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A New Fly Repellent and a Blowfly Dressing.

Preliminary Report.

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EVERYONE who is acquainted with the problem of myiasis in sheep and other animals and also in man, realises the importance of a satisfactory fly repellent, and much work has been done, particularly by Bishopp and his associates in the United States, to find a suitable substance.

In a search for fly repellents the author has examined some oils of common South African plants and wishes to record in this article some preliminary work on one such oil, which appears to be a very effective blowfly repellent, and also to report on tests of certain chemicals which are very toxic to maggots and which could be used in combination with a repellent for the treatment of myiasis.

TAGETES OIL AS A FLY REPELLENT.

The plant *Tagetes minima* (popularly known in South Africa as "khaki bush") is a common weed which is a cause of trouble in maize and other lands in most parts of the Union. It grows on poor, dry soil to a height of a few inches while, in more favourable circumstances, it may reach a height of seven feet and the main stem bears a number of branches, each developing a cluster of small, pale yellow flowers at its tip. If the plant is cut it develops new branches and usually forms a more dense growth than before. The flowering season is towards the end of summer and during the winter the plants die off.

This plant contains a strongly-smelling volatile oil, or rather a mixture of oils, in its leaves, flowers and seeds. The oil can easily be obtained by steam distillation and the yield of the flowering plant is about 0·5 per cent. of the total weight.

A few years ago it was maintained by a certain writer in the daily press that this plant contains an active principle which was effective for treatment against internal and external parasites. The present author made careful tests with the distilled oil and also with infusions and decoctions of the whole plant against internal parasites such as *Haemonchus contortus* and other gastro-intestinal parasites of sheep and, together with Mr. Bedford of this Institute, tested the material against *Psoroptes communis ovis* and *Melophagus ovinus* on sheep and ticks on calves. In all cases the results were entirely negative with the exception of a slight anthelminthic effect in some cases.

In order to test the fly repellent properties of the oil various experiments were made.

A. Tests with Treated Bait.

1. The first test was made by dividing the liver of a slightly decomposed sheep carcass into two equal parts and placing each half into a large, shallow glass dish. Specimen A was the untreated control; 10 drops of Tagetes oil were dropped on different parts of B and the oil soon spread over the whole surface of the liver. The two dishes were placed in the broken shade of trees where blowflies were plentiful. Two days later specimen A was attracting large numbers of flies and contained numerous eggs and young maggots, while B was still quite free of flies, eggs and maggots and the smell of the oil was still quite strong.

2. The second test was made in a way similar to the method used by Bishopp and his co-workers. Glass beakers of one litre capacity were used as containers. Into each was placed moist sand to a height of 5 cm., and on this 60 gm. liver and 60 gm. intestines of a slightly decomposed sheep carcass. The bait was sprinkled over with 1 c.c. of the repellent tested, attempting to spread the fluid as evenly as possible over the bait. The beakers were placed 6 feet apart under a thatch roof open on all sides. Two beakers were prepared for each repellent and there were three controls; they were so arranged as to get the best possible balance, taking into account the effect of light and the possible influence of one specimen on the other. The results are given in the following table:—

		Tagetes Oil.			Oleum Picis (Commercial)			Controls.		
		Flies.	Eggs.	Maggots.	Flies.	Eggs.	Maggots.	Flies.	Eggs.	Maggots
22/1/35	12.0 p.m.	Specimens were set out.								
22/1/35	4.0 p.m.	0	0	0	2M.d./2	0	0	5M.d./3	0	0
23/1/35	9.30 a.m.	0	0	0	1M.d./1	0	0	0	0	0
		Weather cloudy, cool wind.								
	12.0 p.m.	0	0	0	2M.d./1	0	0	2L.s./ 8M.d./3	0	0
		Clouds broken, temperature rising.								
	2.0 p.m.	0	0	0	2L.s./2 2M.d./	+/2	0	6L.s./3 10M.d./3 1S.h.	+3	S.h./1
24/1/35	9.30 a.m.	0	0	0	1L.s./1 4M.d./2	+/2	+/1	9M.d./3	+/3	+/3
	12.0 p.m.	1L.s./1 1M.d./	+/1	0	1L.s./1 5M.d./s	+/2	+/1	3L.s./3 11M.d./	+/3	+/3
	2.0 p.m.	2M.d./1	+/1	0	4M.d./2	+/2	+/2	3L.s./ 21M.d./	+/3	+/3
25/1/35	9.30 a.m.	0	+/1		15L.s./ 20M.d./2	+/2	+/2	20L.s./ 25M.d./3	+/3	+/3

M.d. = *Musca domestica.*
L.s. = *Lucilia sericata.*
S.h. = *Sarcophaga haemorrhoidalis.*
2M.d./2 = 2 M.d. at 2 beakers.
+/1 = Eggs or larvae present in one beaker.

