AN INSECTICIDE-BAIT-BOX METHOD FOR THE CONTROL OF SYLVATIC PLAGUE VECTORS

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(With Plate 5 and 3 Figures in the Text)

INTRODUCTION

In the sixth R. E. Dyer Lecture, Meyer (1957*a*) has reminded us that sylvatic plague, *Pasteurella pestis* in wild rodents, persists in a number of vast regions throughout the world. Enzootic foci have been found in extensive tracts of the western United States, in western Canada and in Coahuila, Mexico. In South America, foci have been found in Argentina, Venezuela, Bolivia, Peru and Ecuador. China, Mongolia and Transbaikalia have long been known as centres of infection emanating from wild rodent sources, and the Trans-caucasian and south-eastern areas of the Soviet Union have had a long history of struggles with this form of the disease. Plague in wild rodents has attracted the attention of investigators in South Africa, in the Rift Valley of Kenya, and in the Kurdistan province of Iran. The traditional view of plague in India, as a domestic rat-borne disease, must now be revised since recent studies, mentioned by Meyer (1957*a*), showed that sylvatic plague served as enzootic foci in the United Provinces.

The manifest decline of classical rat-borne urban plague, especially in port cities and centres of world trade, and the relative stability of sylvatic plague has suggested that wild rodent plague should now receive more attention in the International Sanitary Regulations of WHO (Kartman, Prince, Quan & Stark 1958), especially since travellers who fall ill with bubonic plague *en route* from an infected locality 'are particularly prone to secondary lung involvement' (Meyer, 1957*a*). In spite of the continuing importance of the sylvatic form of the disease, methods for the control of wild rodent plague yet remain to be established. For the most part, control has been devoted to the decimation of wild rodent populations and one searches a recent comprehensive monograph (Pollitzer, 1954) in vain for reports on the control of epizootic plague by the control of wild rodent fleas. Although some tests have been made on the control of ground squirrel fleas (Stewart & Mackie, 1938; Miles & Wilcomb, 1953; Ryckman, Ames & Lindt, 1953) and the fleas in easily accessible gerbil burrows (Davis, 1951), there has been no investigation devoted to the control of plague vectors affecting the small cricetine and microtine rodents which are now considered to be of fundamental importance as plague reservoirs (Baltazard, Bahmanyar, Mofidi & Seydian, 1952; Heisch, 1956; Kartman *et al.* 1958).

The most extraordinarily extensive eradication of wild rodent plague foci has been reported from the Soviet Union where literally tens of thousands of farmers

in the Caucasian steppes, of the Ordjhonikidze region, were organized into a gigantic operation to kill ground squirrel populations between 1933 to 1939 (Ioff, 1941). It was claimed that by 1936 almost no plague epizootics occurred, and in 1937 no foci were discovered in spite of a widespread investigation. In commenting on these optimistic claims, Meyer (1947) pointed out the disappointing results of the ground squirrel eradication campaign in California during 1914. Any scepticism in regard to the report of Ioff (1941) appears, however, to be dispelled in the recent review by Meyer (1957*b*) on his trip to the Soviet Union. He stated that in certain areas in European Russia these gigantic anti-plague measures with 'Cyanogas' and poison grain had virtually 'liquidated' the squirrel populations and their fleas over millions of acres in the Rostov-on-Don region.

Although these spectacular anti-plague measures in the U.S.S.R. may have been successful, it is doubtful whether a similar programme in the western United States would eradicate sylvatic plague, because it is now known that the highly susceptible colonial rodents such as ground squirrels and prairie dogs are not the primary reservoirs of *P. pestis*. From investigations in various parts of the world, including studies in the San Francisco Bay region, it is now fairly certain that the voles and meadow mice and their fleas constitute the true reservoirs of sylvatic plague. Thus control measures in the United States must be oriented to deal with the small native microtine rodents and their ectoparasites. It must be mentioned here that the methods employed to eradicate the ground squirrels in the Soviet Union also eliminated the fleas '...known to attach themselves to burrowing animals such as voles...' (Meyer, 1957*b*). Whether or not similar results could be obtained in the United States is a purely hypothetical question, and without the present possibility or need to test control operations on such a tremendous scale the problem of wild rodent plague control becomes a question of special, localized, intensive measures in situations directly or potentially hazardous to humans (Kartman, 1956).

