been presented graphically. Each graph shows samples taken during the run as a solid line and those comparable from pallets as a broken line. The label application rate is also shown. Where duplicate samples were taken ie two at a time, side by side, only one has been chosen by the toss of a coin. The treatment process itself is variable and so it was decided that the two duplicate samples needed to be considered individually and so the mean of the pair could not be used. For each batch the distribution of results is shown as well as the difference in loading between successive samples, in an effort to show any variation during short periods. For each sampling, sack number one was the first sack sampled but was not necessarily the first sack treated.

3.0.1 Sampling from Ely, Fig. 3, 3A

This was the largest batch of samples analysed. A very steady application rate was achieved with 96% of the samples having a loading of $\pm 10\%$ of the mean, 1720 mg/kg. However, none of the samples achieved the label application rate. The pallet samples compared favourably with those from the run.

3.0.2 Sampling BB, Fig. 4, 4A

This was the smallest batch of samples analysed. Results indicated a consistently uniform application rate, with 97% of the samples containing $\pm 10\%$ of the mean 2040 mg/kg loading. Most of the samples, 81%, were above the 2000 mg/kg label application rate. The pallet samples compare well

with those from the run. Differences between successive samples were generally small.

3.0.3 Sampling CC, Fig. 5, 5A

This batch contained a large number of samples from the run but relatively few comparable from pallets. Treatment again seemed uniform with 91% of the samples containing $\pm 10\%$ of the mean 1630 mg/kg. However, all the samples were below the label application rate. The pallet samples compared well but only covered the first half of the sampled run.

3.0.4 Sampling DD, Fig. 6, 6A

A small batch of samples, 85% containing $\pm 10\%$ of the mean 1930 mg/kg loading. 29% reached or exceeded the label application rate. The pallet samples generally had a lower loading. Differences between successive samples were generally small.

3.0.5 Sampling EE, Fig. 7, 7A

Samples were not taken from pallets. Only 83% of the run samples contained $\pm 10\%$ of the mean 1770 mg/kg. 6% reached or exceeded the label application rate.

3.0.6 Sampling One, Fig. 8, 8A

Samples were not taken from pallets. From the run, 82% of the samples contained $\pm 10\%$ of the mean 1770 mg/kg. Only 3% contained more

than the label application rate.

3.0.7 Sampling Two, Fig. 9, 9A

A large batch of samples from the run, but none from pallets. From the run, 90% contained $\pm 10\%$ of the mean 1950 mg/kg loading, with 28% of the samples achieving the label application rate or above.

The above results are summarised in Table 1.

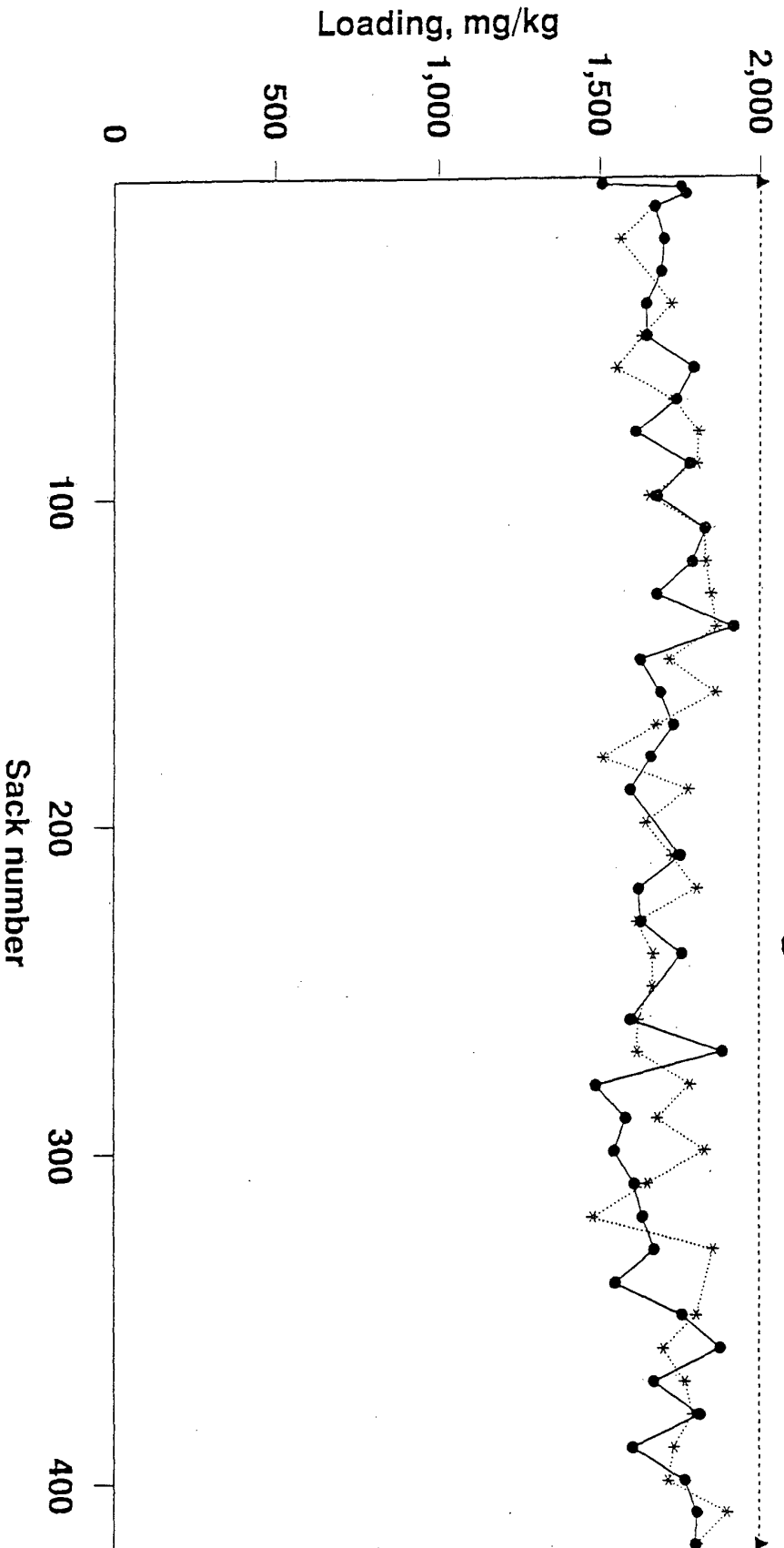
In all the samplings the differences between successive samples were generally low, showing that there was little or no variation between samples over a short period.

Table 1. Proportion of samples containing $\pm 5\%$ and $\pm 10\%$ of the mean dose of ethirimol in each sampling.

Sampling	% of results $\pm 5\%$ of mean	% of results $\pm 10\%$ of mean
Ely	63	96
BB	57	97
CC	69	91
DD	68	85
EE	48	83
One	68	82
Two	66	90