It will be seen that the tagetes oil was very effective, particularly in comparison with oleum picis. The latter does not show up well because the quantities of the repellents used were small, but this was done since it had been determined in other similar tests made previously (not here recorded) that the tagetes oil appeared to be stronger than oleum picis in regard to repellent properties for blowflies.

It is not the intention to record in this preliminary report all the tests that have been made, but to give a few typical results obtained after the first tests made for orientation had been carried out. The work is being continued in various directions and more detail could be given in a later article.

B. Tests in Olfactometer.

Since Tagetes oil has a very strong odour it was thought that this might simply mask the odour of baits used and it was therefore decided to test whether the oil has definite repellent properties for flies.

The type of olfactometer described by Krygsman (1930, 1931) was constructed and tried. It was found, however, that the central box in which the flies are placed was too large and that the flies only accidentally entered the two tubes at the sides. Moreover, it appeared from other tests that the flies do not easily enter into a tube which has no ventilation except at its entrance.

The apparatus shown in Fig. 1 was constructed after several trials and is believed to satisfy the requirements of flies such as blowflies, houseflies and others, which are sufficiently active. The apparatus has also been tried for mosquitoes, but these have the habit of settling down in a suitable spot and do not move about sufficiently to give the desired result.

The apparatus consists of a box A, measuring about 35 by 15 by 15 cm. The entrance is guarded by a gauze " sleeve ", the sides consist of wire screen and the exit is controlled by the sliding door B. This leads into a small box C, measuring 12 by 12 by 12 cm. Its top and front are made of glass, the rest being metal (or wood), and each side contains a circular opening of 7 cm. diameter surrounded on the outside by a short projecting collar. Further are required several tubes, which can most conveniently be made of celluloid, measuring 60 cm. in length with a diameter of 7 cm. They must fit well into the lateral openings of box C. Each tube is provided with four longitudinal openings or " windows " which are covered with gauze cloth and these must be equally large and evenly spaced in all the tubes. These windows provide ventilation and prevent the smell of the repellent tested from spreading through the whole apparatus. When the apparatus is used the windows

