THE PREVALENCE AND CONTROL OF THE SHEEP HEADFLY, HYDROTAEA IRRITANS

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Summary A survey has shown that during the last five years headfly, Hydrotaea irritans, has become a serious pest of sheep in South-West Scotland. Attacks occur mainly on horned breeds of sheep during July and August and are particularly severe on those farms situated close to forests or woodland.

In field trials crotoxyphos gave some protection against headfly. This compound proved to be more effective in a cream containing tar than when used as a spray. The new insecticide 1, 1 - bis (p - ethoxyphenyl) - 2 - nitropropane provided a significant measure of headfly control for the whole of the test period in one of three trials carried out with this compound.

Résumé Une inspection a montré que pendent les cinq derniers ans Hydrotaea irritans a devenu un insecte nuisible des moutons au sud-ouest d'Écosse. Les moutons cornés sont les victimes principales des attaques, quelles ont lieu pendant Juillet et Aôut et sont exceptionnellement grave si la ferme est à proximite d'une forêt où d'un bois.

Aux essais de champ crotoxyphos offrit quelque protection contre H. irritans. Ce composé s'est révélé de faire de l'effet meilleur comme une crème avec gourdon que comme un embrun. Le nouvel insecticide 1, 1, bis (p - ethoxyphenyl) - 2 - nitropropane munit une mesure importante de pouvoir sur les mouches pendant toute la durée de l'épreuve à un de trois essais effectué avec ce produit chimique.

INTRODUCTION

The headfly, Hydrotaea irritans is widely distributed throughout Britain and Europe. During the last five years this fly has been shown to be responsible for the phenomenon of broken heads on certain sheep breeds in the border areas of Scotland and the counties of Morthumberland and Durham (Hunter, 1972; Tarry, 1973; Titchener et al, 1974).

The object of the present investigation was to determine the prevalence and severity of headfly attacks in the West College area and to aid in the development of possible control measures.

METHOD AND MATERIALS

Survey

To determine the prevalence and severity of headfly attacks a survey was carried out in South-West Scotland during the period 1972 to 1974. An even sample distribution was obtained by sampling at least four farms in each 10 km ordnance survey grid square of the area.

Chemical Trials

Trials were conducted on commercial Blackface flocks with a severe headfly problem. Ewe and wether lambs were used for trial work. Whilst attacks were more frequent and severe on tup lambs these could not be used in trials since they were not present in sufficient numbers for valid statistical comparisons. Ewes were included in one trial as they had been particularly seriously affected by headfly in previous years.

Treatments used were :

Flymort 24 (Tuco Chemical Company). A 1% crotoxyphos spray used over the head and body at approximately 2 week intervals.

Headfly repellent dark quality (Robert Young & Co.). A cream containing 0.05% crotoxyphos and pine tar oil. Applications were made at approximately 2 week intervals around the base of the horns.

"C" fluid. The headfly repellent with an increased crotoxyphos content of 0.5%.

GH74 (Wellcome Research Laboratories). The new insecticide 1, 1 bis (p - ethoxyphenyl) - 2 - nitropropane used as a 0.125% dip. Two dippings were carried out with a 4 week interval between them.

Treatments started during the last week in June/first week in July before any broken heads had occurred in the flocks. Trials were then continued until the 3rd week of August. Visual assessment was made of the number and severity of headfly lesions. Statistical analysis of the trial results was by means of the X² test.

RESULTS

Survey

A total of 600 farms were sampled in the survey. Sheep headfly was considered to be a severe problem on 10.9%, a moderate problem on 17.5% and a slight problem on 33.8%. On 37.8% of farms there had been no headfly attacks. The number and severity of headfly attacks showed considerable variation in different shires (Table 1).

Kirkcudbrightshire was the most seriously affected by headfly. Headfly was also a problem in Dumfriesshire and Lanarkshire as well as in some parts of Ayrshire and Wigtownshire. Whilst most headfly attacks occurred on Blackface and other horned breeds some attacks occurred in Border Leicester, Cheviot and Suffolk flocks. Farms situated close to woodland or forestry suffered particularly badly from headfly.

Table 1

Percentage of headfly attacks by Shires in South-West Scotland

Shire	None	Slight	Moderate	Severe
Ayrshire	47.0	32.1	15.5	5•4
Dumfriesshire	31.7	34.5	23.5	10.3
Kirk c udbright- shire	10.7	39.8	24•3	25.2
Lanarkshire	47.4	29.5	12.6	10.5
Renfrewshire & Bute	50.0	35•3	11.8	2.9
Wigtownshire	50.8	32.8	8.2	8.2

Chemical trials

Two available commercial preparations for headfly control, Flymort 24 and Headfly repellent (dark quality) were compared in 1973. The results are given in Table 2.

Table 2

Incidence of broken heads on lambs

Untreated Controls	Flymort 24	Headfly repellent
Trial 1, 1973		
1st observation		
9/110 = 8.2%	4/50 = 8%	0/100 = 0%
2nd observation		
26/110 = 23.6%	4/50 = 8%	0/100 = 0%
3rd observation		
26/110 = 23.6%	5/50 = 10%	1/100 = 1%

A significant degree of protection was afforded by Flymort 24 by the 2nd observation ($X^2 = 4.54$, p < 0.05). By the 3rd observation, however, the protection afforded by Flymort 24 was less conclusive and only approached significance at the 5% level ($X^2 = 3.3\%$, p < 0.10). Headfly repellent provided significantly more protection than Flymort 24 on the 1st, 2nd (significance probability 2.26%) and 3rd (significance probability 3.20%) observations.

Trials were carried out in 1973 and 1974 to compare the effectiveness of Headfly repellent with an improved repellent known as "C" fluid. The results of these trials are given in Table 3.

Table 3

Incidence of broken heads

Headfly repellent	"C" fluid	x2 (1 d .f.)
Trial 2, 1973		
Lambs $14/98 = 14.3\%$	6/104 = 5.8%	3.20 (p < 0.10)
Trial 3, 1974		
Lambs $3/43 = 7.0\%$	0/74 = 0%	2.87 (p < 0.10)
Trial 4, 1974		
Lambs $66/218 = 30.3\%$	41/210 = 19.5%	6.59 (p < 0.02)
Ewes 92/320 = 28.8%	54/328 = 16.5%	14.0 (p < 0.001)

In trials 2 and 3 there was a reduction of headfly lesions in the groups where "C" fluid was used which approached significance at the 5% level. In the 4th trial the protection afforded by "C" fluid was significantly better than that of the commercial repellent especially in ewes.

The efficacy of the new dip GH74 against headfly was also tested in 1974. The results of these trials are given in Table 4.

Table 4

Percentage incidence of	broken heads on	lambs treated with GH74
Untreated controls	gн74	x ² (1 d .f.)
Trial 5, 1974		6
lst observation		
33/96 = 34.4%	65/340 = 19.1%	9.14 (p < 0.01)
2nd observation		
34/96 = 35.4%	51/340 = 15.0%	18.60 (p <0.001)
Trial 6, 1974		
lst observation	ī	
85/250 = 34.0%	27/200 = 13.5%	23.89 (p <0.001)
2nd observation		
73/250 = 29.2%	46/200 = 23.0%	1.89 (p < 0.20)
Trial 7, 1974		
1st observation		
53/177 = 29.9%	13/73 = 17.8%	3.32 (p < 0.10)

By the first observation a significant degree of protection was afforded by GH74 in trials 5 and 6 but not, however, in Trial 7 where protection only approached significance at the 5% level. In the two trials where a second observation was made only in trial 5 was a significant degree of protection afforded by GH74.

DISCUSSION

Most farmers attribute the recent appearance of the headfly problem to the withdrawal of dieldrin and other persistent organochlorine dips. Treatment of heads with, or dipping in dieldrin has not prevented headfly attack (Titchener, 1975a). There is the possibility, however, that when dieldrin was widely used on farms it was having an indirect environmental effect on headfly numbers (Titchener, 1975b).

Advisory experience shows there is an urgent need to find a solution to the headfly problem. In the West College area recent reports show the incidence of headfly attacks to be increasing and the problem to be spreading northwards into Argyll and Perthshire. Whilst the environmental effects of dieldrin on wildlife and the residue hazard in sheep fat preclude bringing back dieldrin there is at present only one commercial compound, the dark quality headfly repellent, that affords any protection against headfly attacks. This repellent, however, does not afford complete protection and very frequent treatments, every 3 or 4 days, are required in severe headfly areas. Many breeders also object to its use since they feel it stains the head.

Of the new compounds tested, with the exception perhaps of "C" fluid, none are likely to be commercially developed. Even more disturbing is the fact that there are few candidate chemicals available for headfly control. One of the peculiar difficulties of the headfly problem is that all attempts to rear headflies artificially have, so far, failed with the result that large scale screening of potential headfly compounds is not yet possible. This has made applications for Animal Test Certificates a most unattractive financial gamble when the limited potential of the headfly market in relation to world sales is considered.

Recently it has proved possible to keep headflies, captured from field populations, alive for long periods in outdoor insectaries and it is hoped to extend this work to the testing of chemicals under conditions of simulated headfly attack. This could lead to a useful preliminary screening procedure but is, however, likely to prove difficult when it is remembered that most of the damage to sheep that occurs is apparently self-inflicted by the rubbing and scratching of the affected parts.