Sampling from Ely

Ethirimol loading

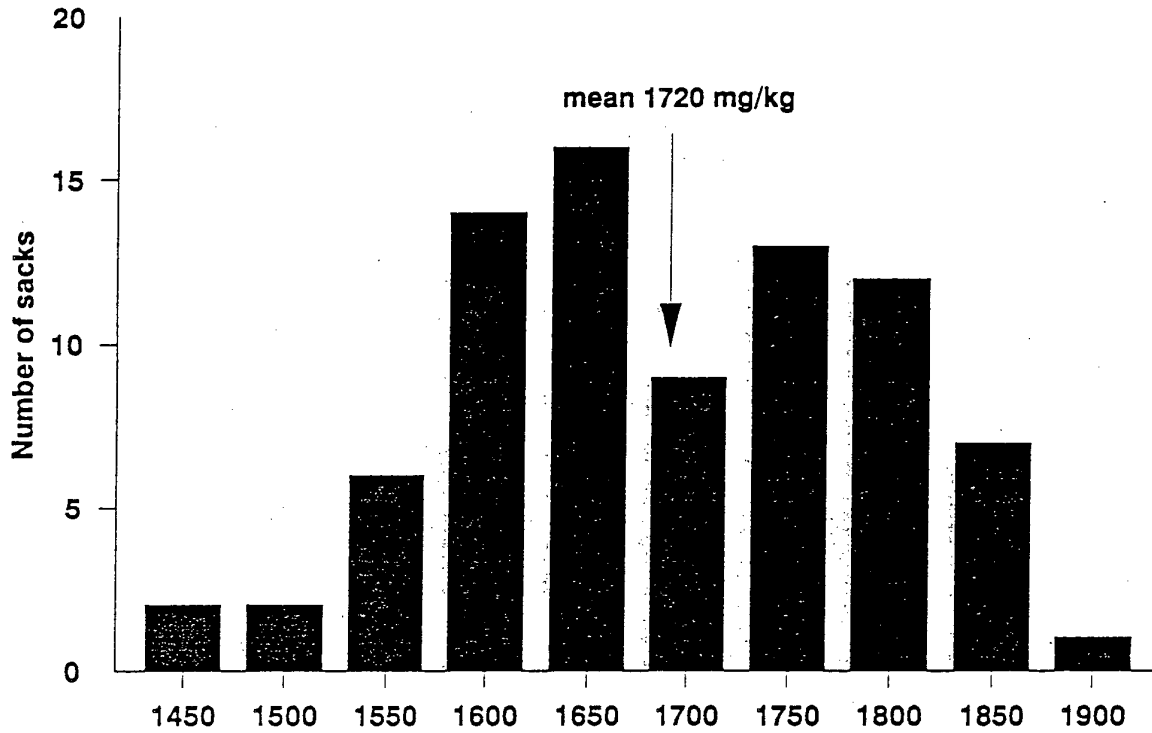


Label application rate Samples taken during treatment Samples from pallets

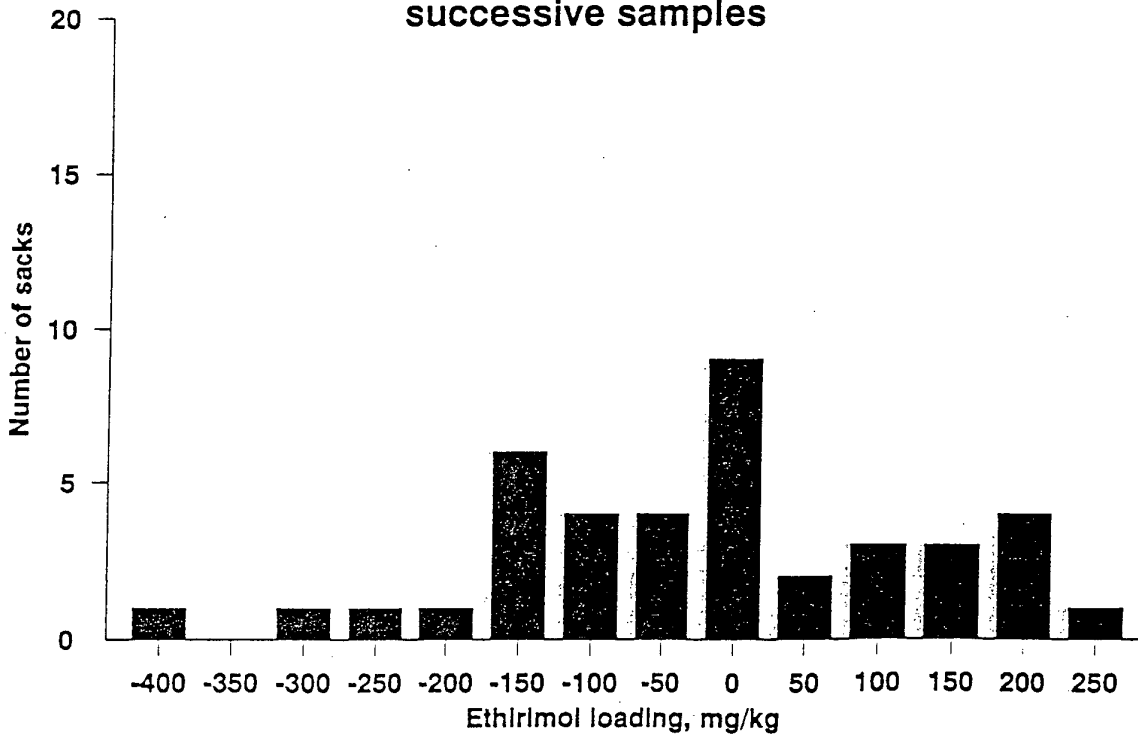
TT
Figure 3

Sampling from Ely

Distribution of results



Distribution of differences between successive samples



Sampling BB

Ethirimol loading

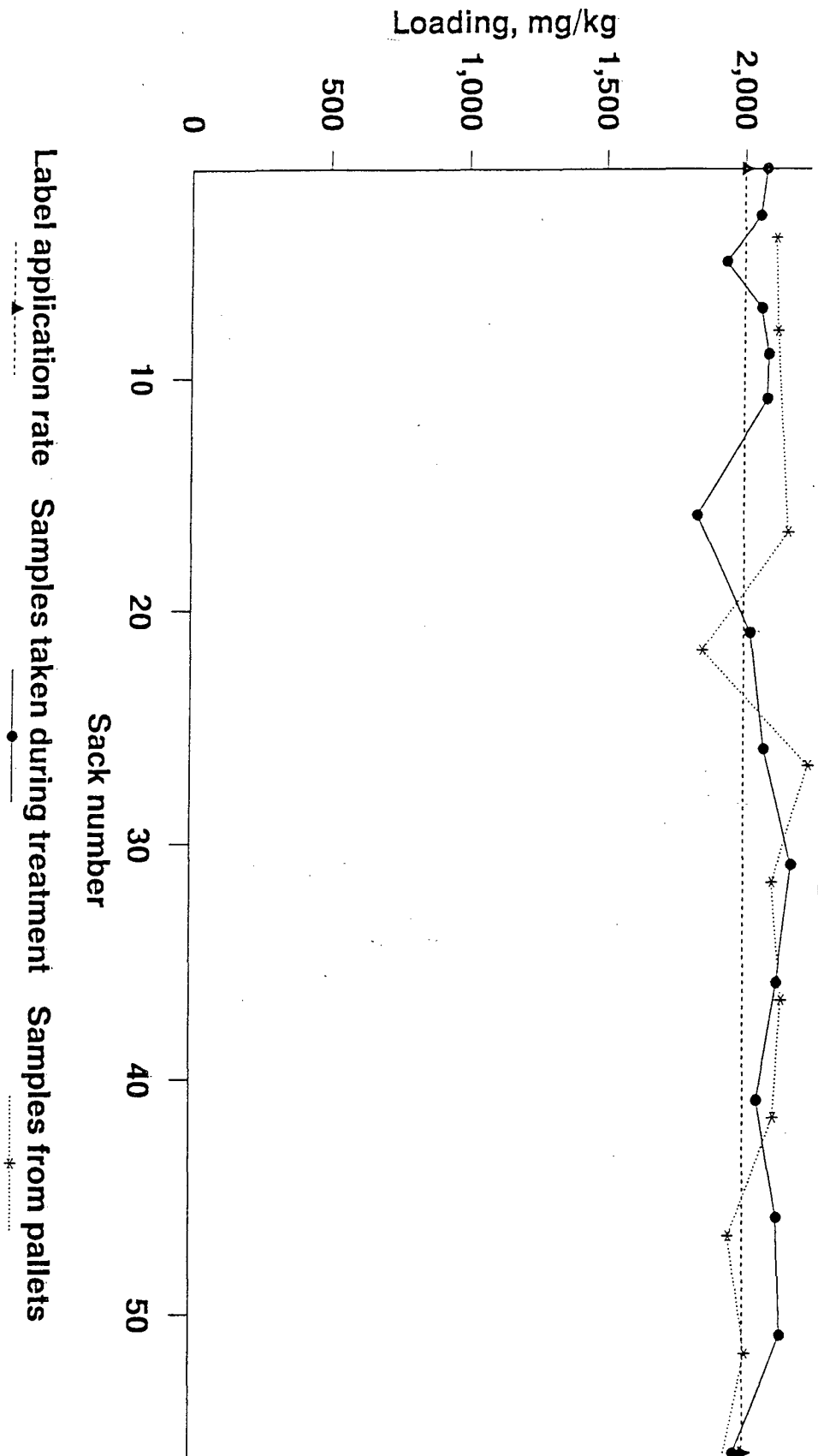
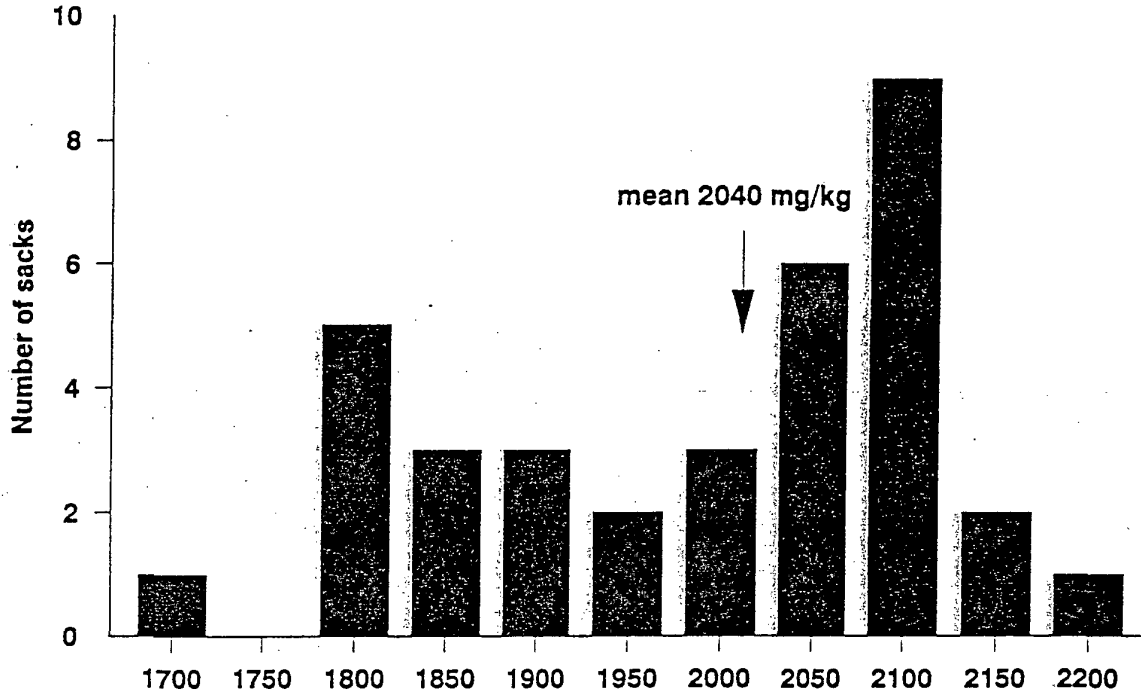