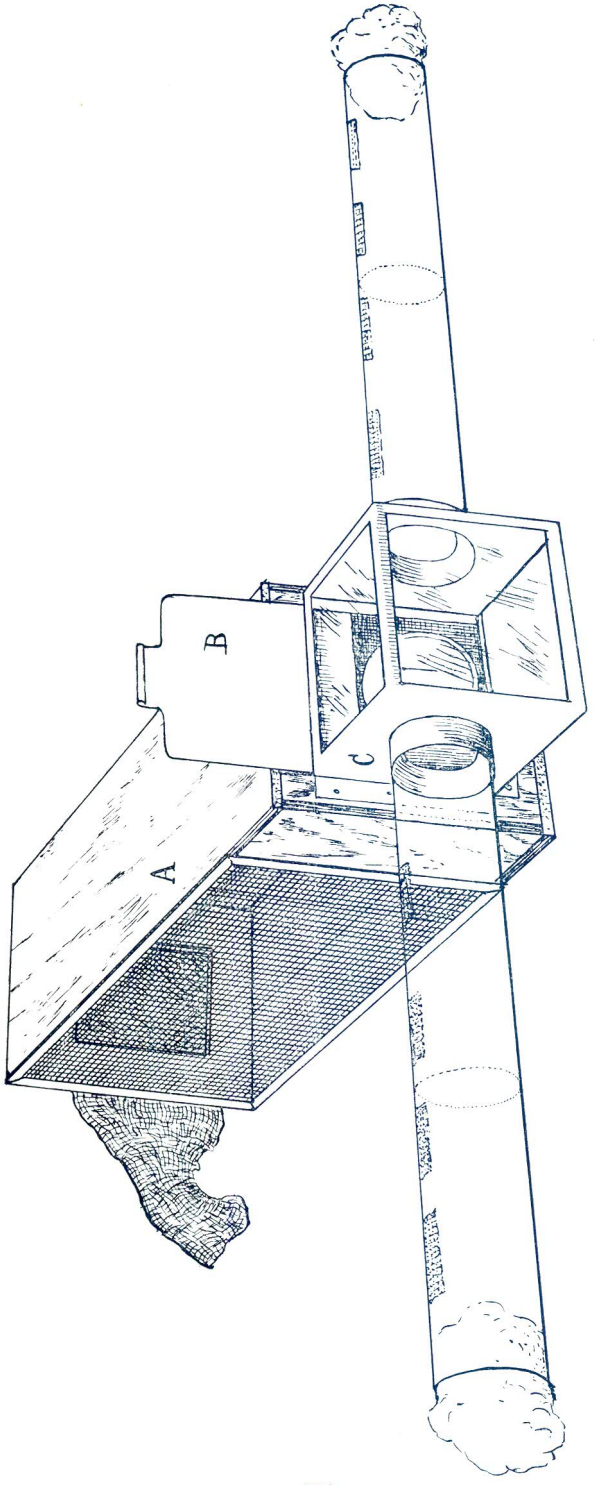

FIGURE 1.

should therefore face upwards. The top and front of box C were at first made of wire gauze, but it was found that the flies would then not readily enter into the tubes.

The apparatus is set up as shown, the ends of the tubes being plugged with cotton wool and the box C facing towards the light. It is best to work in a large room where there is no draught and not too near a window, placing the apparatus in such a position that the light is distributed as evenly as possible over both the tubes. This light factor is very important. About 60-80 flies are placed into box A and they will soon pass through into C. The trapdoor B may now be closed, but as a rule the flies will not return into the darker box A and the open door provides further ventilation, keeping the smell of the repellent from entering the blank tube.

A blank count is first made, after allowing the flies a few minutes to get accustomed to the apparatus. Counts may be made every 30 or 60 seconds and 20 counts are made in all. Only the flies in the distal half of each tube should be counted, and for this purpose it is necessary to draw a line around the middle of each tube. It is convenient in the interval between counts to keep count of the flies in one tube so that at the correct moment attention can be given to the other to register the number there. When repellent has been introduced into one tube the flies in this tube should be watched in order to see how they react to the material when they come near it.

The preliminary blank count will show whether the distribution of light is sufficiently even on both sides. Necessary adjustments should be made. A drop of the repellent is now placed on to a plug of cotton wool and this is slipped into the place of a plug on one side. Beginning after a few minutes 20 counts are made. The plug with the repellent is replaced by a clean one and the flies are then chased back into C by pushing the plugs into the proximal ends of the two tubes; they are further brought into A by turning this side to the light and placing a dark cloth over C if necessary and then closing the door B. Fresh tubes are inserted, the repellent being now placed on the other side, and the flies are allowed to return. The apparatus should be replaced exactly in its previous position. Another 20 counts are made, fresh tubes again inserted and finally 20 blank counts complete the test. Instead of inserting fresh tubes for the second count with repellent the whole apparatus may simply be turned upside down, so that the repellent is now on the other side, but care must be taken to regain the exact previous position with reference to the source of light. The counts are finally summed up and, if necessary, corrections are made as indicated by the blank counts.

Tests with tagetes oil were made as follows:—

1. Flies: *Musca domestica*, *Lucilia sericata*, *Chrysomyia albiceps*, *Chrysomyia chloropyga* and *Sarcophaga haemorrhoidalis*: The blank counts were sufficiently even. Repellent, 68; empty tube, 200.

2. Flies: *Lucilia sericata, Chrysomyia chlorophyga:* Repellent, 55; empty side, 255.

3. Flies: *Lucilia sericata:* Repellent, 75; empty side, 216. In this case a few flies were apparently overcome by the tagetes oil and they remained in the end of the tube in a dazed condition during the rest of the count, being counted each time.

4. Flies: *Lucilia sericata:* Repellent, 65; empty side, 749.

5. Comparative test with oleum picis and *Lucilia sericata:* Repellent, 248; empty side, 347. The oleum picis does not show up well in this apparatus. Flies will even settle down on the plug carrying the material.

It appears therefore that the tagetes oil has definite repellent properties, and it has also been noticed that blowflies approaching a wound which has been treated with mixtures containing the oil will rapidly fly off without settling down.

C. Tests with Larvicides.

It is not necessary here to recount all the tests made with different materials, most of which had been tested before by other workers. No other substance that could be used on a wound was found to be even nearly as effective as carbon tetrachloride and tetrachlorethylene, both known to be strong insecticides.

The tests were made as follows: *Lucilia sericata* eggs were collected by allowing the flies to lay on small pieces of liver. The larvae were allowed to develop in suitable surroundings until they began to migrate from the food material. These fully-developed third stage larvae are known to be the most resistant stage and were used in order to maintain this factor as constant as possible.