The following report presents the results of preliminary investigations with an insecticide-bait-box designed to control wild rodent flea vectors of plague under field conditions in the San Francisco Bay region. The approach to the problem was based upon methods which were developed to control plague vectors affecting Hawaiian field rats (Kartman & Lonergan, 1955). Small portions of the data have been presented elsewhere (Kartman, 1957; Kartman *et al.* 1958), but the present report gives the detailed findings.

METHODS AND MATERIALS

The study was done between June 1956 and January 1958 in a grassy meadow, approximately 485 ft. in elevation, located at the northern end of San Andreas Lake in northern San Mateo County (Pl. 5*a*). This area is a portion of a wildlife refuge and watershed approximately 5 miles south of the San Francisco City and County limits, 1½ miles west of the San Francisco International Airport on San Francisco Bay, and about 2 miles east of the Pacific Ocean. Wild rodent plague has been studied in other portions of the wildlife refuge where epizootics occurred in 1955 and 1957 among the native meadow mice. In general, the area was not

subjected to extremes of temperature and humidity during the study; the monthly mean temperature ranged from 49° F. in January to 61° F. in July, and the monthly mean relative humidity throughout the period fluctuated from 67 % in November to 79 % in July. The rainfall exhibited a much wider range, varying from traces during the summer months (June to August) to about 3 or more inches during each winter month (December to February).

Work commenced on 13 June 1956 when bait-boxes were placed in the field in a grid pattern of 5 lines, each having 5 boxes spaced at 50 ft. intervals (250 × 250 ft.) A check plot, 150 ft. from the bait-box plot, was set up with an identical grid system of live-catch traps. The check plot was used only during the periods in which the effects of DDT were evaluated.

The bait-box (Pl. 5*c*, *d*) consisted of a pine floor board ($\frac{1}{2}$ in. thick, 12 in. long, and 8 in. wide) covered by a U-shaped roof made by cutting a Lard tin ($9\frac{1}{2}$ in. in diameter and $12\frac{1}{2}$ in. deep) in half lengthwise. The tin roof was either nailed to the sides of the floor board or worked into the soil at the sides of the floor. Bait-pans, made from small rectangular sardine-type or round tins, were secured to the middle of the floor board either by a removable nail inserted through their centres into a hole in the floor or by a ring of small nails projecting from the floor. The bait-pans were large enough to accommodate about 100 g. of rolled oats. In contrast to the types of bait-boxes used in Hawaii (Kartman & Lonergan, 1955) the present type emphasized simplicity, ease of handling, and low cost.

From 13 June to 31 July observations were made on the readiness with which wild rodents would enter into and feed in the bait-boxes on rolled oats. During this period, on 3 July, talc powder was placed in a fine layer on the floor boards of the bait-boxes. From 31 July to 9 August rolled oats stained with a dye (DuPont Oil Red; 1–1500 g. bait) were used in the bait-boxes, and live-catch trapping was done to indicate which rodent species were feeding; the methods used to detect stained faeces have been described in a previous paper (Kartman & Lonergan, 1955). On 14 August, two live-catch traps were placed at each bait-box site and rodents were trapped, anaesthetized with ether, and combed over a white enamel pan to obtain flea indices.

On 27 August, a layer of 92 g. of 5 % DDT powder in pyrophyllite was placed on the floor boards of each bait-box. Unstained rolled oats were placed in the bait-pans and trapping was begun on 5 September to observe the effects on flea indices. The bait-boxes were supplied with additional bait and DDT as needed. On 27 September all bait-boxes and DDT were removed from the plot and trapping was continued to observe the residual effects on flea indices. At various times after removal of the DDT, rodent nests were collected, their fleas removed in a modified Berlese apparatus (Miles & Kinney, 1957), and the nest material was sent to the 'Communicable Disease Center's Technical Development Laboratories', in Savannah, Georgia, for DDT analysis. Few nests were collected at any one time because they were difficult to locate and because it was desirable to disturb the plots as little as possible.

On 9 August 1957, 10 % DDT powder was placed in the bait-boxes; the boxes with DDT were removed on 25 September. Observations of flea indices on rodents

were made until 31 January 1958 when the study was terminated. No rodent nests were collected during the period in which the effects of 10% DDT were being evaluated.