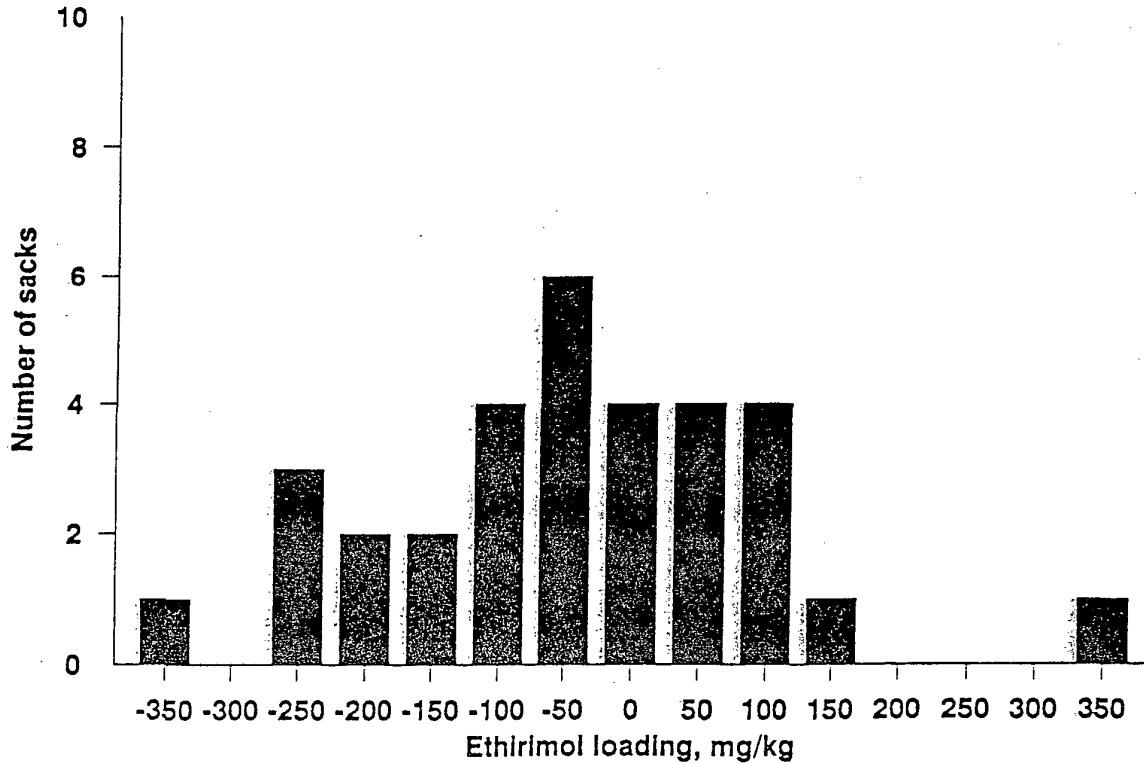
Figure 4

Sampling BB

Distribution of results



Distribution of differences between successive samples



Sampling CC Ethirimol loading

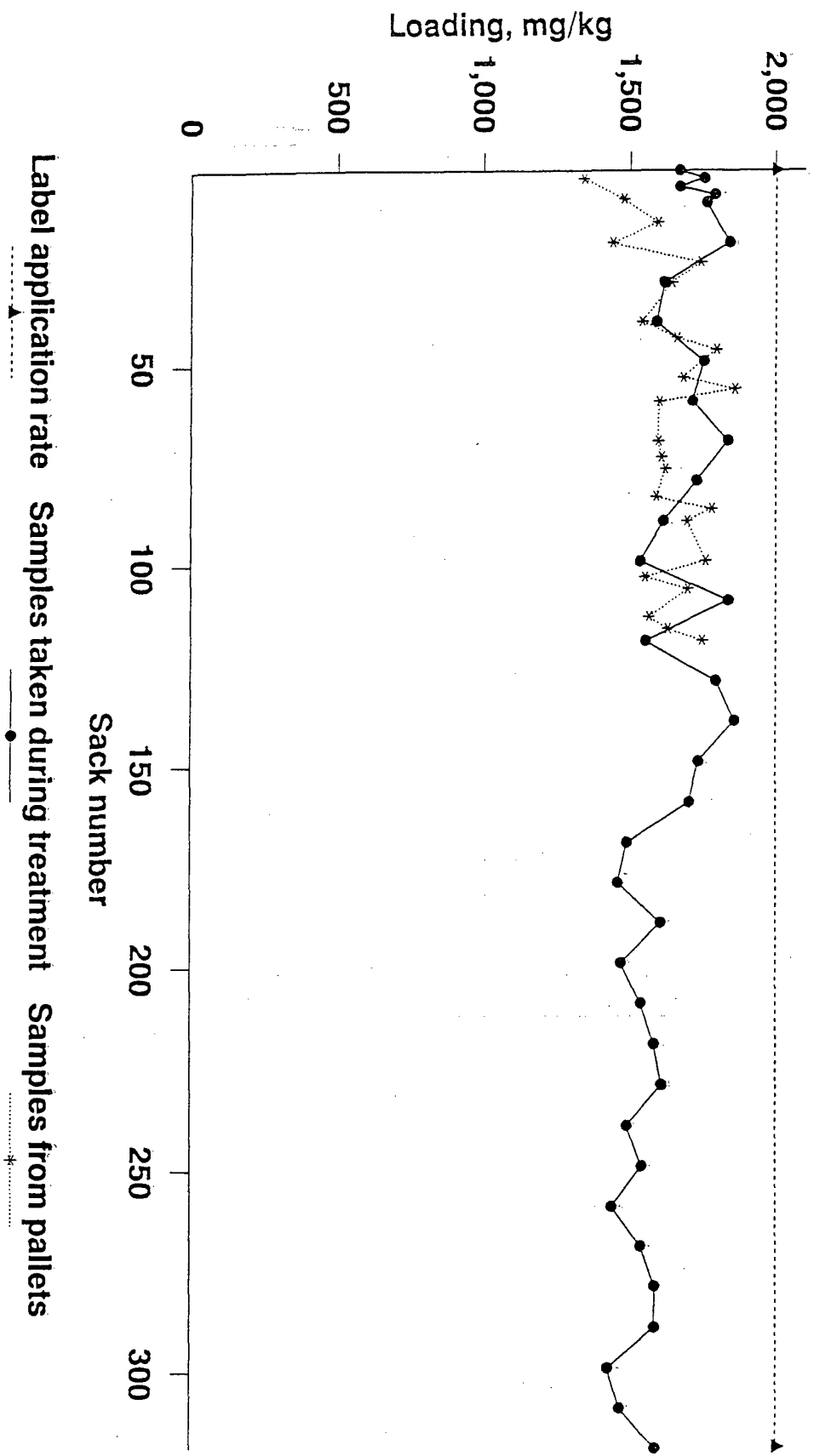
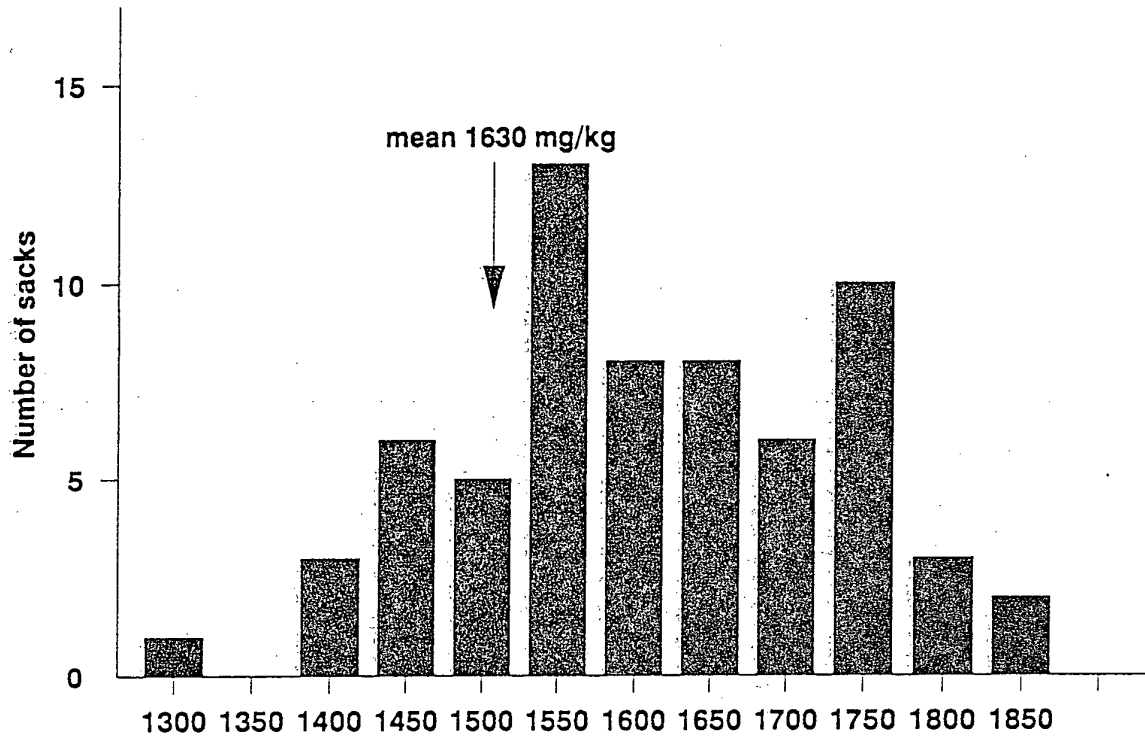