As a rule 20 larvae were placed in a suitable tube and the mixture to be tested was poured over them to a depth of about 2·5 cm. The tube was gently shaken and the period of reaction timed by means of a stopwatch. At the end of the time the mouth of the tube was covered with a piece of gauze cloth, the tube turned upside down and shaken so as to drain the fluid off quickly. The larvae were then transferred to tubes containing clean sand and these tubes were closed with gauze cloth. After allowing sufficient time for pupation and emergence of the flies and after the latter had died, the contents of each tube were sived out and the number of dead larvae, dead pupae and flies counted, as recorded below.

In order to ensure satisfactory contact between the material tested and the larvae it was decided to emulsify the volatile substances such as carbon tetrachloride, tetrachlorethylene and

benzine. Oily mixtures, when applied to wounds, have the undesirable effect of softening the tissues and preventing the formation of a healthy scab. Watery emulsions dry off rapidly and only the less volatile portions remain on and around the wound. The mixtures used in the tests recorded below were not all satisfactory emulsions as will be discussed later.

For the sake of brevity the following abbreviations are used:— CCl_4 = Carbon tetrachloride, C_2Cl_4 = Tetrachlorethylene, T.ol. = Tagetes oil, G.ar. = gum arabic, Aq. = Water (distilled).

I.

Mixture.	Time of Reaction in Mins.	Dead Larvae.	Pupae.	Flies.
1. Benzine 10, T.ol. 10, G.ar. 5, Aq. 75.	1	1	2	12
2. ,, 10, ,, 10, ,, 5, ,, 75.	3	0	0	20
3. ,, 20, ,, 10. ,, 5, ,, 65.	1	18	1	1
4. ,, 20, ,, 10, ,, 5, lead acetate 3 % 65.....	3	20	0	0
5. ,, 20, G.ar. 5, Aq. 75.....	3	18	2	0
6. C_2Cl_4 10, T.ol. 10, G.ar. 5, Aq. 75	3	18	0	2
7. Aq. 100 (42 larvae).....	3	0	3	39

It is seen that 10 per cent. benzine even for 3 minutes is not sufficiently effective and compares rather unfavourably with tetrachlorethylene; 20 per cent. gave better results but was disappointing in subsequent tests. The lead acetate was added on account of its favourable effect on wounds and it did not break the gum arabic emulsion.

II.

Mixture.	Time of Reaction in Mins.	Dead Larvae.	Pupae.	Flies.
8. Benzine 20, G.ar. 5, Aq. 75.....	2	12	6	2
9. ,, 20, ,, 5, 3% lead acetate 75...	2	1	0	19
10. C_2Cl_4 20, ,, 5, Aq. 75.....	2	20	0	0
11. ,, 20, ,, 5, 3% lead acetate 75...	2	20	0	0
12. CCl_4 20, ,, 5, Aq. 75.....	2	19	1	0
13. ,, 20, ,, 3, 3% lead acetate 75...	2	12	6	2
14. T.ol. 10, ,, 5, Aq. 85.....	1	1	0	19
15. 3% lead acetate 100.....	2	0	0	20

In this case the lead acetate appears to have counteracted the effect of benzine and to a slight extent also that of carbon tetrachloride, but this is probably not the correct interpretation of the result—see 3 and 4 above and other tests below.
Tagetes oil has no appreciable larvicidal effect. It was decided to try woolgrease as emulsifier, since this would be particularly suitable for use on sheep. Emulsions with lead acetate were, however, not satisfactory and apparently did not bring about satisfactory contact between the insecticide and the larvae:—

III.

Mixture.	Time of Reaction in Mins.	Dead Larvae.	Pupae.	Flies.
16. C_2Cl_4 20, T.ol. 10, woolgrease 5, 3% lead acetate 65	2	5	0	15
17. ,, 20, T.ol. 10, G.ar. 5, 3% lead acetate 65	2	20	0	0
18. ,, 20, woolgrease 5, 3% lead acetate 75	2	0	2	18
19. Benzine 20, T.ol. 10, woolgrease 5, 3% lead acetate 65	2	0	1	19
20. ,, 20, T.ol. 10, G.ar. 5, 3% lead acetate 65	2	3	1	16
21. ,, 20, woolgrease 5, 3% lead acetate 75	2	0	0	20
22. Aq. 100	2	0	0	20

Soap emulsions were now tried and it was found that the substances tested could very easily be emulsified as follows:—Benzine, CCl_4 or C_2Cl_4 50 cc.; Tagetes oil, 25 c.c.—A. Soft soap, 9 gm.; water to make 25 cc.—B. Add successive small quantities of A to B and shake. The thick emulsion is diluted with 150 cc. water, giving the following concentrations: CCl_4, 20 per cent.; Tagetes oil, 10 per cent.; Soap, 3·6 per cent. In these tests 40 larvae were used in each case.