Acknowledgements

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A LABORATORY TECHNIQUE FOR THE EVALUATION OF COMPOUNDS APPLIED TO CATTLE FOR THE CONTROL OF THE STABLE FLY, STOMOXYS CALCITRANS

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Summary A method for evaluating compounds for control of Stomoxys calcitrans is described. Fly repellency is assessed on pairs of Friesian steers which are exposed at intervals after treatment with the candidate compound to 100 starved Stomoxys in a fly chamber. The technique is illustrated by results obtained with 1, 1 bis (p-ethoxyphenyl) - 2 nitropropane and some preliminary tests with the new synthetic pyrethroid 3 - phenoxybenzyl ± cis trans-2, 2 - dimethyl - 3 (2, 2 dichlorovinyl) cyclopropane - 1 - carboxylate.

Resumé Déscription d'un procédé pour évaluer les composés appliqués dans la lutte contre le Stomoxys. L'effet repoussant sur les mouches est évalué sur des paires de bouvillons Fresians qui sont exposés, par intervalles aprés traitement avec un composé aspirant, à 100 Stomoxys affamés dans une chambre à mouche. La technique est illustrée par des resultats obtenu avec 1, 1 bis (p-ethoxyphenyl) - 2 nitropropane et des essais preliminaires avec une nouvelle pyréthroid synthétique 3 - phenoxybenzyl ± cis trans-2, 2 - dimethyl - 3 (2, 2 dichlorovinyl) cyclopropane - 1 - carboxylate.

INTRODUCTION

The commercial development of a repellent or rapid toxicant for biting fly control on cattle will ultimately depend on successful field trials against the target species. Laboratory techniques are needed however, for the screening and initial development of such compounds.

We have used for many years a mouse/Stomoxys calcitrans test for screening purposes. Mice are sprayed with the candidate compound and four hours after treatment they are exposed to starved Stomoxys for a period of 30 minutes. The absence of feeding is taken as an indication of repellency. The residual effectiveness of any active compound is then examined by immersion dipping of mice followed by exposure to starved flies as before at 24 hours.

There are relatively few published reports of techniques using a bovine as a bait animal. Roberts et al (1960) and Yeoman and Warren (1968) have reported spot tests in which cages of starved Stomoxys are attached for set periods to treated areas on cattle. The percentage repellency and mortality are determined at intervals after treatment. Such tests undoubtedly give a more realistic assessment of the efficiency of the candidate material than would be obtained with the

mouse/Stomoxys test or in vitro techniques. However they suffer from the drawback that the flies cannot escape the influence of the treated area if they wish to do so. Only Roberts $\underline{\text{et}}$ $\underline{\text{al}}$ (1960) has reported a technique against free flying Stomoxys. A treated cow was placed in a screened cage and exposed to 100 starved flies. The repellency was determined after two hours and mortality determinations carried out after 24 hours. The size of the cage was not stated but Blume $\underline{\text{et}}$ al (1971) with the same technique used a cage 2 x 4 x 2.3 metres high.

In the technique reported here, fly repellency is assessed on pairs of Friesian steers which are exposed to free flying Stomoxys in a large fly chamber, at intervals after treatment with the candidate compound. The technique is illustrated by results obtained with 1, 1 bis (p-ethoxypheny1) - 2 nitro propane, viz. GH74 (Holan, G. 1971) and some preliminary tests with the new synthetic pyrethroid 3-phenoxybenzy1± cis trans-2, 2-dimethy1-3- (2, 2-dichloroviny1) cyclopropane-1-carboxylate, viz. NRDC 143 (Elliot et al 1973).

METHOD AND MATERIALS

The basic test was developed with due consideration of the behaviour of the flies in the chamber and the susceptibility of the bait animals.

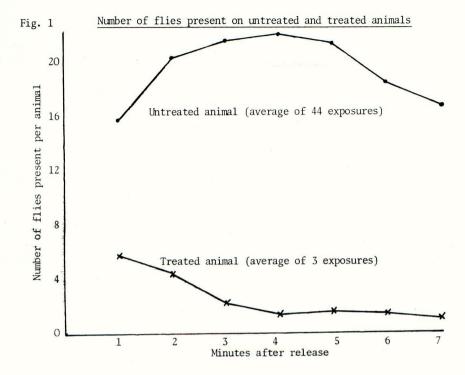
a) Behaviour of flies in the chamber

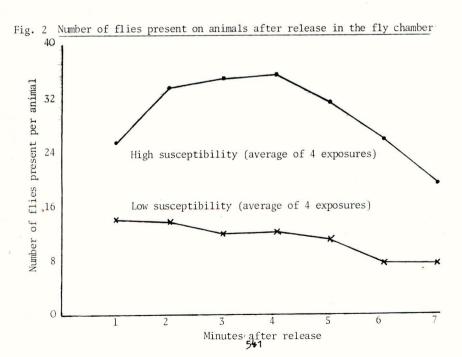
The fly chamber is a nonagonal fly proof room of the following dimensions - ceiling height 2.53 m, floor area 70.50 m and cubic capacity 178.37 m. Banks of thermostatically controlled heaters ensure the temperature is kept above 18°C for optimum fly activity. Testing is carried out in the range 18°C - 32°C. No humidity control is maintained. Ventilation is provided by three extractor fans which can produce a rapid air change. The chamber has translucent glass sides which, together with three pairs of 80w fluorescent lights, ensure even illumination in the chamber. A completely susceptible strain of $\underline{\text{Stomoxys}}$ is bred by the method of Stones (1966). Three-to four-day-old $\underline{\text{flies}}$ are available each working day for testing purposes. The flies are starved for 24 hours prior to use.

Observations on untreated animals have shown that starved Stomoxys released in the chamber locate their host rapidly. The flies land predominantly on the legs and begin to feed. The maximum number present on each animal is reached by the third or fourth minute. Seven minutes after release the number has fallen considerably as fed flies have left. This period is thus significant for the test. On treated animals if the compound acts as a contact repellent or rapid toxicant, the flies will land, become affected and fly or drop from the host. It may take some time for a compound to have an effect on the flies. Therefore observations from 3 - 7 minutes after release of the flies would give a more reliable guide to the performance of the material than those taken over the full seven minute period. Figure 1 compares the number of flies present per minute on untreated animals (average 44 exposures) and on animals 1 day after treatment with 1 litre 0.5% GH74 (average of 3 replicates). The rapid effect on the flies is clearly demonstrated.

b) Susceptibility

Some cattle are very attractive to <u>Stomoxys</u>, others are not. The reason is not fully understood but various physiological and psychological factors such as odour, colouration, hair length and the reaction of the animal to fly





attack are all possible factors determining susceptibility.

Friesian steers of similar size, i.e. around 200 kg are generally used in the tests. The animals are bought in batches of ten or twelve. The susceptibility of each animal is examined initially by exposures to Stomoxys in random pairs. Any with very low susceptibility are discarded. Further assessment is carried out to produce pairs of matched susceptibility as far as possible for any test, i.e. one animal of the pair may have a high susceptibility (Figure 2) and another low, but the average will match the susceptibility of another pair used in the same test.

Assessment of Fly Repellency

A pair of Friesian steers are held in standings 1.8 metres apart. One hundred starved Stomoxys are released on the opposite side of the chamber. Counts are made of the number of flies present on the animals for 7 minutes at one minute intervals. On each occasion the two observers make counts of the flies present from the tail to the head of first one animal and then the other. At the end of the exposure period, the animals are removed from the chamber. A fresh pair is introduced and a further 100 flies released. In this way it is possible to test four pairs of animals comfortably in a 1 - 2 hour period. After testing the animals are housed and not subjected to external weathering as this would introduce a variable factor to the test.

Four pretreatment counts are generally carried out on each pair of animals. After treatment fly exposures are made each working day. Any flies remaining alive in the chamber are allowed to feed on untreated bait animals before each day's testing commences. The order of the pairs exposed in the chamber is changed on each occasion to nullify the effect of flies that have not fed during the previous exposure. In the tests described, treatments took place on Mondays and thus fly exposures were carried out on 1, 2, 3, 4 and 7 or more days after treatment.

After each seven minute exposure the average number of flies present per minute is determined for each animal from the counts taken at 3 - 7 minutes. The average for one pair is calculated and the treatment average is determined if this is replicated on more than one pair of animals. The average post-treatment count on each occasion is related to the average pretreatment count and a $\$ repellency figure obtained.

RESULTS

The technique is illustrated with some results obtained with $\hbox{GH74}$ and the new synthetic pyrethroid NRDC 143.

GH74

The fly repellency obtained by handspraying 1 litre 0.5% GH74 spray diluted from a 25% w/v e.c. over the entire body (i.e. 5g active ingredient per animal) was assessed on three occasions. Two pairs of animals were used in each test. Table 1 gives the fly repellency figures obtained (average for each test).

Table 1
% fly repellency obtained after spraying with 1 litre 0.5% GH74

Days after treatment	Test 1	Test 2	Test 3	Mean
1	94	94	90	93
2	82	91	88	87
3	87	71	86	81
4	82	75	81	79
7	70	43	67	60
8	30	42	44	39
9	41	49	16	35
10	6	32	7	15
11	28	13	4	15

With such a technique variations in fly repellency from test to test must be expected due predominantly to the variability inherent in different fly batches. Large variations in the size of the bait animals could add further complications. It is thus essential to use animals of similar size.

The three tests with GH74 produced similar results. The compound showed a progressive loss of activity every day after treatment. Over the first four days a high level of protection was observed but at seven days the % repellency had fallen to 60%.

NRDC 143

The three tests with 1 litre 0.5% GH74 provided a standard comparison for tests with 0.025%, 0.05% and 0.1% NRDC 143. Two pairs of animals were used per treatment. Each animal was handsprayed with 1 litre of wash diluted from a 20% w/v e.c. The amount of active ingredient applied per animal was thus 0.25, 0.5 and 1.0g respectively. The fly repellency figures obtained are given in Table 2.