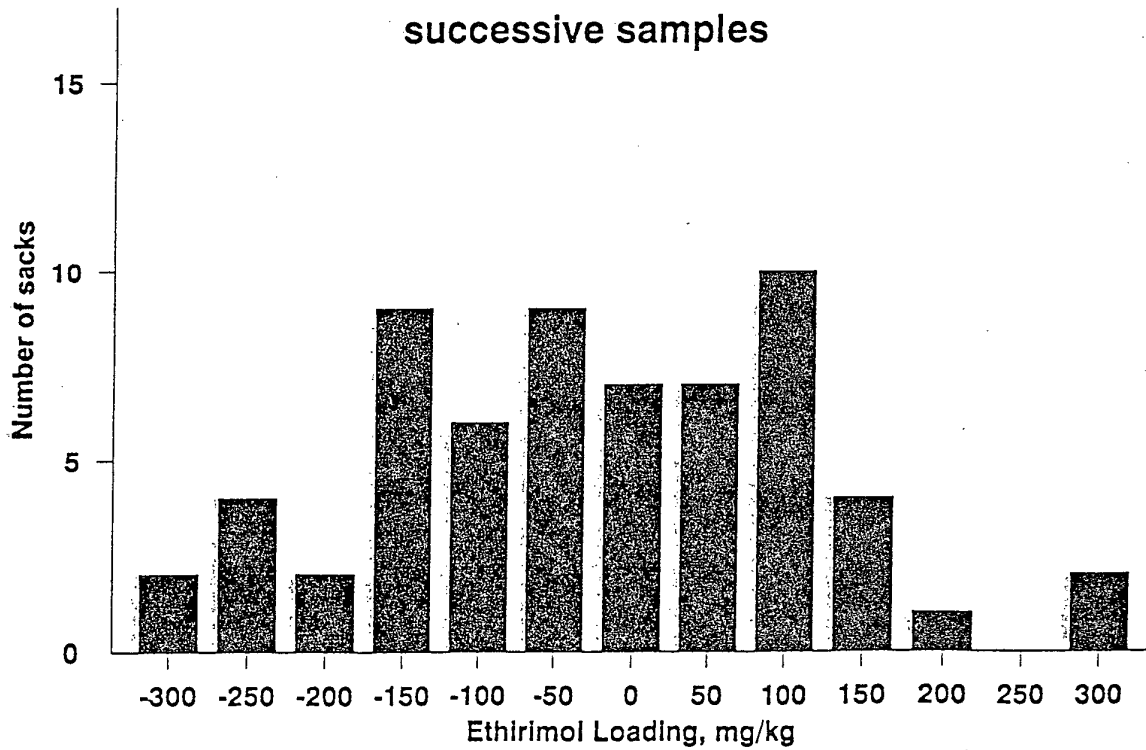
Figure 51

Sampling CC

Distribution of results



Distribution of differences between successive samples



Sampling DD Ethirimol loading

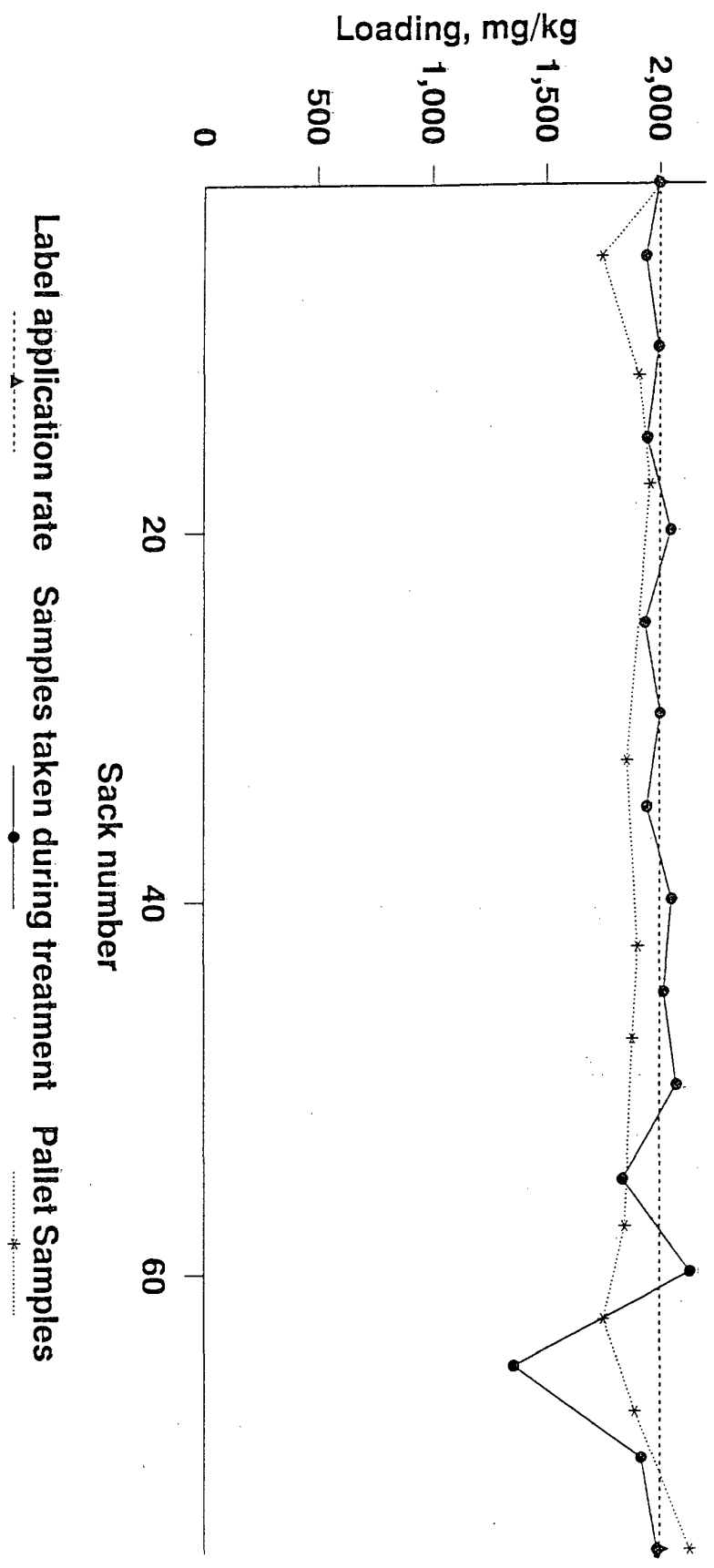
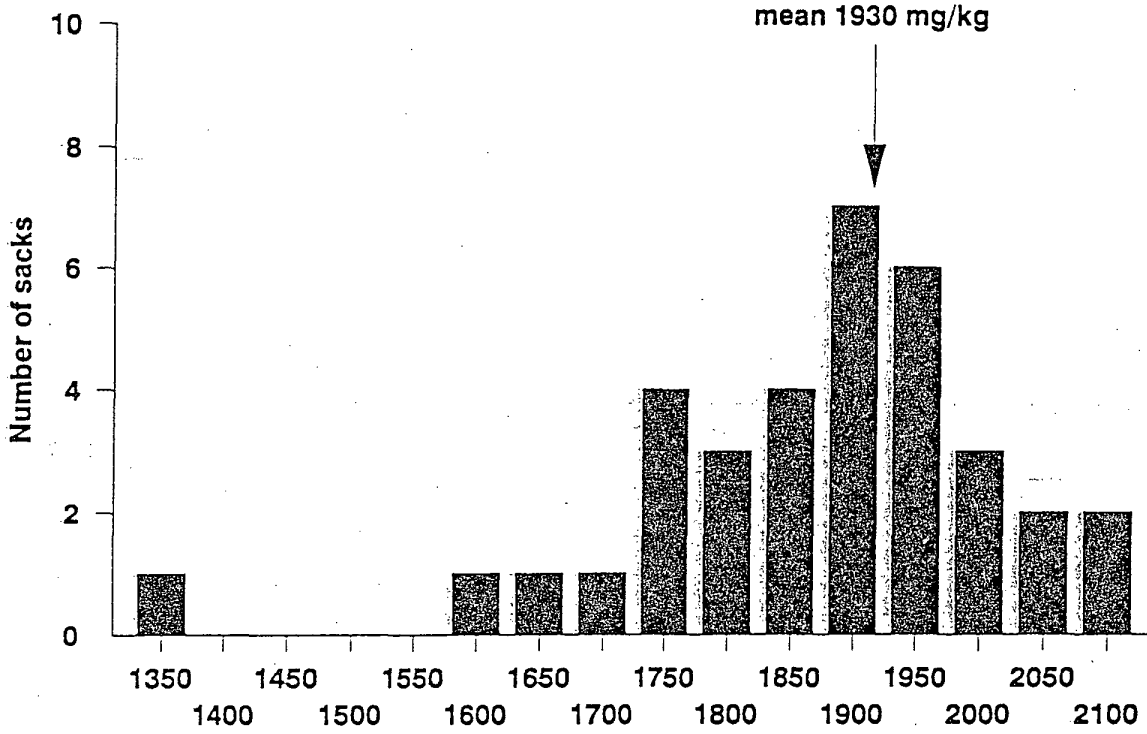