IV.

Mixture.	Time of Reaction in Mins.	Dead Larvae.	Pupae.	Flies.
23. C_2Cl_4 20, T.ol. 10, soap 3·6, Aq. ad 100	1	5	0	35
24. ,, ,, ,, ,,	2	27	3	10
25. ,, ,, ,, ,,	3	24	6	10
26. CCl_4 20, ,, ,, ,,	1	10	7	23
27. ,, ,, ,, ,,	2	34	5	1
28. ,, ,, ,, ,,	3	39	1	0
29. Benzine 20, ,, ,, ,,	1	2	1	37
30. ,, ,, ,, ,,	2	0	3	37
31. ,, ,, ,, ,,	3	2	1	37
32. Water	3	1	2	37

The above test showed, as had been noted before, that CCl_4 and C_2Cl_4 were more effective larvicides than benzine. Since the temperature of the sheep's body would probably have an influence on

the reaction by increasing the respiration of the larvae, the above test was repeated with the modification that the emulsions were warmed in a water-bath to 28° C. before being poured on to the larvae. In each case 20 larvae were used.

V.

Mixture.	Time of Reaction in Mins.	Dead Larvae.	Pupae.	Flies.
33. C_2Cl_4 20, T.ol. 10, soap 3·6, Aq. ad. 100...	1	7	1	12
34. ,, ,, ,, ...	2	19	1	0
35. ,, ,, ,, ,, ...	3	20	0	0
36. CCl_4 20, ,, ,, ,, ...	1	4	11	5
37. ,, ,, ,, ,, ...	2	20	0	0
38. ,, ,, ,, ,, ...	3	20	0	0
39. Benzine 20, ,, ,, ,, ...	1	1	1	18
40. ,, ,, ,, ,, ...	2	0	1	19
41. ,, ,, ,, ,, ...	3	2	1	17
42. Water.....	3	0	0	20

With regard to the reactions of the larvae under the influence of these emulsions it was observed that CCl_4 stunned them almost immediately, while C_2Cl_4 appeared to irritate them strongly for a few seconds before they quietened down and became motionless. It was thought that this property of the latter drug might be favourable, since it would cause the maggots to crawl out of deep wounds before they were killed. Tests made on sheep, however, showed that C_2Cl_4 unfortunately irritates the wound and the animal to a similar degree and that it had to be discarded for this reason.

It was further found in testing various emulsions on sheep that the free alkali of soft soap was irritating and the soap emulsions had to be given up. Woolgrease was again tried and satisfactory emulsions could be made in the following way:—Weigh off into a suitable flask 60 gm. woolgrease; measure off (A) 200 c.c. CCl_4 + 50 c.c. Tagetes oil (it had been found that 5 per cent. of this oil is sufficient as a repellent in the mixture); measure off also (B) 700 c.c. water. Add about 20 cc. of A and 100 of B to the woolgrease and shake or stir to emulsify, then add more water and, if necessary, further small quantities of A up to 60 c.c., shake to emulsify and go on adding water until all of B has been incorporated. Then add the rest of A and shake. Owing to the fact that woolgrease contains small quantities of free acid, the emulsion will not keep well. A few drops of phenolphthalein are therefore added—about 10 drops to the above quantity—and the emulsion is neutralised by adding a small quantity of 10 per cent. NaOH solution. After a few days the pink colour of the indicator may disappear on account of the liberation of further small quantities of acid and alkali should be again added in sufficient quantity to neutralise, or sufficient may be added the first time to give a definite pink colour. Since different samples of woolgrease vary in acid content the amount necessary cannot be stated definitely, but 12 c.c. of 10 per cent. NaOH to a litre of emulsion may be an average quantity to go by.

The emulsion may also be made by leaving out the Tagetes oil at first and adding it later to the neutralised emulsion of woolgrease, CCl_4 and water.

Tests with the above emulsion gave the following results:—

VI.