Table 2
% fly repellency obtained after spraying with
1 litre NRIX 143 at different concentrations

Days after treatment	Test 1 0.025%	Test 2 0.05%	Test 3 0.1%
1	90	87	91
2	78	79	86
3	81	75	82
4	70	78	79
7	57	53	79
8	62	50	71
9	52	50	62
10	0	27	43
11	52	2	45

In comparison with the standard 0.5% GH74 the following points emerge. The initial protective effect of 0.1% NRDC 143 is similar to that of 0.5% GH74 but the residual effectiveness of the NRDC 143 appears better. 0.025% and 0.05% NRDC 143 were not as effective initially as 0.5% GH74 but gave a similar residual effect.

DISCUSSION

The evaluation of treatments for the control of biting flies on cattle under field conditions is beset with difficulties. For example, the fly challenge can vary tremendously within a comparatively small locality and can also fluctuate from day to day according to climatic conditions. It should therefore be constantly monitored by the use of control animals. However in any trial the greater the number of animals treated, the greater the effect will be on the local fly population. This in turn can depress the challenge seen on the untreated animals. The problems serve to illustrate the usefulness of a controlled laboratory technique which can distinguish between the effectiveness of such treatments applied to cattle.

The experiments described were designed to investigate the duration of the protective effect against Stomoxys obtained with NRDC 143 in comparison with a standard GH74 treatment. For that reason a 1, 2, 3, 4, 7, 8, 9, 10, 11 day exposure routine was used. The particular choice of treatment day will allow the effect to be examined on selected days within a five day working week.

The technique can be employed for a variety of purposes. Direct comparisons can be made between compounds tested together. Different application rates can be compared and the effect of repeated treatments on the duration of protection examined. Various formulations can be tested and different methods of treatment used, e.g. handspraying or a mechanical spray treatment.

The results with GH74 show there is a considerable repellent effect seven days after treatment. Extensive field tests, some of which are reported by Wood (1973) have shown that this application rate will give 5 - 10 days protection against a number of species of biting flies. The test thus does give a reasonable guide to the field activity of the compound. Unfortunately the compound failed to meet other equally important criteria concerning the cost of treatments and the level of residues found in milk and beef fat after treatment.

The preliminary results with the synthetic pyrethroid NRDC 143 show that it is more active than GH74. It must therefore hold great promise as a control agent for biting flies on cattle.

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DEVELOPMENT AND ASSESSMENT OF A TEST-METHOD SUITABLE TO SELECT SUBSTANCES EFFECTIVE AGAINST MANGE MITES

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Summary The development of a test method is reported designed to select substances effective against mange. The test consists of three parts. The primary screening is carried out in vitro with Psoroptes cuniculi. The secondary screening is conducted with pieces of skin removed from sheep infested with <u>Psoroptes ovis</u>. The <u>in vivo</u> tests are carried out with <u>Psoroptes ovis</u> of sheep or <u>Chorioptes bovis</u> of cattle. A qualitative evaluation of the <u>effect</u> of treatment against mange mites is difficult, a quantitative assessment is impossible. Therefore the effect of treatment on the animals is assessed by indirect methods. The parasites are counted before and after treatment on certain skin areas of treated and untreated animals. Additionally the number of " scratch-movements " of test animals before treatment is compared with that after treatment. By these methods it is possible to evaluate precisely the effect of treatment. In numerous trials it has been established that by the use of these test methods an accurate selection of substances effective against Psoroptes spp. and Chorioptes spp. is possible.

Résumé Ce rapport concerne le développement d'une méthode d'épreuve permettant le choix des substances actives contre la gale. Le test se déroule en trois degrés: Le screening primaire est effectué in vitro sur des Psoroptes cuniculi. Le screening secondaire se fait au moyen de morceaux de peau atteints de Psoroptes ovis, prélèvés de moutons étant infesté pour le test. Les essais experimentaux chez les animaux sont effectués sur des P.ovis du mouton ou bien sur des Chorioptes bovis du boeuf. Une évaluation qualitative du succès de traitement étant difficile, et une évaluation quantitative étant impossible, le contrôle du succès de traitement se fait chez l'animal par des méthodes indirectes. A cette fin, on compte les parasites à des endroits bien certains de la peau, avant et après le traitement, chez des animaux traités et not traités. En outre, le nombre des mouvements défense des animaux est comparé avant et après le traitement. L'application de ces deux méthodes permet un bon jugement du succès de traitement.

Au cours de larges séries d'experiences effectuées, on pouvait constater, que la méthode de test en plusiaurs degrés que nous avons appliquée, permet une sélection précise de substances actives contre des espèces de <u>Psoroptes</u> et <u>Chorioptes</u>. Les méthodes décrites étant concluantes et pas chères, ils sont concus pour effecteur des méthodes de test routinier.

INTRODUCTION

The importance of mange infestation in sheep and cattle is well known. Government measures such as isolations of infested animals or compulsory treatments have been organized to eradicate this disease. The losses caused by mange are tremendous. The yearly loss caused by Psoroptic mange in sheep in the Argentine is US\$40 M. This is more than by tick infestations in that country. (Endrejat, 1967). The weight gain in cattle, was reduced by about 250 g/day/animal due to mange infestation (Tobin, 1962). Our own trials showed that with heavy Chorioptes bovis infestation of bulls the normal fattening time of five months is prolonged to eight months. There are two reasons for the increasing importance of mange. First, the intensive livestock rearing predisposes to its spread and second, in some countries where it was thought that the disease was being eradicated, it has persisted. For example, Tarry (1974) reported 48 cases of mange in England during 1973 and 1974 and Bone (1964) reported that it was still present in the USA in 1964.

An intensive control of mange infestation in our domestic animals is absolutely necessary. In the following, test methods are described suitable for selecting substances effective against mange.

METHOD AND MATERIALS

Primary screening For primary screening a natural population (larvae, nymphs, adults) of ear mange of rabbits (Psoroptes cuniculi) is used. Scabs contaminated with numerous mange mites are collected one hour before commencement of trial from the ear of infested rabbits and stored at 28°C± 1°C and 80% humidity. 10-25 mites are transferred to "blister-packagings" of an appropriate size which are charged with the ingredients to be tested. These "blister-packagings" are stored under controlled conditions at 28°C ± 1°C and 80% ± 10% humidity. The evaluation of the effect of the test substances is examined after 24 hours microscopically (magnification X 12.5).

The criterion of efficacy is the incidence of death of treated mites. For evaluation of findings the following key is used:

100% efficacy = all mites dead >50% " = >50% of mites dead <50% " = <50% of mites dead 0% " = all mites alive. Secondary screening From sheep naturally infested with Psoroptes ovis heavily infested pieces of skin of 1 cm² are cut out. 15 minutes prior to section the appropriate sheep is treated intramuscularly with Rompun(R) at 0.15 mg/kg in order to tranquilize the animal and to anaesthetise its skin. The wound afterwards is coagulated with thermocautery. The infested skin section is transferred into a Petri dish. Thereafter 1 ml of the ingredient to be tested is pipetted onto the infested piece of skin. The Petri dish is then covered and stored at 28°C $^{\pm}$ 1°C and 80% $^{\pm}$ 10% humidity. The evaluation of the effect of the substance is examined after 24 hours microscopically (magnification x 12.5). The criterion of efficacy is the incidence of death of treated mites.

In vivo trials The test is carried out against all stages of P.ovis (larvae, nymphs, adults) on naturally infested sheep. To estimate the grade of infestation before treatment 5 pieces of skin of 1 cm² are cut out from sheep and the mites are counted microscopically. The degree of infestation is classified as follows:

+ = less than 10 mites per skin area

++ = 10-100 mites per skin area +++ = numerous mites per skin area

Furthermore the scratching movements of the animals are counted over a 1h period on day-1 and $\frac{1}{2}$ 0.

The animals are treated by dipping in a plunge dip. A second treatment is carried out 10 days after the first treatment. The effect of treatment is observed at intervals. To evaluate the grade of infestation after treatment 5 pieces of skin are taken from the sheep and evaluated on day, 1,7,28 and 56. A further indirect evaluation of the effect of treatment is the reduction of scratching movements of treated animals in comparison to untreated animals. Therefore the animals are observed over a 1h period at various intervals after treatment and the number of scratching movements is noted. These observations are always made before taking the skin samples. The treatment is regarded as efficient if no live mites are found after treatment and no scratching is observed. The treatment is regarded as partly efficient if the number of mites on the skin areas is reduced by more than 80% and the number of scratching movements is decreased. A substance is regarded as ineffective if more than 20% of mites survive and the number of scratching movements is only slightly reduced.

Trials to determine the effect of treatment against <u>Chorioptes bovis</u> are carried out after the same method. To estimate the grade of infestation before and after treatment 5 skin areas of $4~\rm cm^2$ on predelection sites are investigated with a magnifying glass.

⁽R) registered trade mark Bayer AG, Leverkusen, West Germany

RESULTS

To determine if the described method is suitable to select substances effective against mange <u>in vitro</u> and <u>in vivo</u> trials were carried out with different remedies against various mite species.

The following substances were used: γ -BHC, trichlorfon, fenthion, coumaphos, phoxim, quintiofos, oxythioquinox.

V-BHC = 1,2,3,4,5,6-hexachlorocyclohexane, 99% or more gamma isomer

trichlorfon = dimethyl (2,2,2,-trichloro-l-hydroxyethyl)
phosphonate

fenthion = 0,0-dimethyl 0- 4-(methylthio)-m-tolyl phosphoro-thioate

coumaphos = 0-(3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl) 0,0-diethyl phosphorothioate

phoxim = phenylglyoxylonitrile oxime 0,0-diethyl phosphorothioate

quintiofos = 0-ethyl 0-quinolyl-8 benzenephosphono-thioate

oxythioquinox = 6-methyl-2,3-quinoxalinedithiol cyclic S,Sdithiocarbonate.