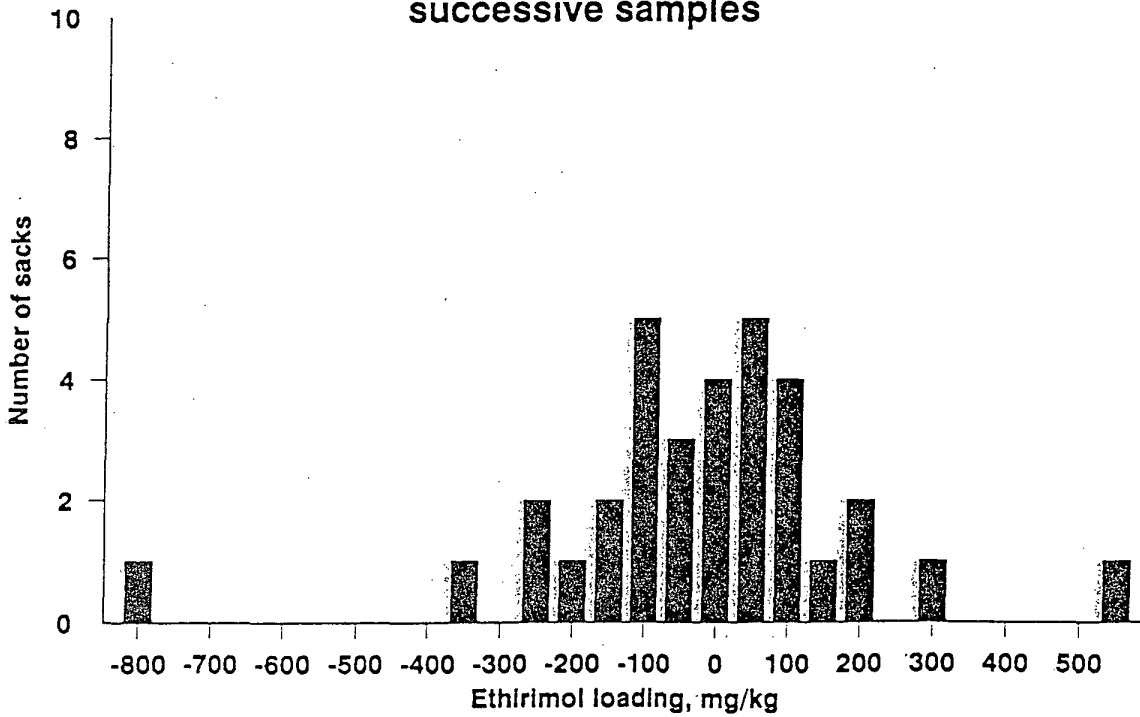
Figure 6

Sampling DD

Distribution of results



Distribution of differences between successive samples



Sampling EE Ethirimol loading

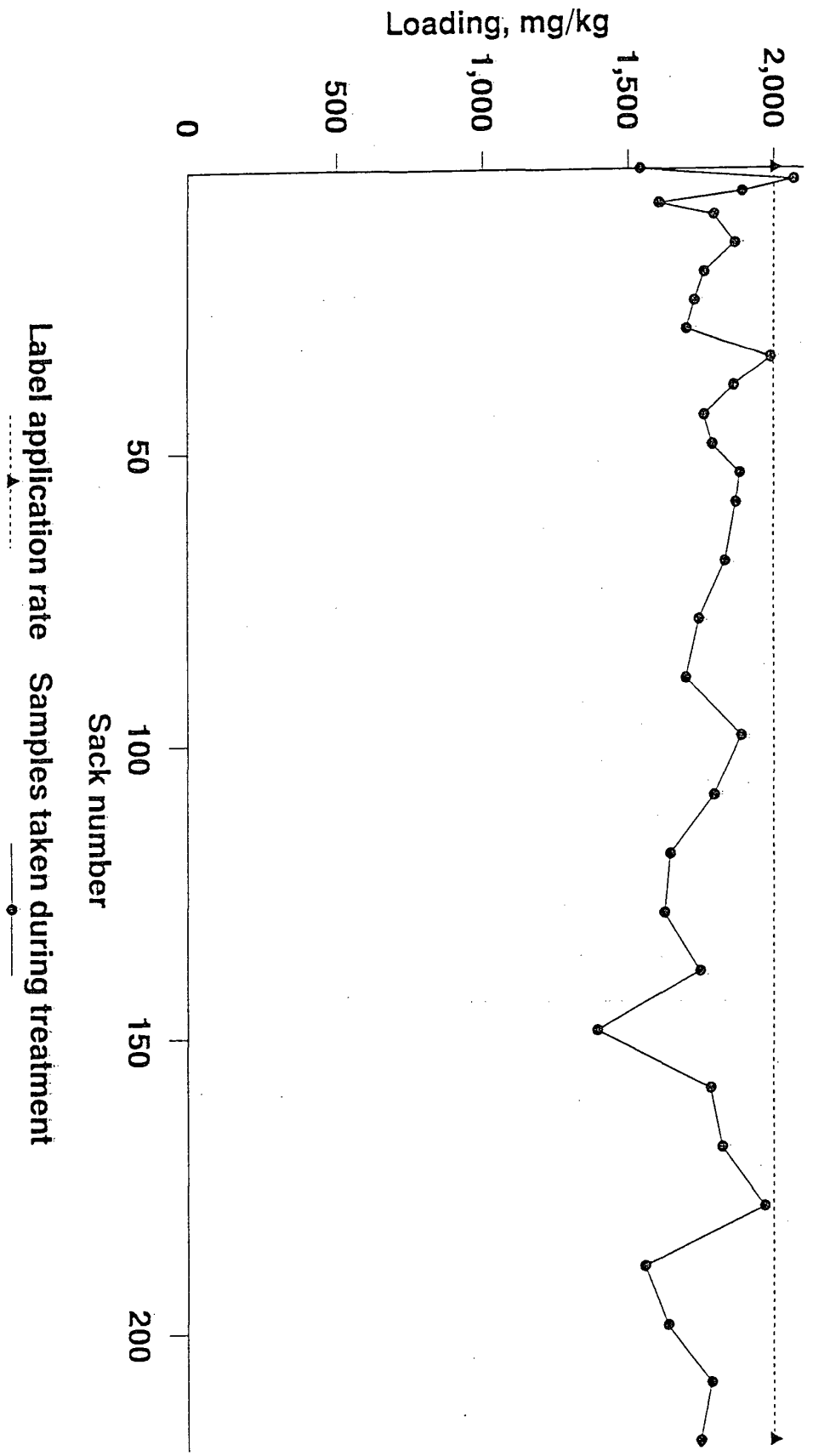
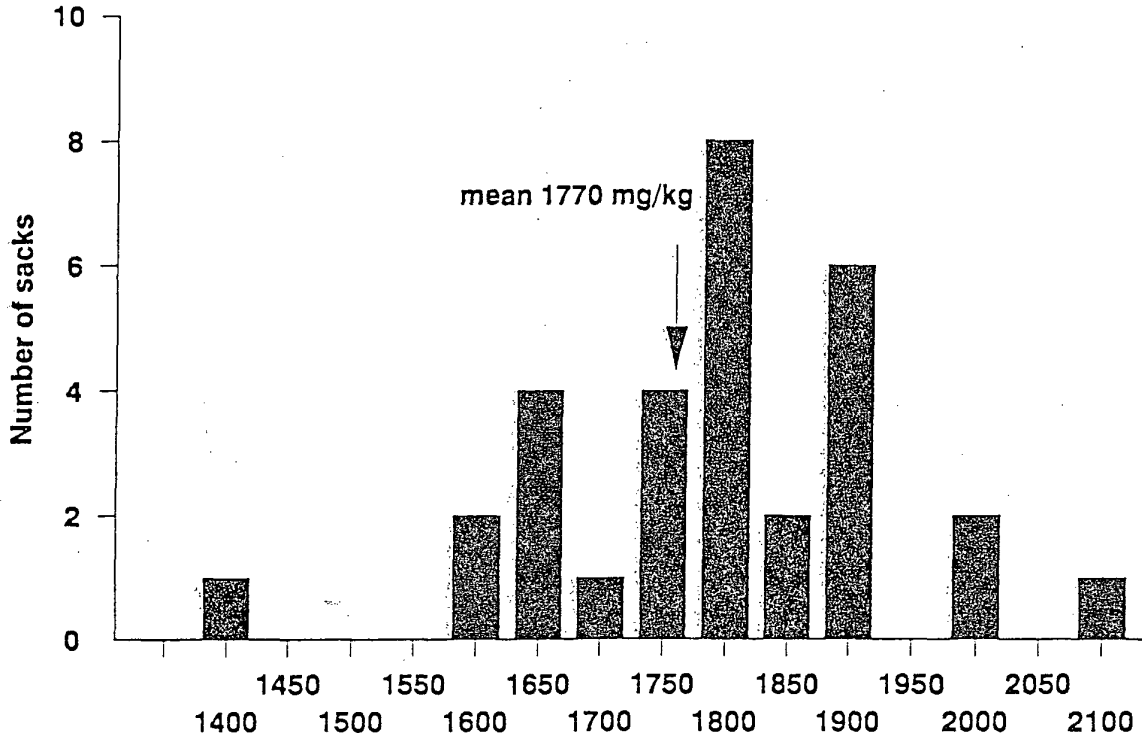