DATA AND DISCUSSION

Table 1 summarizes the rodent captures throughout the study. Of the three wild rodents captured, the vole, *Microtus californicus*, was so predominant that the observations must of necessity deal almost solely with this species. The significance

Table 1. *Numbers of rodent captures by species in the DDT bait-box plot and in the check plot from* 15 *August* 1956 *to* 31 *January* 1958

Rodent species	Plot*	No. of captures	% of total by plot	Plots B+C	
				No.	% of total
Microtus californicus	B	1233	75·3	1879	74·7
	C	646	73·6		
Peromyscus maniculatus	B	163	9·9	262	10·4
	C	99	11·2		
Reithrodontomys megalotis	B	240	14·8	372	14·9
	C	132	15·2		
Totals	B	1636	100·0	2513	100·0
	C	877	100·0		

* B, DDT bait-box plot; C, check plot.

Table 2. *Numbers of fleas by species taken off rodents in the DDT bait-box plot and in the check plot from* 15 *August* 1956 *to* 31 *January* 1958

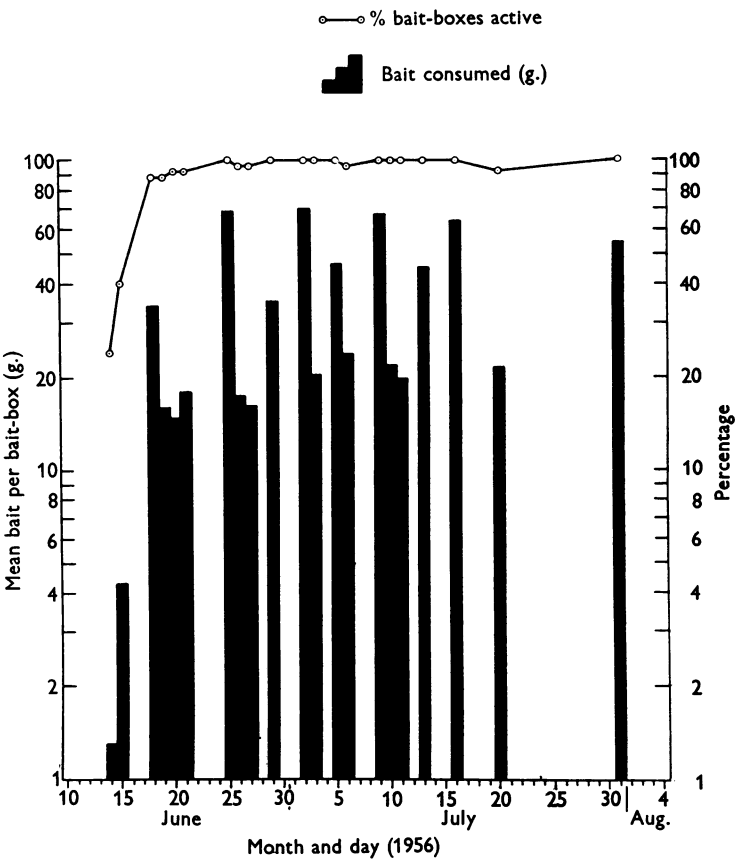
Flea species	Plot*	No. taken	% of total by plot	On *Microtus*		On other rodents†	
				No.	%	No.	%
Malaraeus telchinum	B	1038	68·4	1005	96·6	33	3·4
	C	1879	79·8	1821	96·9	58	3·1
Hystrichopsylla linsdalei	B	331	21·8	315	95·1	16	4·9
	C	245	10·4	234	95·5	11	4·5
Atyphloceras m. multidentatus	B	76	5·1	74	97·3	2	2·7
	C	115	4·8	107	93·0	8	7·0
Catallagia wymani	B	28	1·8	27	96·4	1	3·6
	C	32	1·3	29	90·6	3	9·4
Opisodasys keeni nesiotus	B	44	2·9	2	4·6	42	95·4
	C	81	3·7	6	7·5	75	92·5
Totals	B	1517	100·0	1423	93·8	94	6·2
	C	2352	100·0	2197	93·4	155	6·6

* B, DDT bait-box plot; C, Check plot.
† *Peromyscus maniculatus* and *Reithrodontomys megalotis*.

of this is evident in the light of recent studies which suggest that *M. californicus* is the primary enzootic plague reservoir in the San Francisco Bay region (Kartman *et al.* 1958). The summary of fleas taken from the rodents (Table 2) shows that *M. californicus* was host to over 90% of all fleas collected and was the preferred

host to four of the five flea species encountered. Two of these species, *Malaraeus telchinum* and *Hystrichopsylla linsdalei*, are considered to be the main plague vectors in the area; these two species not only predominated on *Microtus* but accounted for 90 % of all fleas collected. Thus the main consideration was given to these two flea species when evaluating the effects of DDT.