Table 1

P.cuniculi/all developmental stages in vitro
Killing effect of different compounds in %

Concn of a.i. in ppm	у -В НС	killing trichlor- fon		couma-	24 h aft phoxim	er appl quintion fos	ication coxythio- quinox
1000	100	>50	>50	100	100	100	0
300	100	< 50	>50	100	100	100	0
100	100	0	450	50	100	100	0
30	100	0	0	50	100	100	0
10	>50	0	0	50	>50	0	0
3	< 50	0	0	0	<50	0	0
1	0	0	0	0	0	0	0
0.3	0	0	0	0	0	0	0
control	0	0	0	0	0	0	O

The results demonstrate that the in vitro efficacy of BHC, phoxim and quintiofos against P.cuniculi is good, that of coumaphos is moderate and that of the other compounds is poor.

In Table 2 results are stated which are obtained with the above mentioned substances against $\underline{P.ovis}$ when using the test at the isolated skin part taken from infested sheep.

P.ovis/all developmental stages on pieces of sheep skin killing effect of different compounds in %

Concn of a.i. in ppm	ү-ВНС	killing trichlor- fon	effect fen- thion	couma-	24 h after phoxim	applicat quintio- fos	tion oxythio- quinox
1000	100	∠ 50	>50	100	100	>50	0
300	100	<50	<50	100	100	<50	0
100	100	0	0	450	100	0	0
30	100	0	0	0	>50	0	0
10	<50	0	0	0	0	0	0
3	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
0.3	0	0	0	0	0	0	0
control	Ö	Ö	0	0	0	0	0

The results show that the efficiency of the tested substances against $\underline{P.ovis}$ is similar to that against $\underline{P.cuniculi}$. The efficiency of \overline{quinti} of against $\underline{P.ovis}$ is poor.

In table 3 the results of a plunge dip trial in sheep naturally infested with $\underline{\text{P.ovis}}$ are summarized.

Table 3

P.ovis/all developmental stages in vivo (sheep) Killing effect in % after plunge-dipping with different compounds

Concn of a.i. in ppm	-ВНС	killing trichlor- fon	effect fen- thion	couma-	48 h afte:	r second a quintio- fos	
1000	100	0	>50	100	100	< 50	О
300	100	0	0	>50	100	0	0
100	>50	0	0	0	> 50	0	0
30	<50	0	0	0	<50	0	0
10	0	0	0	0	0	0	0
control	0	0 ,	0	0	0	0	0

The results indicate that the findings obtained against $\frac{P.ovis}{trial}$ in isolated pieces of skin can be transferred to this $\frac{P.ovis}{trial}$. The efficiency of γ -BHC and phoxim against $\frac{P.ovis}{trial}$ is good, that of coumaphos moderate, trichlorfon, fenthion, quintiofos and oxythioquinox are inefficient.

Table 4 shows the results of a hand spray trial against C.bovis in naturally infested cattle.

Table 4

C.bovis/all developmental stages in vivo (cattle)
Killing effect in % after handspraying with different compounds

Concn of a.i. in ppm	ү-внс	killing trichlor- fon	fen-	in %, 48 couma- phos	h after phoxim	second quintio- fos	application oxythio- quinox
1000	100	0	>50	>5 0	100	0	0
300	100	0	0	0	100	0	0
100	0	0	0	0	450	0	0
30	0	0	0	0	0	0	0
control	0	0	0	0	0	0	0

The results show that the findings obtained against $\underline{P.bovis}$ in isolated pieces of skin can be transferred also to $\underline{C.bovis}$.

Table 5 shows the clinical and parasitological findings obtained with a test substance against $\underline{P.ovis}$ in naturally infested sheep.

Table 5

P.ovis/all developmental stages in vivo (groups of 24 sheep) Clinical and parasitological findings before and at different times after application of a test substance

Concn of a.i. in ppm	Nu:	Numbers mbers of ± 0	of scratch living mit + 1	ings/sheepes5 skin property 4 7	p/hour (ran ieces on day + 28	ge)* + 56
1000	15* (6-21) >10**	12 (6–15) >10	6 (0-9) <10	0.05 (0-0.5) 0	0	0
500	18 (9-21) >10	18 (9-24) >10	6 (0-9) >10	(0-9) <10	0.5 (0-3) <10	1 (0-16) <10
250	12 (6-15) >100	9 (6-15) >10	6 (0-9) >10	0.5 (0-3) >10	9 (3-12) >10	12 (9-15) >10
control	30 (15-42) >10	30 (12-42) >100	30 (15-48) >10	18 (15-42) >10	30 (15-45) >100	27 (15-48) >100

The results indicate that the indirect assessment of efficacy is suitable to determine the effect of treatment against P.ovis.

In table 6 the appropriate trials in cattle naturally infested with ${\tt C.bovis}$ are stated.

Table 6

C.bovis/all developmental stages in vivo (groups of 18 cattle)
Clinical and parasitological findings before and at different
times after application of a test substance

Concn of a.i. in ppm	Nun -1	Numbers of	of scrat	cchings/cat nites/skin + 7	tle/hour area on da + 28	ay + 56
500	14* (12-18)	12 (9-21) >100	0	0	0	0
	>100**	>100	<10	0	0	O
control	11 (8-16) >100	12 (8-18) >100	10 (8-18) >100	12 (6-14) >10	8 (6-16) >100	13 (8-18) >100

^{*} mean number of scratchings/cattle/1 h (range)

The results show that the clinical improvement of treated animals corresponds to the reduction of parasites.

In table 7 the results of different substances against various mite species are shown.

Table 7

Different species of mange mites/all stages in vivo Control of mites after application of different compounds

species	ү~ВНС	Efficatrichlor- fon		differen couma- phos		unds quintio- fos	oxythio- quinox
Ps.cun.	+	(+)	(+)	+	+	+	_
Ps.ovis	+		(+)	(+)	+	+	-
Chor.bovis	5 +	-	(+)	(+)	+	+	_
Ps.bovis	+	_	(+)	(+)	+	+	_
Sarc.spp.	+	+	_	_	(+)	(+)	_
Myob.musc.	+	-	_	_	_	-	+
Myoc.musc.	+	-	-	-	-	-	+

⁺ good efficacy, (+) moderate efficacy,
- no efficacy

^{**} mean number of living mites/5 skin areas

The results demonstrate that the test model used (P.cuniculi in vitro, P.ovis in isolated skin) is suitable to select substances effective against Psoroptes spp. and C.bovis. These results cannot be transferred to Sarcoptes spp. Myobia musculi and Myocoptes musculinus are not suitable to select substances effective against P.ovis or C.bovis.

DISCUSSION

There are relatively few publications dealing with the development of test methods to select substances effective against mange. This is in contrast to the importance of mange disease.

The reason for this could be the difficulty in experimental work with mange mites. The selection of substances effective against mange using this obligate parasite by means of in vitro tests is more difficult than with other ectoparasites which are facultative parasites (blowfly-larvae) or which are temporary parasites with a longer non-parasitic phase (ticks). The in vivo test is very difficult due to the size of the parasite. Qualitative assessment is difficult and counts of parasites are impossible.

A reliable selection of substances effective against <u>Pso-roptes</u> and <u>Chorioptes</u> can be made using the described test model:

1. Pre-selection with P.cuniculi in vitro

Precise selection with <u>P.ovis</u> in isolated pieces of skin

3. In vivo trials with indirect proof of parasites in target species

The results obtained with <u>P.cuniculi</u> and <u>P.ovis</u> can be transferred to <u>Chorioptes spp.</u> but not to <u>Sarcoptes spp.</u>

In our trials the method described by Heine (1962) for the treatment of $\underline{\text{Myobia musculi}}$ and $\underline{\text{Myocoptes musculinus}}$ proved to be unsuitable for the selection of substances effective against $\underline{\text{P.ovis}}$ and $\underline{\text{C.bovis.}}$

Our test model has proved its efficiency for the selection of substances effective against these parasites in a routine screening programme for several years.

Acknowledgements

The author wishes to thank his co-workers who have helped him in this work.

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AMITRAZ - FOR THE CONTROL OF ANIMAL ECTOPARASITES

WITH PARTICULAR REFERENCE TO SHEEP TICK (IXODES

RICINUS) AND PIG MANGE (SARCOPTES SCABIEI)

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Summary In preliminary field trials, amitraz (1,5-di-(2,4-dimethylphenyl)-3-methyl-1,3,5-triazapenta-1,4-diene) has shown encouraging activity against cattle and pig lice, sheep tick and pig mange.

Further trials are in progress which it is hoped will confirm that amitraz will find application against these and other ectoparasites, both in this country and other parts of the world.

INTRODUCTION

Amitraz (1,5-di(2,4-dimethylphenyl)-3-methyl-1,3,5-triazapenta-1,4-diene), a new compound has shown activity against the Australian cattle tick (Palmer et al 1971) and against ticks of major importance in South Africa (Harrison et al 1972; Baker et al 1973). Good activity has also been obtained against cattle ticks in South American trials (unpublished data). In addition, trials against sheep scab in Argentina are giving promising results.

This paper describes preliminary field trials designed to determine the activity of amitraz against a number of animal ectoparasites of importance in the United Kingdom, namely, cattle lice (Linognathus vituli and Damalinia bovis), pig lice (Haematopinus suis), sheep tick (Ixodes ricinus) and pig mange (Sarcoptes scabiei var. suis). These trials are part of the programme of work to determine the spectrum of activity of amitraz and its potential for use both in this country and in other parts of the world.