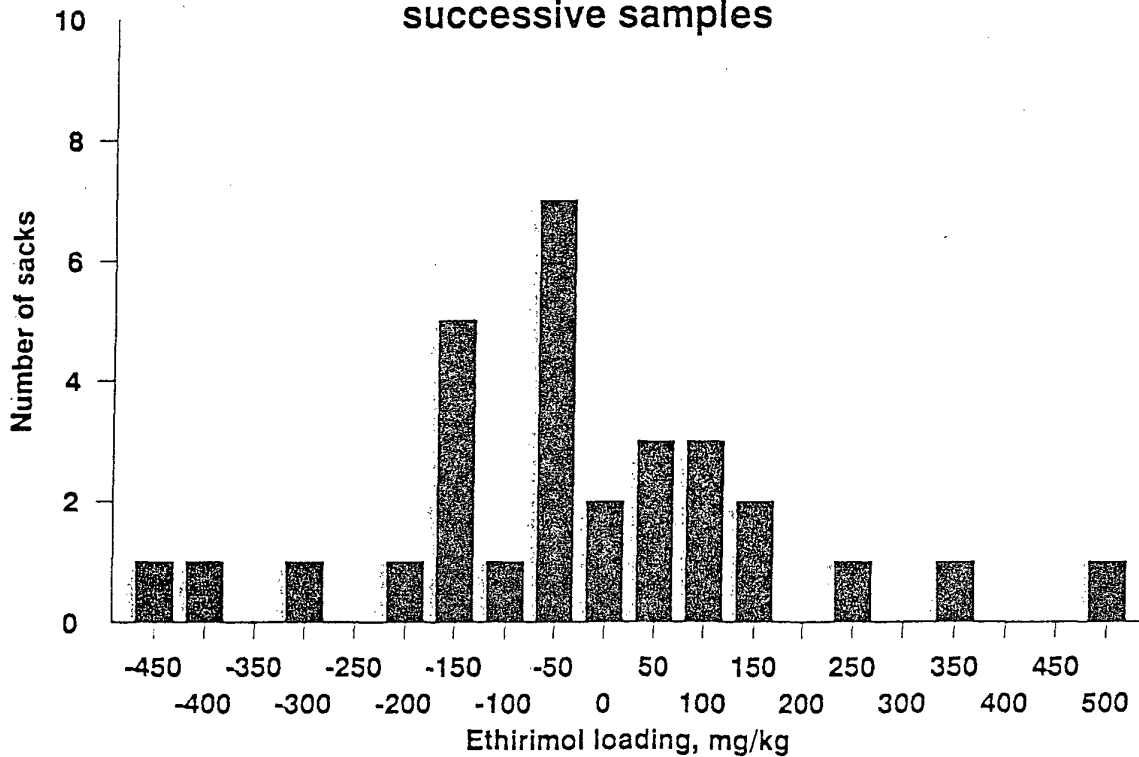
Figure 7

Sampling EE

Distribution of results



Distribution of differences between successive samples



Sampling One

Ethirimol loading

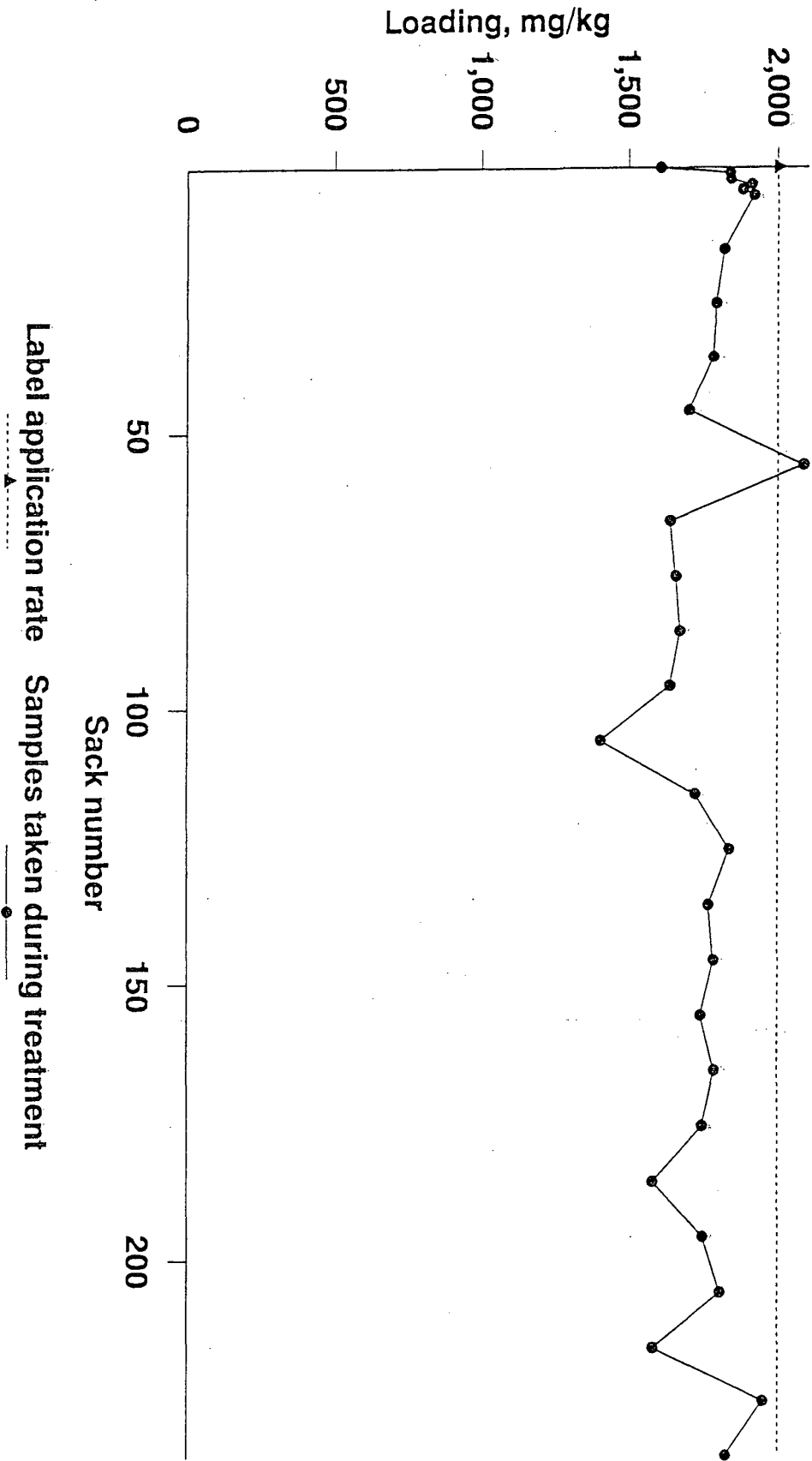
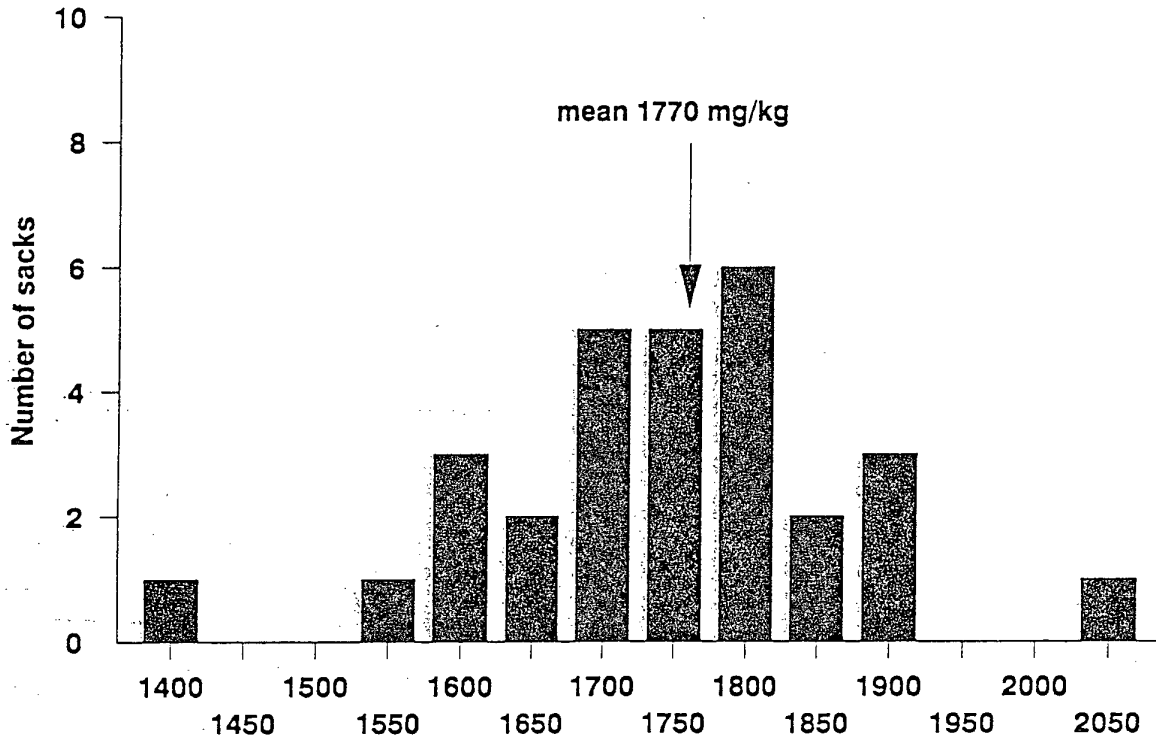


