

DEVELOPMENT OF RESISTANCE TO DDT BY ANOPHELES SACHAROVİ IN GREECE

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SYNOPSIS

The DDT house-spraying programme carried out throughout Greece between 1946 and 1950 proved highly effective against both the local malaria-vectors (*Anopheles sacharovi* and *A. superpictus*) and other domestic pests. However, during 1947 the housefly (*Musca domestica*) began to reappear in large numbers, and in subsequent years the development of a similar resistance to DDT was noticed among other domestic pests, such as *Culex molestus*, fleas, bed-bugs, and cockroaches. In 1951, signs of a weakening of DDT efficacy against the local malaria-vector *A. sacharovi* were noted for the first time.

A series of controlled observations was made by the authors, from July to October 1952, in the Mórnos district of Navpaktos and the Skála district of Lakonia (Peloponnese). These showed that while the effect of DDT on *A. sacharovi* in the Mórnos district, although presenting signs of weakening, is still sufficiently strong, in the Skála district the vector has developed a considerable degree of resistance to DDT and, it seems, an even higher degree of resistance to Gammexane, although the latter insecticide has never been used for house-spraying in the area. In the authors' opinion, the development of this resistance in the Skála district can be attributed mainly to the systematic application of insecticide by house spraying since 1946.

House spraying with DDT, as shown from its first experimental application in Greece in 1945,⁵ as well as from its subsequent general application throughout the entire country between 1946 and 1950, is highly effective against the local malaria-vectors (*Anopheles sacharovi* and *A. superpictus*) and other domestic pests.

During these years, anopheline catches within sprayed quarters were, as a rule, negative from the day following the spraying, and remained so

during the entire breeding-season of these insects (May to October). Occasionally, a few specimens were caught within sprayed quarters, especially during the early mornings; these anophelines showed obvious toxic symptoms (kinetic ataxia, convulsions, etc.) and died a few hours after being caught.¹⁰ On the other hand, it was noted that anopheline density in the few premises left unsprayed (control stations) in each treated village was usually low and that, not infrequently, despite some fluctuations, complete annihilation of the anophelines was attained.⁹ The immediate consequence was the rapid decline of malaria incidence in Greece to a point when malaria transmission was almost completely checked.^{8, 11}

However, during the second year of the nationwide application of DDT by house spraying, the housefly (*Musca domestica*)—which, in the previous year, had completely disappeared from sprayed quarters—reappeared in high density, being entirely unaffected by the insecticide.^{3, 6} During the following years the same phenomenon occurred successively with other domestic pests (*Culex molestus*, fleas, bedbugs, cockroaches, etc.). However, until the end of the 1950 malaria season, there was not the slightest indication of the development of a similar situation with regard to anopheline mosquitos. During the 1951 malaria season, signs of the lower efficacy of DDT against *A. sacharovi* were observed for the first time in some areas of the Peloponnese.^{1, 2} This fact was reported by Livadas in a brief report submitted by him in October 1951 to the World Health Organization.⁷ The present paper includes more-recent observations made in certain areas—in particular in the district of Skála, Lakonia, in the Peloponnese—from July to September 1952.