METHODS AND MATERIALS

Cattle Lice Trial

A pour-on formulation of amitraz was applied to four pairs of beef calves, naturally infested with lice, to give a range of dose levels from 0.375 to 3g active ingredient per animal. All treatment groups and a pair of untreated controls were kept in separate pens throughout the trial. Before, and at intervals after treatment, lice were counted on the following areas - the whole of the mane, and standard 150 sq cm areas of the right and left shoulders and rump. At 4 weeks after treatment, those animals which were found to be free of lice infestation were challenged with infested donors.

Pig Lice Trial

The efficacy of a 12.5% emulsifiable concentrate (e.c.) formulation of amitraz was determined, using sows infested with lice. The treatment groups were as follows:-

Two groups were treated with amitraz at 0.1%; one was treated at day 0, and the other treated twice, at day 0 and 14.

Three groups were treated with amitraz at 0.05% - one at day 0, another at day 0 and 7, and the final group at day 0 and 14.

The final group was treated with gamma - BHC, a single application at 0.05%.

The animals were sprayed individually with 2 litres of wash by means of a hand sprayer. A group of untreated controls were examined at intervals to confirm the persistence of the infestation.

All treated animals were examined for the presence of lice at weekly intervals after treatment for at least 3 weeks.

Sheep Tick Trial

50% wettable powder (w.p.) and 12.5% e.c. formulations of amitraz were evaluated against tick infestations on sheep at five sites in Eastern Scotland. A dipping rate of 0.05% was employed for both formulations. Where necessary replenishment was carried out at $1\frac{1}{2}$ times the initial wash concentration for every 50 gallon drop in volume.

The activity of amitraz was compared at each site with that obtained for the product normally used on the farm. This gave comparisons from roughly equal groups of animals chosen at random between amitraz and dioxathion, chlorpyriphos, chlorfenvinphos and coumaphos. The standard products were all used at a concentration of 0.05%.

Comparison of amitraz activity with small groups of untreated animals was possible on two farms. A total of 2,500 animals was used in the trials.

The efficacy was determined by making counts of attached nymphs and adult ticks on representative numbers of animals from each group, at intervals up to 6 weeks after dipping.

Pig Mange Trial

A 12.5% e.c. formulation of amitraz has been evaluated using a litter of infested piglets housed in the experimental unit. The presence of mites (Sarcoptes scabiei) in the ears of all pigs was established by microscopic examination of skin scrapings taken from inside the ears - the only area of the body infested. The animals were assigned to three groups of equal infestation and treated as follows:-

Group 1 - animals were sprayed once over the whole body area by means of a small pressurised hand sprayer with amitraz at a concentration of 0.1%.

Group 2 - animals were sprayed with amitraz at 0.05% on days 0 and 5.

Group 3 - animals were sprayed with a blank formulation on days 0 and 5.

At day 19 the three separately housed groups were mixed to present a mite challenge to the treated animals.

At 27 days after the mix, all the animals were sprayed with amitraz at 0.1%.

The volume of spray applied to each animal at each treatment was $500~\mathrm{mls}$.

Samples from the ears of all the animals were examined microscopically at intervals up to 14 days after the final treatment.

A second trial using infested animals on outside farms is in progress.

RESULTS

Cattle Lice

The results obtained in this preliminary trial indicate that amitraz applied at 3 and 1.5g/animal, effectively cleared an existing mixed louse population and prevented reinfestation, i.e. from hatching eggs, for 4 weeks after treatment. The animals were, however, susceptible to reinfestation when challenged after this time. The 0.75 and 0.375g levels were effective against the original lice infestation but could not prevent reinfestation with lice originating from eggs present on the animals at the time of treatment.

Pig Lice

Good activity was obtained with the initial treatment of amitraz at 0.1% but a second treatment at 14 days was found necessary to clear the animals. The results obtained with the 0.05% spray showed that two treatments were necessary, preferably 14 days apart. The standard mange dressing containing gamma - BHC was effective for up to 5 weeks after a single treatment.

Sheep Tick

Tables 1 and 2 record the results of the sheep tick trials in which both formulations of amitraz used at 0.05% compared favourably with the standard products. Activity was better against adult female ticks than against the nymphs.

Pig Mange

The initial trial results are recorded in Table 3. They show that one treatment of amitraz at 0.1%, or two treatments at 0.05%, were effective. Upon challenge 19 days after treatment, one animal from the group treated at 0.1% became reinfested. A single treatment of amitraz at a rate of 0.1% was again effective when the animals were treated to complete the trial.

DISCUSSION

The activity of amitraz as an effective acaracide against cattle tick in many parts of the world is now well established. The results of our initial trials suggest that amitraz could find application against other ectoparasites of economic importance in the temperate regions of the world.

Site Sheep		Standard	Weeks	Mean No. Attached Ticks/Sheep						
		Product	After Dipping	Live A	<u> </u>	Live A	Nymphs C	Dead A	op _s	
F	Ewes and	Coumaphos	2	0	1.4	0.5**	29.3	0	0.1	
	Hoggs	• 300 000	6	8.6	9.6	84.1	99.0	5•9**	28.0	
G	Hoggs	Chlorphen- vinphos	3	0.1	0.1	9•3**	15.7	0.4**	6.0	
		VInpnos	6	0.5	1.8	11.3	16.1	0.3**	2.8	
Н	Ewes	Dioxathion	6	0.5	0.1	7.6	19•5	0.1	1.5	
			2	0.5	0.6	29.2	45.0	1.0*	3.6	260
J	Ewes	Dioxathion	4	0.5	3.3	14.4	25.9	0.4*	2.3	10
		Treated at 0 and 4 weeks	6	6.1	3.4	6.8	4.0	1.1	1.1	

A = Amitraz treated sheep

C = Standard product treated sheep

* = Significant difference between mean K.05

** = Significant difference between mean P<.01

26.1

Table 2

The efficacy of amitraz (12.5% e.c.) against Sheep Tick used at 0.05%

				М	ean No	 Attache 	d Ticks/S	heep	
Site	Sheep	Standard Product	Weeks After Dipping	Live A	0 +s C	Live A	Nymphs C	Dead A	<u> </u>
Н	Hoggs	Dioxathion	2	0	0.1	2.4**	17.7	0.3**	1.8
			6	0.7	0.9	30.6	61.0	0.7*	3.0
J	Hoggs Amitraz Treated at O and 6 weeks	Dioxathion Treated at 0, 2 and 6 weeks	2 4 6 8 10	0* 0.8* 6.8 0 4.7	0.4 2.2 7.5 3.7 7.5	0.3** 15.7 54.5 14.3 5.2	12.5 22.7 76.5 23.1 7.1	0** 0.4 0.4 0.4**	1.8 2.2 1.5 2.8 1.5
К	Hoggs	Chlorpyriphos	2	0	0.1	0.9*	2.5	0	0
			6	0	0.1	6.6	6.1	.0	0

A = Amitraz treated sheep

C = Standard product treated sheep

^{* =} Significant difference between mean P<.05

^{** =} Significant difference between mean PC.01

Table 3

The efficacy of amitraz (12.5% e.c.) against Pig Mange

							Numbe	rs of	live m	ites a	nd eggs	per e	ar sc	raping				
Group	Conc.	Pig No.	Pre-tr	eatment	5	Days		treatm 2	ent 1	0		ys aft			Days	after	respre	aying
	Spray	110.	Eggs	Alive	_	Alive		Alive		Alive		5 Alive		Alive	Eggs	Alive	Eggs	Alive
		9	1	11	0	1	0	0	0	0	0	0	0	0	0	0	0	0
_		20	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0.1%	22	8	3	1	0	0	0	0	0	2	25	2	4	0	1	0	0
		32	0	2	0	0	0	0	0	0	0	0	Ó	0	0	0	0	0
							Days	after										
							Eggs	Alive	Eggs	4 Alive								262
		15	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0.05%	19	0	12	5	14	0	0	0	0	0	0	0	0	0	0	0	0
		21	0	3	6	3	0	0	0	0	0	0	0	0	0	0	0	0
		16	0	3	6	23	1	8	9	2	5	35	9	109	0	0	0	0
_		25	4	11	0	1	1	3	0	0	1	5	1	0	0	0	0	0
3	Blank	31	1	1	0	2	3	5	6	5	1	22	3	15	0	0	0	0
		33	0	0	0	13	14	20	4	1	0	6	0	1	0	0	0	0

Gps. 2 and 3 Respray

Gps. 1, 2 and 3 mixed

All sprayed amitraz 0.1% conc.

The activity of the pour-on formulation of amitraz against cattle lice is encouraging and further trials are planned.

The persistence of the e.c. formulation used in the pig louse trial was not satisfactory. However, the activity of amitraz shown in these trials indicates that effective control may be possible using an improved or different formulation.

Sheep ticks are a major problem in certain hill areas of the United Kingdom both as potential vectors of disease and as active blood suckers. Amitraz has been shown to be at least as effective as the commercially available products in reducing the tick burden. Our trials also indicated a reduction in the number of cases of tick borne disease with amitraz treatment. Further large scale trials are planned in other areas of the country when lower user rates of amitraz will be evaluated and particular attention paid to the very important tick transmitted diseases such as louping ill and pyaemia.

Opinions differ on the economic importance of sarcoptic mange in pigs. It is agreed, however, that it is an increasing problem that can flare up given the right environmental and husbandry conditions. In the initial trials, amitraz has shown good activity against the mange mite. The results to date the field trials confirm this fact.

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AMITRAZ - AUSTRALIAN FIELD TRIALS

AGAINST THE CATTLE TICK (Boophilus microplus)

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Summary A series of field trials is described carried out in Queensland using amitraz for the control of the cattle tick (Boophilus microplus). Excellent acaricidal efficacy with rapid tick clearance was demonstrated. The addition of 0.5% calcium hydroxide effectively stabilised amitraz in polluted spray race and plunge dip vats. A simple dip management technique for stabilisation and reinforcement is described.