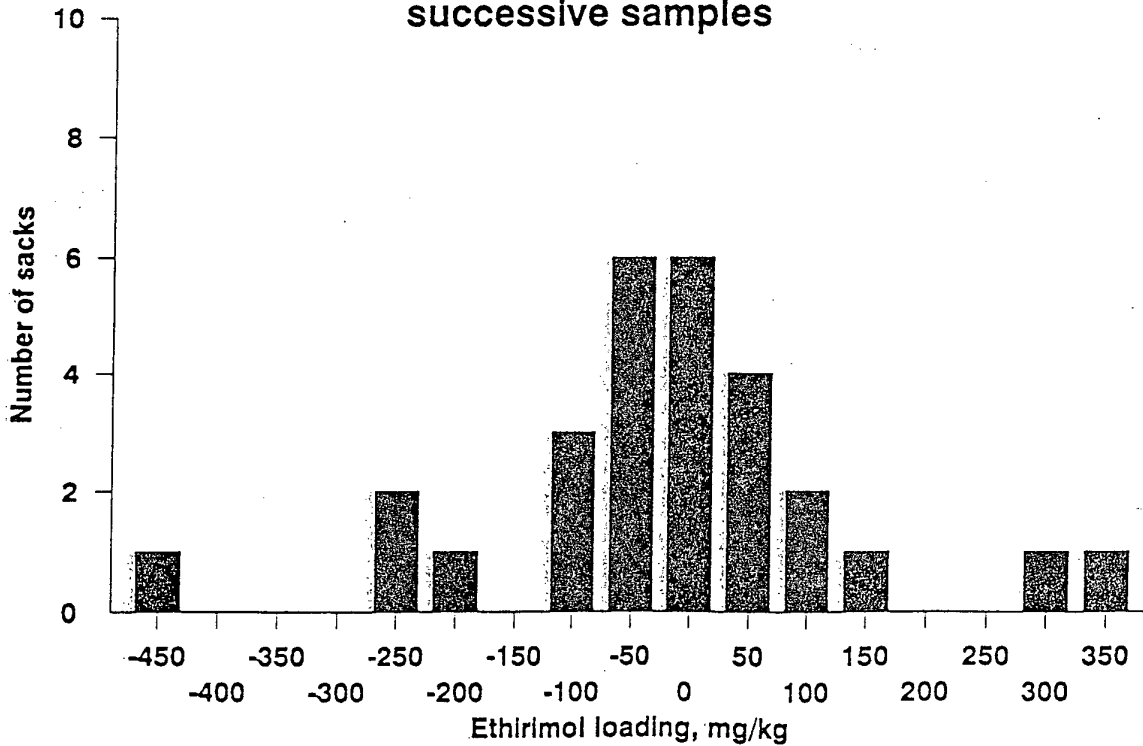
Figure 8

Sampling One

Distribution of results



Distribution of differences between successive samples



Sampling Two

Ethirimol loading

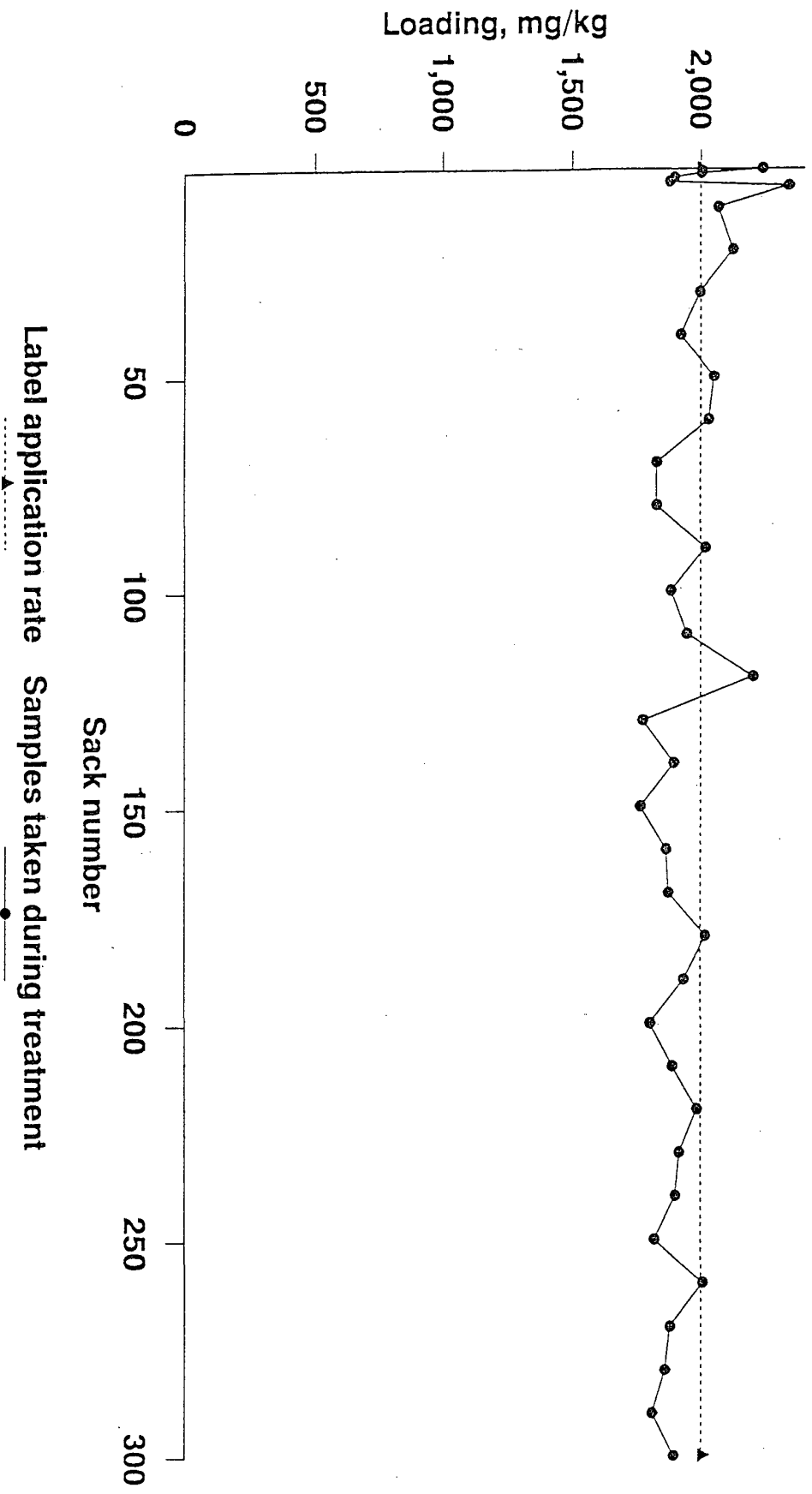
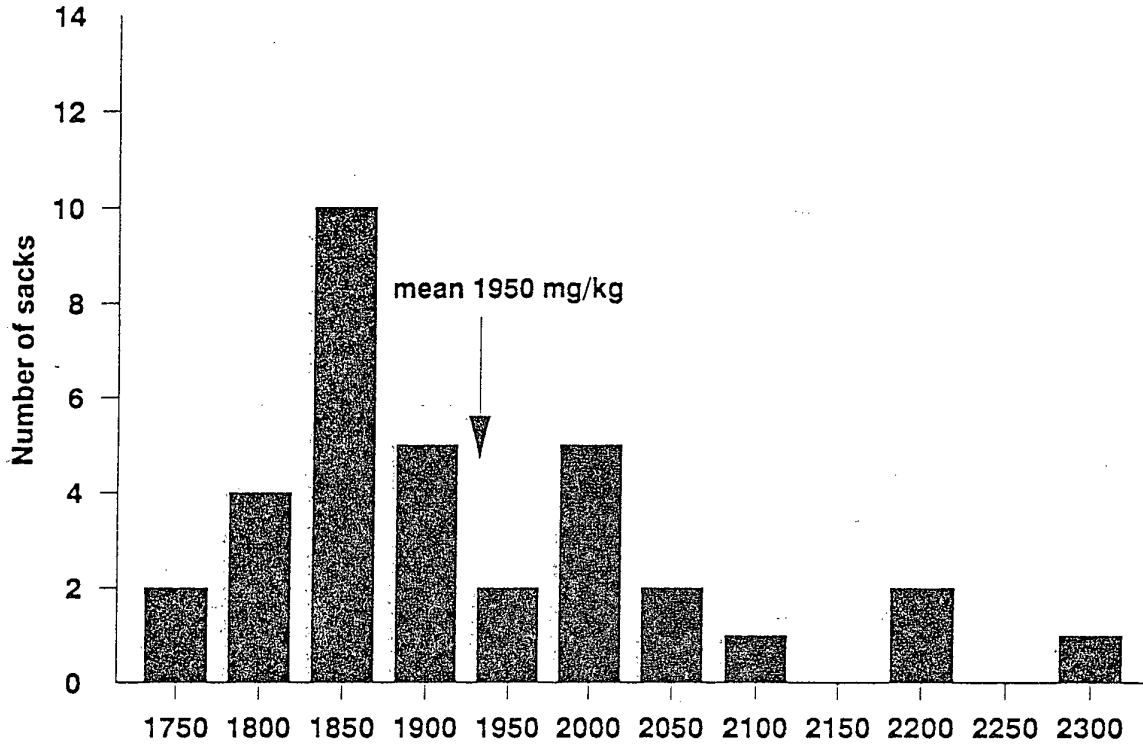


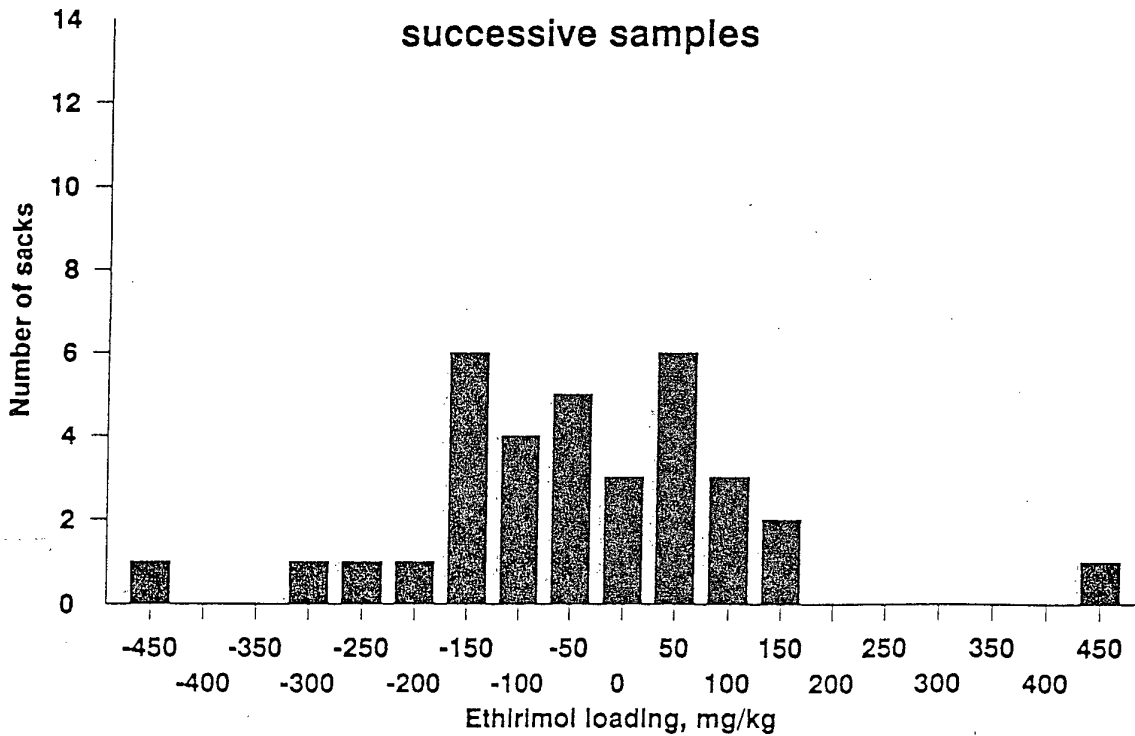
Figure 9

Sampling Two

Distribution of results



Distribution of differences between successive samples



3.0.8 Samples from one-tonne and half-tonne sacks

Table 2 shows the mean loading of ethirimol mg/kg of the four replicate samples taken at each level.