Observations

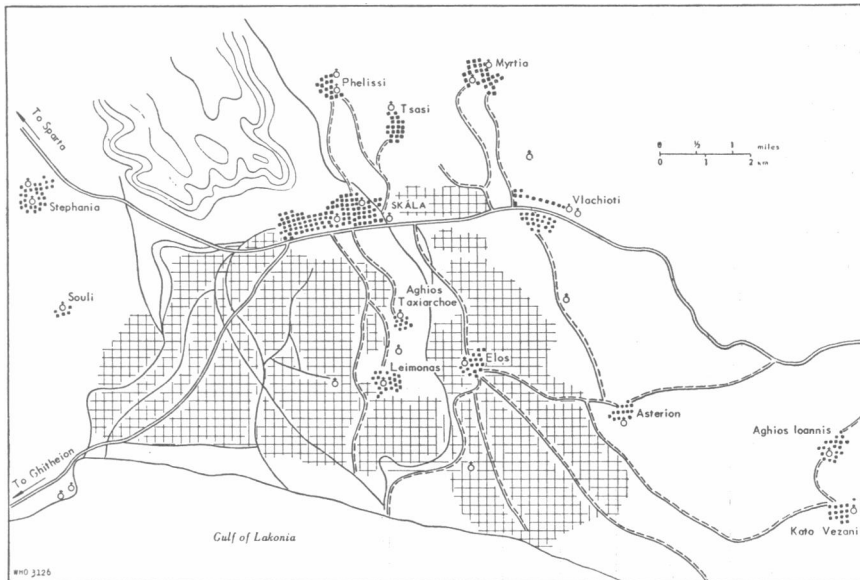
Mórnos, Navpaktos

In various villages sprayed with DDT during 1952, a weakening of DDT efficacy against anophelines was noticed. In the villages of the Mórnos district of Navpaktos, where systematic daily catches were made, it was observed that catches within treated quarters remained negative for from 8 to 12 days after spraying. Subsequently, a few specimens of *A. sacharovi* appeared; and about four weeks after the spraying, the number of mosquitos of this species caught daily within sprayed quarters ranged from 45 to 106 per ten catching-stations. It should be noted that this anopheline density was markedly lower than that observed either in the same villages before spraying or during the same period in unsprayed villages of the area.

Skála, Lakonia (Peloponnese)

The district of Skála is located near the outlet of the river Evrótas, approximately 45 km (28 miles) from Sparta. It includes 13 villages with a total population of 7,182 (see fig. 1). The population is mainly engaged

FIG. 1. DISTRICT OF SKÁLA, LAKONIA (PELOPONNESE)



Rice-fields Indicated by hatching

in rice cultivation, which has assumed great importance in the district during recent years, and in the cultivation of cotton.

Before the DDT-spraying operation, malaria was widely prevalent in this district. Table I shows the fluctuations which occurred in the spleen-rates and parasite-rates of schoolchildren in the village of Skála during the years 1933-8;¹² these figures can be considered as representative of conditions throughout the whole district.

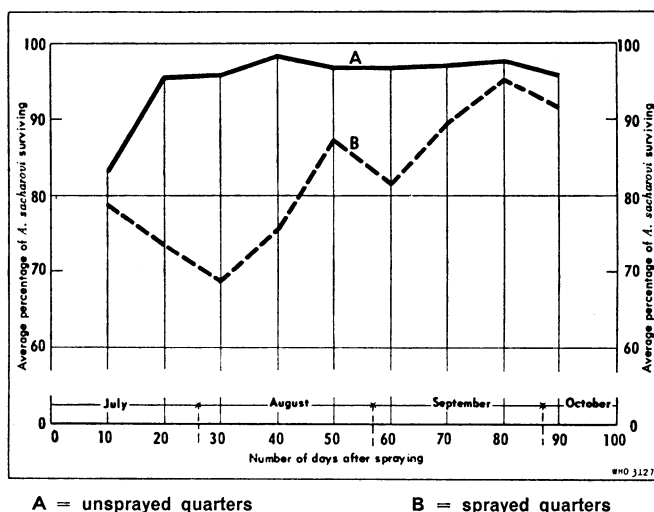
After the application of DDT by house spraying in 1946, malaria rapidly declined, and the parasite-rates of the infant and school-age groups in the village of Skála dropped to zero.¹¹

TABLE I. SPLEEN-RATES AND PARASITE-RATES AMONG SCHOOLCHILDREN IN THE VILLAGE OF SKÁLA, 1933-8

Year	Spleen survey		Parasite survey	
	number examined	spleen-rate (%)	number examined	parasite-rate (%)
1933	99	80	99	38
1934	95	89	95	58
1935	94	89	94	64
1936	100	77	100	34
1937	84	63	84	25
1938	100	83	98	54

Early in 1951, difficulties connected with the procurement of the necessary quantity of insecticides made advisable a reduction in the extended spraying-programme previously applied in Greece. It was thus decided that two areas—namely, Peloponnese and Crete—should, in principle, be excluded from the house-spraying programme and that spraying should be carried out only in areas where careful epidemiological investigations had shown it necessary to take immediate suppressive measures. Accordingly, house spraying with DDT was not applied to the Skála district at first. A little later—about the middle of the 1951 malaria season—the high anopheline density observed in the district, which resulted from the over-developed rice cultivation, indicated the necessity for resuming house