In searching for *Microtus* nests it was observed that the nests were built either above ground within dense clumps of vegetation (Pl. 5*b*) or to about 1 ft. beneath



Text-figure 1. Consumption of rolled oats mainly by *Microtus californicus* and by *Peromyscus maniculatus* and *Reithrodontomys megalotis* in bait-boxes prior to DDT application.

the ground within an intricate tunnel system. Their runways were usually found beneath grass and heavy vegetation. The nests of *Peromyscus* and *Reithrodontomys* were unusually difficult to find. These were the main conditions which dictated the testing of an insecticide-bait-box scheme since other means of dispensing insecticides would have kept most of the material away from contact with the rodent, its burrow, or its nest. As in Hawaii, it appeared that the use of insecticides in the field should be developed on the basis of utilizing the rodent itself as the contactor and disseminator of the insecticide (Kartman & Lonergan, 1955).

The consumption of bait and the degree of activity in the bait-boxes are shown in Text-fig. 1. In most instances enough bait was placed in the boxes to prevent

its complete consumption before additional bait was added. However, when several days intervened between baitings, the initial consumption after the next baiting usually reached a higher level than on subsequent days. This can be seen in Text-fig. 1. In general the graph indicates that, after an initial period of adjustment, the bait consumption rose and was maintained at about 20 g. per box per day. Activity at the bait-boxes was over 90 % throughout the major portion of the test. The use of stained bait during a 10-day period (Table 3) indicated that all three species of rodents were entering the bait-boxes. The use of talc powder on the floor boards had no noticeable effect on the willingness of the rodents to enter the boxes.

The results of these tests suggested that little difficulty would be encountered with either shyness to the bait-boxes or to the use of DDT powder unless the insecticide itself had some repellent effect upon the rodents. DDT was chosen for the initial tests since it still remains a residual insecticide of choice in flea control and since no significant evidence of flea resistance to DDT has been reported (Simmons, 1954).

Table 3. *Test with stained* bait (rolled oats) to determine the proportion of captured rodents in the DDT plot which had entered the bait-boxes (results of six trappings from* 31 *July to* 9 *August* 1956; *prior to DDT treatment*)

Rodent species	No. of captures	With stained faeces	
		No.	%
Microtus californicus	30	23	76·6
Peromyscus maniculatus	12	8	66·6
Reithrodontomys megalotis	3	3	100
Totals	45	34	75·5

* DuPont 'Oil Red' (No. 49875); 1–1500 g. bait.

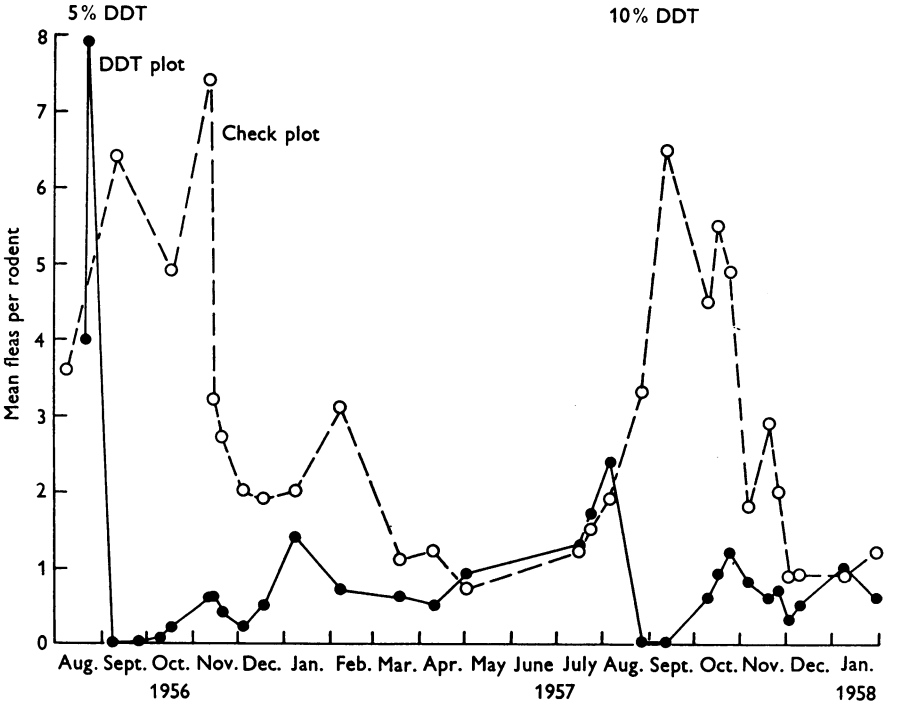
The effects of 5 and 10 % DDT powder on all flea species parasitizing *Microtus californicus* are shown in Table 4. In the case of both insecticidal levels, there was a highly significant immediate drop in the flea indices followed by a gradual return to normal indices over a period of several months. For some unexplained reason, the residual effect of the 5 % appeared somewhat superior to the effect of the 10 % powder. That this was not due to any unusual build-up of fleas is shown in the check-plot data where normal fluctuations appear fairly regular; the indices were at their peaks during the autumn and remained low during the winter-spring period. On the other hand, it may be possible that 10 % DDT had an adverse effect upon the rodents which was not present at the lower insecticide level. Nevertheless, the data show effective flea control on *Microtus* by the bait-box method when exposure to DDT lasted for periods of from 4 to 6 weeks.