Table 2. Mean ethirimol loadings (mg/kg) in different parts of one-tonne and half-tonne sacks

Sack	Top mean CV%	Middle mean CV%	Bottom mean CV%	Upper mean CV%	Lower mean CV%	Overall mean CV%
1	2380 7.8	2270 6.7	2140 5.9	2170 1.9	1970 3.6	2210 8.2
2	1940 8.7	1780 6.7	1920 3.4	2100 6.4	1980 0.7	1920 7.4
3	2060 4.5	2050 2.7	1950 7.2	2090 3.1	1900 11	2010 5.9
4	1980 6.4	1960 4.3	1810 12	1840 3.8	1920 0.3	1900 7.4
5	1930 4.5	1900 5.3	1840 5.7	2050 0.6	1970 1.7	1920 5.3
6	2070 7.4	1960 9.8	1850 5.3	1910 0.7	1940 7.8	1950 8.9
7	1930 10	1950 8.7	1940 15	1920 6.6	1740 6.5	1910 10.0
8	1990 7.3	1830 6.2	1880 2.8	1810 9.0	1790 3.9	1870 6.5
9	2070 10	1810 7.6	2100 4.5	1700 3.7	1600 2.9	1910 12.1
10	2000 2.5	1910 4.7	1850 5.0	2070 4.7	1920 2.8	1940 5.4
11	1120 12	1100 12	1090 4.1			1100 9.5
12	1110 2.8	1110 16	1080 10			1100 10.4
13	1180 10	1140 5.1	1000 5.4			1110 8.9
14	1240 8.9	1150 12	1010 9.5			1100 12.4
15	1400 5.6	1320 11	1370 6.9			1360 7.9
16	1050 8.8	1000 8.6	1070 7.6			1040 8.2
17	1170 16	1080 6.2	1070 12			1100 11.9
18	1630 8.8	1870 34	1450 5.6			1650 23.9

Examination of the data in Table 2 and ranking each level of the sacks in order of loading, largest to smallest, suggested that samples from the top appeared to have the largest loading and the bottom the smallest. An analysis

of variance was carried out on the data by the statistics department and it was concluded that when sack eighteen was ignored it could be said that the loading at the top was higher than at the bottom. If sack eighteen was included in the analysis no differences could be concluded.

3.2 Seed to seed Variability

Table 3 shows the loading (mg/kg) and mass (mg) for each seed from the two samples.

From Table 3 of it can be seen that both the loading and mass between seeds was very variable, but the loading more so. Therefore it would seem that the weight of individual seed was not the cause for variability in loading between samples from the same batch.

Table 3. Ethirimol loadings (mg/kg) and seed weight of 25 individual seeds from two samples

	Sample 1		Sample 2	
	mg/kg	mg	mg/kg	mg
1	1950	46.6	2660	60.9
2	1740	36.5	2390	42.1
3	1930	50.6	1680	56.2
4	2790	59.8	2410	51.6
5	2160	61.2	2020	53.1
6	2290	56.2	5950	39.6
7	2350	54.9	2550	37.7
8	1550	57.7	3030	56.1
9	1970	62.1	1080	40.9
10	2010	55.1	2900	41.1
11	3790	46.9	2130	40.4
12	3000	28.3	2240	44.2
13	1480	53.9	1640	58.9
14	1980	50.9	3220	33.7
15	2080	51.1	2870	50.4
16	3210	40.7	1910	37.4
17	2360	45.4	1900	24.8
18	1100	62.9	2120	53.9
19	1520	45.1	4390	32.1
20	2180	62.9	2140	37.4
21	1840	45.1	2950	34.3
22	1530	38.1	1800	41.6
23	1970	60.9	1860	47.9
24	1490	42.1	1960	39.3
25	1830	67.4	2040	37.9
Mean	2080	51.3	2470	43.7
CV%	28.7	18.9	39.6	21.1

3.3 Loss of ethirimol during drilling

After this test a lot of the powdery pink dressing could be seen along the hopper and the drill. Table 4 shows the results of this test with loading of ethirimol as mg/kg.

Table 4. Changes in seed loadings (mg ethirimol/kg) during passage through a commercial drill unit

Sack		Hopper		Drill Outlet	
Position	mg/kg	Position	mg/kg	Position	mg/kg
Top 1	2038	1	1778	1	1874
2	1825	2	1913	5	1776
3	1903	3	1976	10	1820
Middle 1	2004	4	1967	15	1765
2	1846	5	1803	20	1765
3	1913			25	1781
Bottom 1	2008				
2	2063				
3	1921				
Mean	1946	Mean	1887	Mean	1796

From the results shown in Table 4, there would seem to have been a loss of fungicide during the passage of seed through the drill. A t-test was used to determine if there was any significant differences between the sack, hopper and drill outlets.

At the 95% confidence level there was;

No significant difference between the sack and the hopper,

A significant difference between the sack, hopper and the drill outlet,

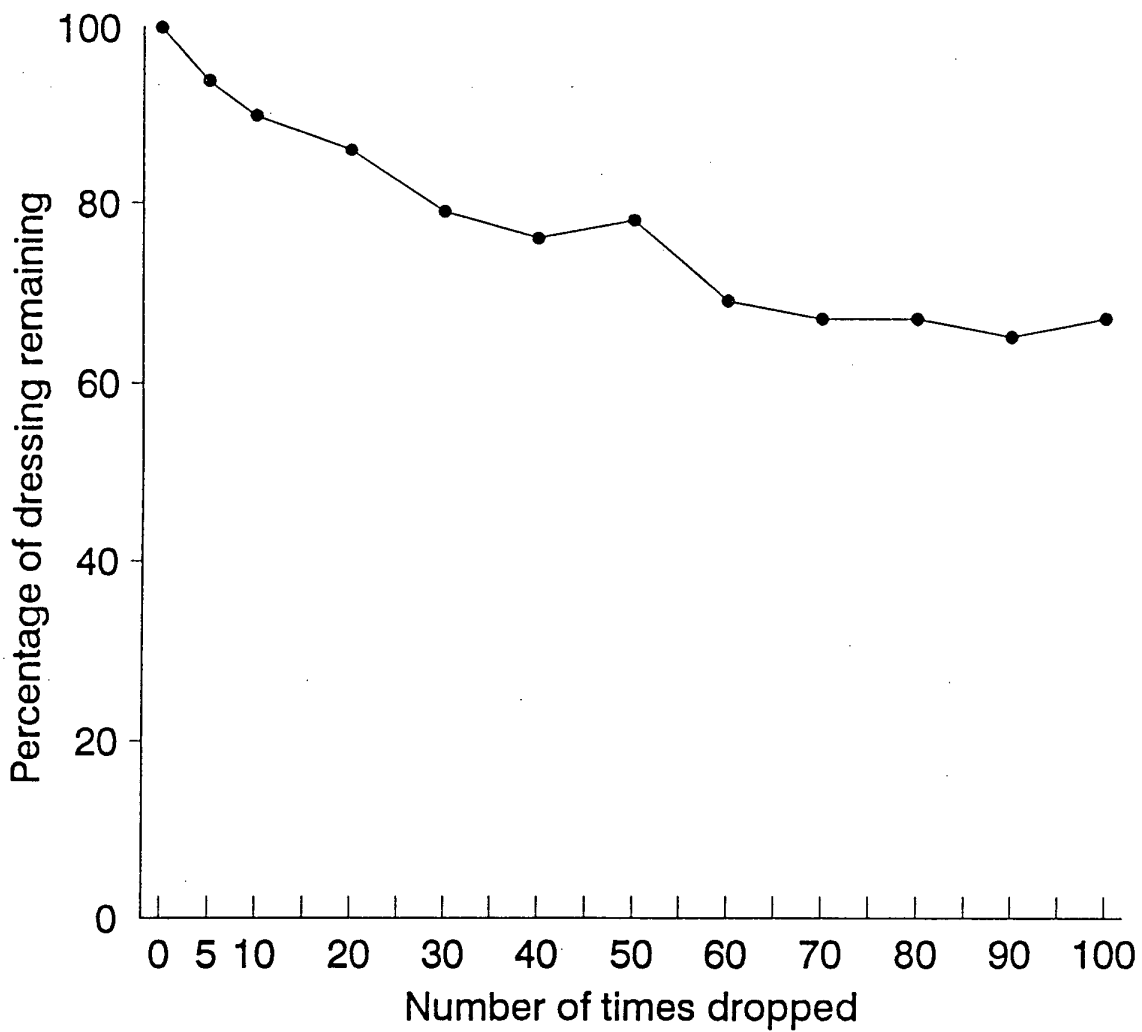
A significant difference between the sack and the drill outlets,

No significant difference between the hopper and the drill outlet.

3.4 Retention Test

Fig. 10 shows the results of the retention test as a graph of percentage of treatment remaining on the seed against the number of times dropped.

FIGURE 10: Proportion of treatment remaining on seeds during drop test



After approximately sixty drops the seed had lost over 30% of the treatment and appeared to lose no more. The sample dropped did have a high loading initially (2170 mg/kg) compared with other samples considered in previous analyses. Further work on a lower-loaded sample would need to be undertaken to see if the same trend was followed.

In practice the seed is handled a lot, during transportation, storage, etc, but not nearly as much as being dropped sixty times. It may therefore be assumed that more than 65% should still be remaining following delivery to the customer, storage and drilling. This instability may be relatively unimportant when the mean loading is high but could become increasingly significant as doses decline.

4.0 SUMMARY OF RESULTS

1. There was, in general, close agreement between samples taken during a run and the equivalent post-run samples taken from sacks on pallets. It should not therefore be necessary to sample both of these sources in order to assess treatment quality.
2. Only one sampling (BB, mean dose 2040 mg/kg seed) produced a mean dose greater than the label application rate of 2000 mg ethirimol/kg. Mean doses of other samplings ranged from 1630 to 1950 mg/kg.
3. In every sampling, at least 80% (82 - 97%) of samples contained $\pm 10\%$ of the respective mean dose. The corresponding proportions of samples containing $\pm 5\%$ of the mean dose ranged from 48 - 69%.
4. Loadings on individual seeds range from doses equivalent to 54 - 297% of the label application rate.
5. Significant proportions of loadings were lost from treated seeds following transfer to, and passage through, a seed drill.

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