INTRODUCTION

Amitraz has been accepted as the common name for 1,5-di-(2,4-dimethylphenyl)-3-methyl-1,3,5-triazapenta-1,4-diene, previously known as BTS 27419.

Harrison et al (1972) reported its activity against $\underline{B.microplus}$ and referred to the prevention of its degradation in the dipping bath.

About 9 million head, or approximately one-third of Australia's cattle population live in an area infested with the cattle tick which is considered to be one of the greatest factors limiting cattle production. Cattlemen in northern Australia probably spend more time and money on tick control than any other phase of management. Highly effective acaricides are therefore essential and the growing incidence of organophosphorus resistant strains of B.microplus increases the importance of new types of acaricide.

The majority of cattle in tick infested areas are treated in plunge dipping vats of which there are about 6000 in Queensland and 1300 in northern New South Wales. There are also about 1000 mechanical spray race installations and approximately 4000 properties applying acaricides by hand-spraying.

Before the introduction of a new acaricide it is therefore necessary to establish its suitability for application by these three methods. For hand-spraying the chemical does not need to be stable in the spray wash for any length of time but must be safe and effective. When the other more important methods of application are involved it must also be stable for long periods under conditions of heavy pollution with faeces, urine, soil and a complex bacterial flora.

The series of field trials described in this paper was designed to examine these aspects of amitraz formulations. They were all carried out under field conditions with natural infestations of B-microplus.

METHOD AND MATERIALS

A 50% wettable powder (w.p.) and two different 12.5% emulsifiable concentrate (e.c.) MK I and MK II formulations of amitraz have been used.

In hand-spraying trials the acaricide was applied by means of a portable power spray unit with a hand-lance. Approximately 12 litres were applied while the animal was suitably restrained in a crush.

A variety of mechanical spray race plants were used all of the high volume, low pressure, recirculatory type with sumps capable of holding about 2000 litres of wash.

Plunge dipping vats were of the usual design varying in capacity from 9500 litres to 14000 litres.

The calcium hydroxide used in stabilisation trials was commercial hydrated lime containing approximately 83% Ca(OH)₂. Dip wash concentration of calcium hydroxide was estimated by titration against N/10 hydrochloric acid using thymolphthalein indicator papers.

The cattle involved in the trials were both $\underline{\text{Bos taurus}}$ and $\underline{\text{B. indicus}}$ breeds with a variety of crossbreds.

Where counts of parasitic female ticks were carried out only those individuals measuring 4.5 mm - 8.0 mm in length were included.

RESULTS

Hand-spraying Trials

Two small scale dose response trials were conducted using a 12.5% amitraz emulsifiable concentrate.

In each trial cattle were inspected daily for three days prior to treatment and adult female ticks counted on one side of each beast. On the day of treatment animals were allocated to one of four groups in such a manner as to produce an approximately equal infestation in each group. Three groups were treated at 0.0125%, 0.025% or 0.0375% and animals in one group remained untreated.

Following treatment tick counts were made at approximately the same hour on three alternate days each week.

Results were similar for each trial and are typified in Tables 1 and 2.

Table 1

Average tick counts per side before and after spraying

Day	0.0125%	0.025%	0.0375%	Untreated
- 2	10	11	22	11
-1	16	27	27	35
0	28	30	33	31
1	0	0	0	20

Table 1 (continued)

Day	0.0125%	0.025%	0.0375%	Untreated
3	0	0	0	10
5	0	0	0	3
7	0	2	0	7
10	0	0	1	1 5
12	1	0	0	16
14	1	0	0	17
17	0	0	0	6
19	1	0	0	7
21	1	0	0	7
24	2	1	5	9
26	6	16	8	18
28	15	30	14	30

Table 2

Progr	essive to	otals and p	ercentage	e control	(Calcula	ted from av.	count/side/
0.0125%			0.0	025%	0.0	Untreated	
	Prog.	%	Prog.	%	Prog.	%	Progressive
Day	Total	Control	Total	Control	Total	Control	Total
1	-	100	-	100	-	100	20
3	_	100	-	100	-	100	30
5	-	100	-	100	-	100	3 3
7	-	100	2	95	=	100	40
10	_	100	2	96.4	-	98.2	55
12	1	98.6	2	99.2	1	98.6	71
14	2	97.6	2	97.6	1	98.8	88
17	2	97.9	2	97.9	1	99.0	94
19	3	97.0	2	98.0	1	99.0	101
21	4	96.2	2	98.1	1	99.1	108
24	6	94.8	3	97.4	6	94.8	117
26	12	81.1	19	85.9	14	89.6	136
28	27	83.7	49	70.9	28	83.5	165

In both trials there was a high level of control at the three concentrations employed. It is of interest to note the fall in level of infestation on untreated animals. The impression is that these infestations were affected by contact with treated animals, particularly where treated calves were suckling untreated control cows. The period of residual protection appears to be of the order of 2-3 days.

No symptoms of toxicity were observed in treated cows or calves.

Spray Race Trials

A number of trials have been conducted involving the application of amitraz formulations by mechanical spray races to study both acaricidal efficiency, safety and 'stripping' behaviour or exhaustion.

In all cases a wash concentration of 0.025% amitraz was used. The herds involved averaged 182 head with a range of 70-483.

In each trial a representative number of animals were taken at random and adult female ticks counted on one side. Half of these animals remained untreated but ran with the treated herd. Inspections were carried out 14, 21 and in some cases 28 days after spraying, when ticks were counted on the untreated animals and on an equal number of randomly selected treated animals.

Table 3 referring to one trial (50% w.p.) illustrates typical figures.

Table 3

Mean counts of adult female ticks per side (Mechanical Spray)

Pre-treatment	14 days Post-treatment	21 days Post-treatment Untreated 27 (2-47)		
54 (23-122)	Untreated 14 (7-19)			
	Treated 0.6 (0-3)	Treated 0		
	96% control	100% control		

Wash samples were taken at intervals during the spraying process. These were submitted for chemical assay in order to study 'stripping' behaviour.

In all trials tick control was of a high order, being 100% in those installations which gave best spray coverage. Where the Mk I e.c. was used however, there was insufficient stability of the wash samples to permit a study of the exhaustion behaviour of the wash, in spite of the addition of calcium hydroxide to the samples. In those instances where the w.p. or the Mk II e.c. were used however, stability was excellent and stripping was shown to be in the order of 30%.

Two trials were maintained to observe the use of the w.p. formulation in spray races over an extended period. In each case the sump was recharged on the occasion of each treatment, assuming complete degradation of amitraz over the period of approximately three weeks between treatments. Tick control was excellent throughout the trial period; the only adverse comment from the co-operators referred to the inconvenience caused by not being able to utilise wash remaining in the sump for treatment of individuals or small groups of animals requiring spraying prior to movement off the property.

In an attempt to overcome this objection and to economise generally in chemical usage long term spray race trials were set up in which calcium hydroxide was added to the wash to raise pH to about 12.0. These trials have given very satisfactory results.

Stabilised Plunge Dip Trials

The first dip was charged in April 1973 and has been studied continuously since that time. A further six trials were set up shortly afterwards. These were located so as to include a range of geographical locations, cattle management systems and standards, and resistant strains of B.microplus.

The 50% w.p. formulation has been used in all seven trials. The initial target concentration was 0.025% amitraz and 0.5% calcium hydroxide. Tick infestation and dip wash concentrations have been monitored at frequent intervals. Dipping programmes and procedures have been those normally employed by the co-operators. Detailed dip logs have been maintained for each trial site.

To the end of June 1975 a total of 133,371 cattle have been dipped including animals of all ages from day-old upwards.

a) Acaricidal efficiency

This has been uniformly excellent throughout; on one property for instance treatment was originally carried out at 21-day intervals. However the extremely high level of tick control achieved with amitraz has led to the adoption of 28-day intervals with continued excellent control. Another trial involves an extensive property (7,000 cattle on 90,000 acres) with a severe shortage of labour. A strategic dipping programme is therefore impossible and many cattle can only be mustered at 6-8 week intervals. Consequently tick burdens have in the past been heavy and during the dry season such infestations combined with poor feed have caused considerable tick worry with a number of deaths. During the trial period there has been a marked reduction in tick burdens with a consequent improvement in condition of the cattle which have gone through two dry seasons with little or no tick worry.

Table 4, referring to trials 15 and 16, shows typical tick counts before and after the commencement of those trials.

Table 4

Mean counts of adult female ticks per side (Plunge dip)

	Tria	1 15	Trial 16			
Pre-treatment	33	5.3	7.0			
	Untreated	Treated	Untreated	Treated		
7 days post-treatment	13	0	4.4	0		
14 days post-treatment	20.6	0	0.6	0		
21 days post-treatment	16.2	0.2	1.4	0		
28 days post-treatment	-	-	0.4	0		
Control	99	9%	100%			

b) Chemical Stability

The stability of amitraz in aqueous media is dependent upon the maintenance of a high pH. In the early stages of these trials measurement of pH of the dip wash was the only method of monitoring used in an attempt to ensure the presence of sufficient calcium hydroxide. It was realised that with the expiry of a positive balance, by stripping and acid-alkali reaction, pH could drop rapidly causing undesirable conditions.

Such an eventuality occurred in trial 15 when, at an inspection seven weeks after commencement and after treatment of over 4000 cattle, there was an apparent change in the dip fluid which, in contrast to the normal sweet smelling brown wash,

had become black in colour with a strong putrid smell and a pH of 7.3.