The effects on the principal flea species concerned are demonstrated in Text-figs. 2 and 3. Under normal conditions *Malaraeus telchinum* exhibits a more extreme seasonal variation on *Microtus* than does *Hystrichopsylla linsdalei*. The latter species is primarily a nest flea and its index shows peaks of as high as 38 fleas per nest during the winter-spring period (unpublished data), whereas its host index

Table 4. *Effect of* 5 *and* 10 % *DDT powder in bait-boxes on the incidence of fleas* on the native meadow vole,* Microtus californicus (*bait-boxes with* 5 % *DDT from* 27 *August to* 27 *September* 1956; *with* 10 % *DDT from* 9 *August to* 25 *September* 1957)

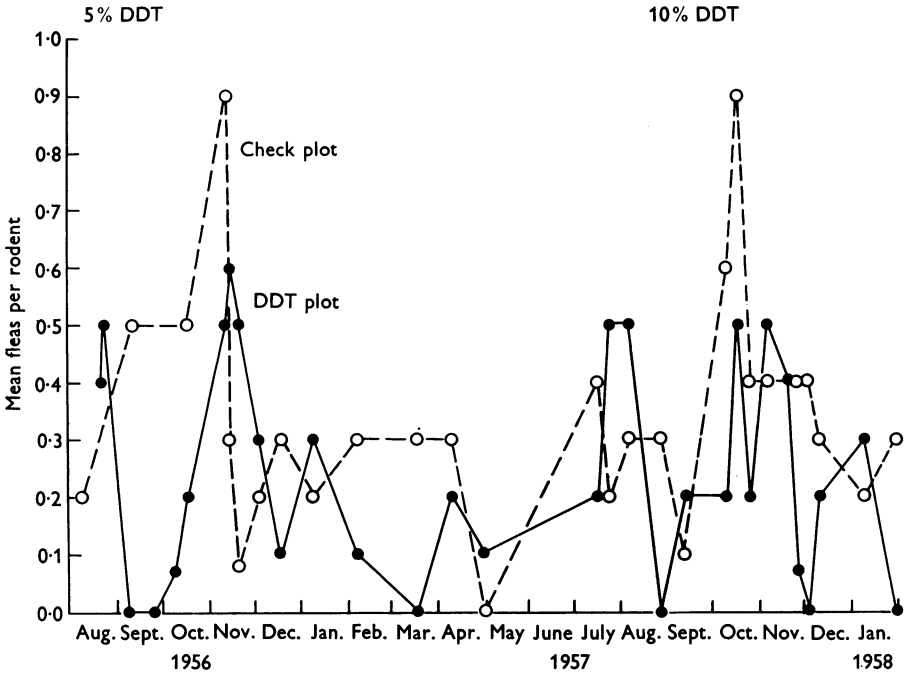
Treated plot				Check plot			
Dates (1956–58)	No. hosts/ no. fleas	Mean fleas	% Hosts infested	Dates (1956–58)	No. hosts/ no. fleas	Mean fleas	% Hosts infested
15–23 Aug.	64/342	5·3	92·2	8–9 Aug.	11/49	4·4	54·5
5–26 Sept.	162/2	0·01	1·2	11–13 Sept.	27/200	7·4	78·3
2–17 Oct.	206/63	0·3	21·4	9–17 Oct.	32/176	5·5	99·9
23 Oct.–27 Nov.	239/274	1·1	50·6	23 Oct.–27 Nov.	109/564	5·2	88·9
11–18 Dec.	36/24	0·7	44·4	11–18 Dec.	31/71	2·3	70·9
8 Jan.–7 Feb.	56/59	1·0	41·4	8 Jan.–7 Feb.	40/126	3·2	82·5
19 Mar.–2 May	63/58	0·9	42·8	19 Mar.–2 May	34/46	1·3	52·9
9–24 July	83/180	2·2	75·9	9–24 July	81/146	1·8	67·9
30 July–6 Aug.	60/189	3·1	85·0	30 July–6 Aug.	51/131	2·5	82·3
21–27 Aug.	27/0	0·0	0·0	13–27 Aug.	39/159	4·1	84·6
12 Sept.–10 Oct.	47/33	0·7	42·5	12 Sept.–10 Oct.	26/160	6·2	88·3
17–25 Oct.	44/71	1·6	52·2	17–25 Oct.	31/192	6·2	93·5
31 Oct.–6 Nov.	36/48	1·3	55·5	31 Oct.–6 Nov.	31/76	2·4	80·6
20–26 Nov.	30/29	0·9	40·0	20–26 Nov.	28/90	3·2	71·4
3–10 Dec.	36/21	0·6	41·6	3–10 Dec.	29/37	1·3	58·6
9–31 Jan.	20/21	1·1	60·0	9–31 Jan.	23/29	1·3	61·9