	Mixture.	Time of Reaction in Mins.	Dead Larvae.	Pupae.	Flies.
43.	CCl_4 20, T.ol. 5, woolgrease 6, Aq.ad. 100 (25°C.)	1	20	0	0
44.	,, ,, ,, ,,	2	20	0	0
45.	,, ,, ,, ,,	3	20	0	0
46.	,, ,, ,, (30°C.)	1	20	0	0
47.	,, ,, ,, ,,	2	20	0	0
38.	,, ,, ,, ,,	3	20	0	0
49.	Water.....	3	0	0	20

The above result was so satisfactory that it was decided to repeat the test and also to try 10 per cent. CCl_4 in the same emulsion. In test VI, the room temperature had been 25° C., in test VII it was 21° C., and this was also the temperature of the emulsion (40 larvae used in each case):—

VII.

	Mixture.	Time of Reaction in Mins.	Dead Larvae.	Pupae.	Flies.
50.	CCl_4 10, T.ol. 5, woolgrease 6, Aq.ad. 100 (21°C.)	1	22	2	16
51.	,, ,, ,, ,,	2	22	9	9
52.	,, ,, ,, ,,	3	20	13	7
53.	CCl_4 20, T.ol. 5, woolgrease 6, Aq.ad. 100 (21°C.)	½	39	1	0
54.	,, ,, ,, ,,	1	39	1	0
55.	,, ,, ,, ,,	2	36	4	0
56.	,, ,, ,, ,,	3	40	0	0
57.	,, ,, ,, (30°C.)	½	40	0	0
58.	,, ,, ,, ,,	1	40	0	0
59.	,, ,, ,, ,,	2	40	0	0
60.	,, ,, ,, ,,	3	40	0	0
61.	Water.....	3	0	17	23

CCl_4 10 per cent. is apparently not sufficient and it may be advisable to keep to the 20 per cent. emulsion. The fact that 17 pupae failed to emrge in the case of the control may reduce the value of the test to some extent. However, in Nos. 56-60 the larvae never moved after having been immersed but gradually turned black and dried out.

With regard to its larvicidal effect this emulsion may be considered as satisfactory.

D. Tests on Sheep.

While the mixtures and emulsions described above were being tested on larvae, they were also tested on infested sheep, as already indicated, and the satisfactory larvicidal effect of carbon tetrachloride, as well as the fly repellent properties of Tagetes oil was clearly demonstrated.

The requirements of a good blowfly mixture are: (1) Strong larvicidal effect; (2) absence of irritation and interference with the healing of the wound; (3) prevention of re-infestation until the wound is healed.

The emulsion described above satisfies these requirements to a high degree. The larvicidal effect is eminently satisfactory. The carbon tetrachloride-woolgrease emulsion with Tagetes oil has very slight irritant properties, if any. Some sheep will be uneasy for a few moments after the application of the emulsion, but this passes off very quickly and there is no sign of irritation or interference with the healing of the wound. The writer has applied the emulsion to a fresh abrasion on his hand and noticed no irritation whatever.

The emulsion has been tested on a fairly large number of cases with moderate to very large wounds and in no case did re-infestation occur, except in one sheep which lay prostrate and was repeatedly wetted by rain. This case was re-infected five days after the first treatment. The emulsion breaks soon after it has been applied to the wound. The larvae are killed within a minute and the carbon tetrachloride and water evaporate fairly soon, the length of time required depending on the humidity and temperature of the atmosphere. The wool-grease and Tagetes oil settle down in the wool surrounding the wound and the smell of the oil is in evidence for 10-14 days. The wound is usually dry after 24 hours and heals rapidly.

Tests are now being conducted with this emulsion under different conditions with reference to climate, pasture, etc., and also on cattle subject to attack by the screw-worm *Chrysomyia bezziana*. In one case a farmer treated a number of infested cattle with complete success, no re-infestation occurring. The results of other tests are not yet available but will be reported upon at a later date.

It has been found that the 5 per cent. Tagetes oil can be replaced by 10 per cent. Oleum picis, but cases of re-infestation have occurred when such an emulsion was used.

Summary.

It is shown that the steam-distilled oil of the plant *Tagetes minima* has strong repellent properties for blowflies and that it is suitable for use in a blowfly dressing.

Carbon tetrachloride and tetrachlorethylene are excellent larvicides for use against blowfly maggots, but tetrachlorethylene is irritating on wounds. Both these drugs are distinctly more effective than benzine.

Emulsions of the abovementioned substances are suitable as blowfly dressings and woolgrease was found to be the most satisfactory emulsifier.

Particulars are given in regard to the preparation of a suitable emulsion.

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