A few days previously a dip-side assay method for calcium hydroxide had been adopted. This demonstrated complete exhaustion of calcium hydroxide and laboratory assay showed that the amitraz content had fallen to 0.012%. No hydrated lime had been added to the vat since the initial charge with a target concentration of 0.5%.

There was a similar episode in the same vat when floods and monsoon rain had prevented dipping for a period of three months. Whilst pH was apparently maintained over that period there was a marked fall in the calcium hydroxide content with an apparent loss of amitraz. The fluid again became black and foul-smelling, pH had fallen to 10.5, lime content to about 0.03% and amitraz to 0.008%.

However in all cases where adequate calcium hydroxide has been maintained there has been no evidence of amitraz degradation. Management procedures have been evolved to maintain such a level.

c) 'Stripping' behaviour of amitraz and calcium hydroxide

Stripping is considered to have taken place when the fluid draining off a beast has a lower concentration of active ingredient than that used to spray or dip it. This results in the progressive lowering of the concentration of the dipwash during treatment of a number of animals.

In a long-term dipping trial the amount of chemical used to maintain the concentration at the original level i.e. the topping-up rate, can be used to calculate the stripping rate. Naturally any chemical breakdown, in this case of amitraz or hydrated lime, will appear to be due to stripping. The terms 'usage rate' or 'rate of exhaustion' may therefore be more appropriate.

The amounts of fluid and chemical used over the period of these trials is shown in Table 5_{\bullet}

Table 5

Consumption of materials during 7 dipping trials

Total number of animals treated	133,371
Total volume of fluid used	334,611 litres
Total weight of 50% amitraz w.p.	256 kg.
Total weight of hydrated lime	5,710 kg.
Mean volume of fluid per beast	2.5 litres (2.01-4.58)
Mean weight of 50% amitraz w.p./beast	2.0 g (1.59-3.0)
Mean weight of hydrated lime/beast	43.0 g (34.18-71.52)

Recently field studies have commenced on the use of the Mk II 12.5% e.c. in plunge dipping vats. Three vats previously used for extended trials with 50% w.p. were cleaned out and charged with e.c. Target concentration was 0.025% amitraz, stabilised with hydrated lime at 0.5%. At this stage trials have been set up for 3 months only and 22,250 animals treated. Biological efficacy has been indistinguishable from that experienced with the w.p. formulation.

d) Toxicity

At no time during field trials using amitraz for spraying or plunge dipping have

any symptoms of toxicity been shown. At least 158,600 animals have been treated by these means including many calves from day-old upwards.

One animal is known to have drowned and two have been destroyed as a result of spinal injuries received during dipping.

DISCUSSION

The field trials described in this report demonstrate the outstanding acaricidal efficiency of amitraz used at 0.025% and show that this chemical can be satisfactorily stabilised by the addition of calcium hydroxide. As with most field studies there are features which are observed but which cannot be tabulated. The first of these is the rapid rate of clearance of ticks following treatment. From about one hour after application ticks, especially juveniles, can be seen to be detached and moving on the animal to disappear soon afterwards. Cattle have been seen to be clear of ticks as soon as six hours after treatment.

The second notable feature is the high order of efficiency against all parasitic stages of the tick and thirdly the apparent acceleration of the moulting process which combined with a marked residual acaricidal activity causes the ultimate death of most ticks emerging from the moult. This residual activity appears to be operational against infesting larvae for 7-10 days after treatment.

The experimental use of a variety of topping-up rates during plunge dipping and spray race trials eventually led to the adoption of a simple management procedure in which a "twin-pack" of 500 g 50% amitraz w.p. with 10 kg hydrated lime, as stabiliser, is utilised. For initial charging one of these packs is used per 1000 litres of water. Topping up is at the rate of one pack per 700 litres of water added.

An episode has been described in which the stabilising calcium hydroxide became exhausted when the dip was idle for a period of 3 months. This incident occurred in a northern tropical situation with high ambient temperatures which can be expected to accelerate the normal chemical reaction between calcium hydroxide and carbonic acid which results from solution of carbon dioxide from the atmosphere and from putrefaction of organic pollutants. Some depletion of alkali reserves was also noted during idle periods in dips located in cooler sub-tropical areas but there it was not sufficient to be of concern. It is now the practice to add hydrated lime at the rate of 10 kg per 2000 litres to any vat which will be idle for two months.

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THE SIGNIFICANCE OF FEEDING AND BLOOD-MEAL IDENTIFICATION STUDIES IN THE PLANNING OF SHEEP HEAD-FLY CONTROL MEASURES

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Summary Study of the structure of the proboscis of Hydrotaea irritans and of its feeding position on various media and on animals, shows that it is used in several positions appropriate to suction, filtration, scraping, or direct feeding. In the fully extruded positions the prestomal teeth are seen to be very highly developed, and to penetrate some skin by scraping so that fine blood is available. Using serological precipitin techniques, it was shown that 73.6% of recently-fed flies contained cattle blood, 19.3% contained sheep blood and 10.6% rabbit blood. As the proportion fed may be 20% or lower it is suggested that treatment of cattle will have a greater effect on total H. irritans numbers than will treatment of sheep.

INTRODUCTION

Effective control measures against an insect such as the sheep head-fly, Hydrotaea irritans, depend on a correct interpretation of its biology and behaviour. Previous work elucidating the development of this pest species has shown (Tarry, 1973) that, as it both develops and congregates away from farm areas to a great extent, traditional insecticidal control measures concerned with treatment of farm buildings and manure disposal areas, will have very little effect. Either fly contact must be avoided entirely, by grazing livestock from June to August away from affected pastures, or a treatment must be used which affects the flies at the only time of contact with livestock, during feeding excursions.

This report gives a summary of experimental work examining the feeding behaviour of H. irritans, and discusses possible control techniques in the light of the results obtained.

Structure and function of the proboscis

METHODS

The proboscis structure of <u>H. irritans</u> was examined using conventional high power methods to study mounted preparations, and using a scanning electron microscope technique. When study of the evaginated proboscis was carried out, it was found useful to kill the specimens rapidly by placing them in deep-freeze conditions.

The investigations of the functional positions of the mouth parts were based on

a number of techniques. Caged <u>H. irritans</u> adults were allowed to feed on blood smears on microscope slides, and on blood-agar gel plates in petri dishes. Caged wild-caught flies were also studied feeding on restrained guinea-pigs with areas of the abdomen recently shaved and were observed in containers under low power as they fed on sugar and blood films.

RESULTS

Simple anatomical examination reveals that the proboscis of $\underline{\text{H. irritans}}$ is similar superficially to those of most muscid flies. It is an erectile structure consisting of rostrum, haustellum and oral disc. The feeding process is facilitated by adaptation of the position and use of the oral disc, depending on the varying nature of the substrate.

The substrate itself is seen frequently to consist of free liquid, such as would be found on mucous surfaces (as on the face of livestock), but can also consist of non-fluid surfaces such as dried scabs, exposed skin, or, experimentally, dried sugar films. The oral disc at rest is maintained in the closed position, but for all feeding purposes the two 'leaves' of the labella are separated to varying degrees corresponding to different media. When feeding on dried or near-dried films of blood, the 'filtering position', as described by Graham-Smith (1930) for Calliphora erythrocephala, is most usual. Here the labellae are opened to present a flattened suctorial surface, the pseudotracheae being applied to the food which enters the oral aperture by way of the interbifid grooves. Figure 1 is a photomicrograph of an impression made during feeding on a microscope slide bearing a thin and recently-dried blood smear; the labellum is seen to have been so closely applied that the angled fissures of the pseudotracheae are closed and the prestomal sulcus has been obliterated. Where thicker films, or moister ones are used, the thin disc is pressed less closely to the surface and the impression is missing at the centre.

In other muscid species a further series of labellar positions has however been described, brought about by progressive contraction of the retractor muscles, leading finally to the prestomal area being fully extended. The lateral processes are drawn back against the sides of the haustellum to a greater or lesser extent, bringing the prestomal area forward as described by Graham-Smith. This brings about the scraping position, when the prestomal teeth at the centre are brought forward until they are able to make contact with the food surface, and then by full extension the direct-feeding position, when the oral aperture is fully exposed, the filtering apparatus is out of action, and liquid and particulate food is drawn directly into the crop (Fig.2). The experiments carried out have confirmed that these processes are also present in Hydrotaea irritans.

When H. irritans adults are permitted to feed on blood-agar gel plates the response is very different from that first described. Instead of secreting a drop of saliva on the surface, as is seen with Musca and Lucilia, the mouth parts penetrate deeply through the gel, leaving only an almond-shaped puncture. This corresponds to the 'direct-feeding' position used for absorbing liquids. When the shaved abdomen of a guinea-pig was presented the flies eventually located a point of feeding activity at which 'scraping' of the skin was initiated and within 2-3 hours of increasing feeding activity they had penetrated the epidermis entirely, so that blood was available. A spot of blood placed on the unbroken skin accelerated the process as feeding activity was intense from the beginning.

The result of the stereo-scan studies illustrates the reason for the efficiency of this feeding/scraping process. The prestonal teeth are greatly enlarged compared even to those of Calliphora, recognised as a scraping feeder, and although the first

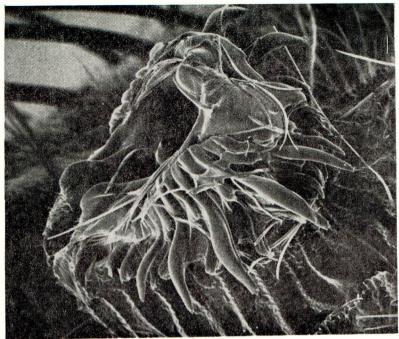
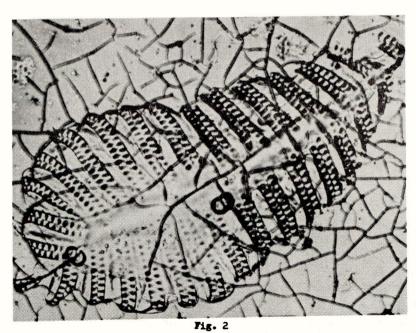


Fig. 1
H.irritans:proboscis,direct-feeding position



H_irritans:imprint on blood smear,filtering position.
575

row is the largest, four rows of decreasing size appear to be present (Fig.2). The open oral aperture is clearly seen in this 'direct-feeding' position. In the scraping position the retractor muscles would be less withdrawn, the prestomal teeth projecting outwards to loosen particles at the surface.