* Fleas were predominantly *Malaraeus telchinum* and *Hystrichopsylla linsdalei* (see Table 2).



Text-fig. 2. Effect of 5 and 10 % DDT powder in bait-boxes on the occurrence of the flea *Malaraeus telchinum* on the native meadow vole *Microtus californicus*.

on *Microtus* remains fairly restricted throughout the year with an annual mean of about 0·6 and a range of from 0·1 to 0·9 fleas per host. The nest index of *Malaraeus* also is known to rise after the autumn rains, but its incidence on *Microtus* ranges from below one flea per host during the spring season to as high as 7 or more fleas per host in the autumn. Text-figs. 2 and 3 indicate a more clear-cut and positive effect of the DDT on host indices of *Malaraeus telchinum* than on *Hystrichopsylla linsdalei*. After an initial drop, the *H. linsdalei* returned rapidly to its normal index on *Microtus*, whereas the residual effect of DDT on *Malaraeus telchinum* lasted for several months.



Text-fig. 3. Effect of 5 and 10% DDT powder in bait-boxes on the occurrence of the flea *Hystrichopsylla linsdalei* on the native meadow vole *Microtus californicus*.

These differences in the degree of control of *M. telchinum* and *Hystrichopsylla linsdalei* on the host may be more apparent than real. It is known that nest fleas like *Hystrichopsylla* jump upon the host for a blood meal and leave very soon after feeding (Ioff, 1941). They parasitize the host in very small numbers at any one time and are relatively sluggish. On the other hand, *Malaraeus* stay on the host for longer periods, are present in large numbers, and are quite active. These differences are also evident from data on host range of the two species. Although both species have been found to lack a high degree of host specificity, the frequency and percentage occurrence of *Hystrichopsylla linsdalei* on hosts other than *Microtus* have been shown to be much more restricted than for *Malaraeus telchinum* (Miles, Kinney & Stark, 1957; Murray, 1957). Under the same conditions, *M. telchinum* was found on 6 host species in addition to *Microtus*, whereas *Hystrichopsylla linsdalei* was found only on 3 additional host species (Kartman *et al.* 1958). The

available evidence suggests that effective control of *H. linsdalei* must take place in the host's nest.