DISCUSSION

It appears from the above experiments that <u>H. irritans</u> cannot penetrate skin, even as thin as that of a guinea-pig, directly. It has, on the other hand, a very efficient scraping armature which may be brought into use. Feeding is a suctorial process, either on simple liquids in the filtering position, or by scraping and the action of salivary enzymes for more solid materials.

From the structure observed, and illustrated (Fig.2), <u>H. irritans</u> falls into Makhanko's third muscid group, (Makhanko 1973) that of haematophagous flies capable of scratching a wound as well as taking free blood. There appear to be, however, in this case more than the single row of specialised teeth which he postulates. The injury seen to be caused by the activity of this fly, where a drop of blood was left by a biting species such as a tabanid, suggests they can be a more serious pest than may have been realised, and are certainly sometimes able to extend existing lesions.

As the secretion of saliva and resorption into the crop is an integral part of the feeding process either in filter (liquid) or in 'scraping' methods of feeding, there is a potential for disease transmission which bears out the claim of the importance of the species in the spread of summer mastitis and other diseases (Sorensen 1974; Pedersen 1973).

Experimental work on food sources

Following preliminary tests in 1973, <u>H. irritans</u> adults were collected selectively from areas of different types to determine the main food sources in these habitats. Identifications of the blood-meals taken by 643 flies were made by Dr. P. Boreham of Imperial College London, using the serological technique developed for mosquitoes.

METHODS

Standard reference antisera, prepared in rabbits, were available to identify most common groups of vertebrate, and tests were carried out by the precipitin-ring method (Boreham 1972) to show whether the collected flies had fed within the previous 4-5 days, on any of the possible hosts within the area of the pasture or woodland from which they were obtained. Before testing they were stored with a silica gel desiccant, to prevent degradation of the protein fractions during transport.

As <u>H. irritans</u> are known to feed eagerly on the facial surfaces of ungulates, tests were carried out to determine whether the presence in their alimentary canal of tears, nasal secretions or saliva, which contain some diluted blood proteins, could affect the precipitin test result. The standard antisera showed no reaction either to extracts of flies fed on eye, nasal or salivary secretions, nor to various dilutions of the secretions themselves.

RESULTS

The results of the 1974 blood-meal identifications are shown in Table 1. The categories are self-explanatory, the "unidentified bovid" representing flies which gave no response to previous categories, but which gave a slight precipitate with a broad-spectrum bovid-sensitive antiserum. It is assumed that this identifies individuals which may have fed some days before on either cattle or sheep. In the results shown, as in the smaller number of 1973 tests, about four times as many flies had fed on cattle as on sheep blood (Tarry & Kirkwood 1975) and it is probably reasonable to divide the residue of 'unidentified bovid' reactors in the same proportion, giving a further 16.8% of cattle-positive and a further 1.2% of sheep-positive flies, and bringing the respective totals to 73.6% and 19.3%, compared with 80.5% and 15.0% in the 1973 test which included only 82 flies.

DISCUSSION

These results show certain major features of interest:

- 1. The great majority of <u>H. irritans</u> studied over two years and a number of areas, had been feeding on blood from cattle, and few had fed on sheep despite the emphasis given by the farming community to the association between sheep and the fly. Sheep are largely fleece-covered, and it may be that this result is a measure of the more ready availability of blood from cattle. Not only are cattle readily available and attractive hosts but are also continually bitten by <u>Tabanidae</u>, <u>Stomoxys</u> and other blood-feeding species. Cattle feeding still predominated even among flies caught one to two miles inside a forest reserve area.
- The result indicates a great potential for contact between H. irritans and cattle blood, so that these insects will inevitably represent a vehicle for disease transmission.
- 3. The high figure of 10.6% of H. irritans feeding on rabbit blood (up to 33.3% in one location) approaches that on sheep and is clearly of interest especially as no other wild host has been identified in over 700 flies. It indicates that widely different mammals may act as normal food sources for H. irritans which, especially in remote areas, will not therefore be dependent on farm livestock for its protein requirements. It is also suggested, on the basis of this result that in the summer months H. irritans must be taken into account in the transmission of myxomatosis (in addition to fleas and mosquitoes) especially as the eye (for example) can be a favoured feeding area.

Significance of results on feeding, to techniques of control

The control of a free-living pest insect such as $\underline{Hydrotaea}$ may be approached in the following ways:

- 1. Interference with the growth cycle, generally during early development.
- Interruption of the cycle by attack on the adult in its natural habitat, for example by treatment of resting sites.
- 3. Attack on the adult in its pest situation, for example by treatment of potential hosts such as cattle or sheep using insecticides or repellents. With H. irritans, as with Lucilia sericata, it is not practicable nor desirable to carry out the extensive treatments which would be required to control larvae in the soil or adults through their resting sites in woodlands. Where the use of

Table 1. H. irritans blood-meal identifications (Northumberland) 1974 results

Sample	Positive		e	Bovid		Sheep/Goat		"Unidentified Bovid"		Rabbit		Unidentified mammal, and horse	
	Total flies	Total	%	Total	Z	Total	%	Total	Z	Total	%	Total	15
2	19	3	15.8	1	33.3	0	0	1	33.3	0	0	1	33.3
4	84	18	21 .4	9	50.0	0	0	5	27.8	3	16.7	1	5.6
5	36	15	41.7	7	46.7	5	33.3	3	20.0	0	0	0	0
6	108	24	22.2	18	75.0	3	12.5	3	12.5	0	0	0	0
8	.177	24	13.6	12	50.0	5	20.8	4	16.7	2	8.3	1	4.2
9	41	18	43.9	10	55.5	3	16.7	4	22.2	1	5.6	1	5.6
10	96	21	21.9	9	42.8	0	0	4	19.0	7	33.3	1	4.8
11	36	11	30.6	5	45.4	1	9.1	3	27.2	1	9.1	1	9.1
12	7	2	28.6	1	50.0	0	0	1	50.0	0	. 0	0	0
13	39	6	15.4	3	50.0	3	50.0	0	0	0	0	0	0
Total	643	142	22.1	75	52.1	20	14.1	28	19.7	14	9.9	6	4.2

a susceptible breed of sheep is unavoidable it will be necessary to attack the flies by the third method, that is by treatment of the livestock themselves.

Nature of treatment

The frequency of feeding by any one fly may be expected to vary depending on climatic conditions and various biological factors, and results which have been given for the proportion of recently fed flies, (varying between 20% and 70%) show how important such factors can be. The low overall feeding rate for the 1974 season, at 20.5% possibly reflects the cold weather of the early summer in that year, the 1973 figure of 53.6% across 6 collecting areas, was obtained during a late warm period.

On the assumption that the precipitin test can be relied upon to show clearly the presence of mammalian blood for a period of only 3 days after feeding, a positive result for cattle blood in 70% of fed flies (equivalent to about 50% when 70% of flies are shown to have fed) indicates that about half the fly population have visited cattle during this 3-day period. In contrast, where 19% of fed flies contained sheep blood only 13.3% of the population would have visited sheep during the three days, and it would take $3\frac{1}{2}$ weeks for the entire population to have visited sheep.

As the proportion of fed flies in the population is generally much less than 70%, the interval between visits to sheep could, on this basis, readily fall to over 7 weeks (35% of flies blood-positive) while cattle were still being visited every 12 days.

As the interval between emergence in mid-June and oviposition in early August is approximately 6 weeks, it is essential to select a treatment which can make contact with all maturing female flies (virtually all the population by August) during that period. In those areas having the highest rate of feeding on sheep blood, only two feeding visits to sheep may have been made during this period; where the rate is lower this will be reduced to a single visit or none at all. To ensure that there is an effective reduction in the number of flies surviving to breeding age, every flock in an 'epidemic' area would need to be effectively treated throughout the whole of the period in question.

A fly has been shown, however, to visit cattle up to 9 times during the preoviposition period, and at the lowest feeding rate at least three visits would be
made. Under the conditions of a normal, mixed grazing area, therefore, the
efficient treatment of grazing cattle herds by only a quarter of farmers (those aware
of the losses to sheep) should be effective in reducing the fly population at once,
and consequently (as it is a single-generation insect) during the following year
also through the limiting of ovipositing flies. Suitable insecticidal treatment of
grazing cattle through the summer may be expected also to reduce greatly the incidence, for example, of summer mastitis. There is strong evidence to link this
seasonal disease with the feeding of H. irritans, (Sorensen 1974, Bahr 1975) and
Achnelt (1955) has shown that the occurrence of this disease can be reduced by 90% by
insecticide treatment.

A number of difficulties must be borne in mind if the treatment of livestock, especially cattle, on a wider scale is considered. For example, where milking herds represent a high proportion of cattle present, there will be widespread limitation on the application of pesticides. There are also residue restrictions where beef animals are concerned. It is still important to bear in mind that, as shown in the border areas (T. Hunter, 1972) and is also evident in Hydrotaea-infested areas of Denmark, sheep of a "downs" type, with thickly-wooled heads, are virtually immune to the development of head-fly lesions.

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