The findings from *Microtus* nests showed that all flea species could be effectively controlled in the nest with 5% DDT powder in the bait-boxes (Tables 5 and 6). As long as DDT was present in the nests, both adult and immature fleas were kept at a significantly low level. This condition lasted for at least 132 days after the DDT was removed from the treated plot. Thus the flea vectors and reservoirs of sylvatic plague were decimated by contact with DDT after its transport by the

Table 5. *Effect of* 5% *DDT powder in bait-boxes on the incidence of fleas* in nests of the native meadow vole,* Microtus californicus

Treated plot					Check plot				
		Fleas					Fleas		
Days after treatment	No. nests	No.	Mean per nest	Mean mg. DDT per nest	Days after treatment	No. nests	No.	Mean per nest	Mean mg. DDT per nest
8	7	0	0	2·1	20	7	63	9·0	0
83	4	1	0·2	1·1	83	3	44	14·6	0
132	3	0	0	1·0	132	4	46	11·5	0
195	4	134	33·5	0	195	4	154	38·5	0

* Fleas include immature stages; adults were mainly *Malaraeus telchinum* and *Hystrichopsylla linsdalei* (see Table 6).

Table 6. *Species and numbers of fleas found in nests* of the native meadow vole,* Microtus californicus, *from the DDT bait-box and check plots at various times after treatment with* 5% *DDT (only adult fleas are included since the larvae found were not identified)*

	Treated plot. Days after treatment								Check plot. Days after treatment							
	8		83		132		195		20		83		132		195	
Flea species†	No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean
Malaraeus telchinum	0	0	0	0	0	0	22	5·5	13	1·8	9	3·0	7	1·7	9	2·2
Hystrichopsylla linsdalei	0	0	1	0·2	0	0	5	1·2	3	0·4	17	5·6	13	3·2	6	1·5
Atyphloceras m. multidentatus	0	0	0	0	0	0	1	0·2	0	0	17	5·6	0	0	2	0·5
Catallagia wymani	0	0	0	0	0	0	0	0	0	0	1	0·3	2	0·5	2	0·5
Totals	0	0	1	0·2	0	0	28	7·0	16	2·3	44	14·6	22	5·5	19	4·7

* For number of nests used in computing the means see Table 5.

† A single male cat flea, *Ctenocephalides felis felis*, was found in one nest at 8 days in the treated plot.

rodents to their nests. The effective control of *Hystrichopsylla linsdalei* in rodent nests suggests that resistance to DDT was probably not a factor in the apparently poor control of this species on the host (Text-fig. 3). In some cases, considerable residues of DDT were found in the *Microtus* nests (Table 5 indicates the mean values). At 8 days after treatment the DDT in nests varied from less than 0·2 to 4·7 mg. of DDT. At 83 days the range was from less than 0·1 to 1·65 mg. Finally, at 132 days, the DDT varied from less than 0·1 to 2·3 mg. It is of interest to note that none of the nests from the check plot, 150 ft. away, gave evidence of DDT residues. This confirms other studies which have shown *Microtus* to have very restricted movements within its home territory.

The promising results obtained suggest that an insecticide-bait-box method can be developed for use in the field control of fleas affecting cricetine and microtine species. The effects of longer application of insecticide, other insecticides, and the possibility of application to other rodent species and conditions need to be investigated.

SUMMARY

Field trials were conducted in the San Francisco Bay region to test the effectiveness of 5 and 10% DDT powder in rodent bait-boxes against fleas on native wild rodents and in the rodent nests. The principal species concerned were known flea vectors of sylvatic plague, *Malaraeus telchinum* and *Hystrichopsylla linsdalei*, and the meadow vole, *Microtus californicus*, an important plague reservoir. Preliminary trials with the bait-boxes showed that *Microtus* and other small native rodents would enter them quite readily and feed upon rolled oats. About 90 g of either 5 or 10% DDT powder, placed on the floor boards of the bait-boxes, was picked up by the rodents and resulted in effective immediate control of all flea species. Residual control of all flea species on *Microtus* (with the possible exception of *Hystrichopsylla linsdalei*) was obtained over a period of several months. Analyses of *Microtus* nests showed considerable amounts of DDT which had been transported to them by the rodents. All flea species in nests were effectively controlled by 5% DDT for at least 132 days after the DDT was removed from the area.

The field work was performed by Alva R. Kinney and Robert L. Martin. The fleas were identified by Harold E. Stark. The DDT analyses of rodent nests were done by the personnel of the Technical Development Laboratories, CDC, Savannah, Georgia, under the direction of J. A. Jensen.

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EXPLANATION OF PLATE 5

a, General view of treated plot showing San Andreas Lake in background; *b*, location of *Microtus californicus* nest in dense cover; *c*, side view of bait-box showing DDT trails at each end and a *Microtus* leaving box; *d*, end view of bait-box showing bait-pan and DDT powder on floor.

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