FIG. 2. PERCENTAGE OF *A. SACHAROVII* SURVIVING IN SPRAYED AND UNSPRAYED QUARTERS IN VILLAGE OF ASTERION — I: AFTER FIRST 24-HOUR PERIOD

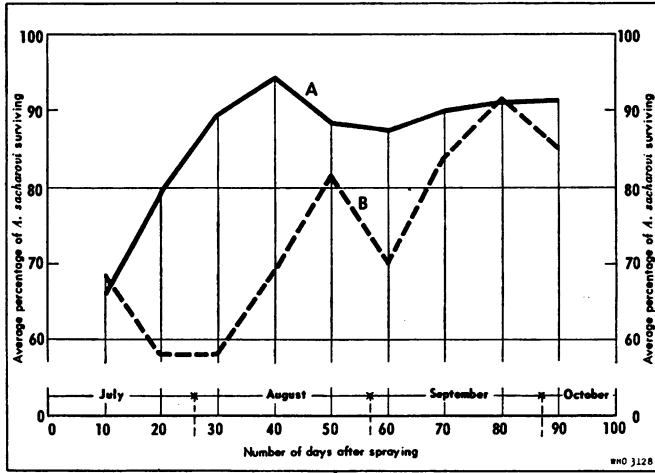


spraying. Nevertheless, it was observed that, in spite of the spraying, the anopheline density in treated premises continued to be unusually high.

The Skála district, therefore, presented appropriate conditions for the carrying-out of more-detailed investigations during 1952. For this purpose, two observation stations with field laboratories were established: the first in the village of Asterion and the second in the village of Souli. Of all the villages in the district, Asterion was the only one where a systematic DDT house-spraying programme was carried out in 1952. The personnel of the stations consisted of two laboratory assistants,^a and three insect-collectors who were provided with a jeep. The work carried out in these two stations was supervised successively by the authors. It should be noted here that

^a Special mention should be made here of the co-operation shown throughout the period of observation by the laboratory assistants, Mr. J. Petrides and Mr. F. Terzis.

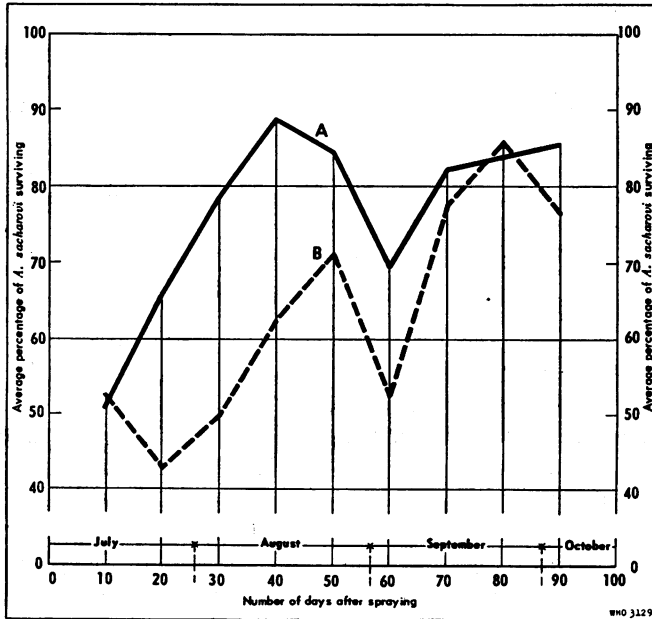
FIG. 3. PERCENTAGE OF *A. SACHAROVI* SURVIVING IN SPRAYED AND UNSPRAYED QUARTERS IN VILLAGE OF ASTERION — II: AFTER SECOND 24-HOUR PERIOD



A = unsprayed quarters

B = sprayed quarters

FIG. 4. PERCENTAGE OF *A. SACHAROVI* SURVIVING IN SPRAYED AND UNSPRAYED QUARTERS IN VILLAGE OF ASTERION — III: AFTER THIRD 24-HOUR PERIOD



A = unsprayed quarters

B = sprayed quarters

TABLE II. SURVIVAL-RATES OF *A. SACHAROVI* CAUGHT IN SPRAYED

Observation period (1952)	Interval between spraying and observation (days)	<i>A. sacharovi</i> caught in sprayed quarters					
		number of tests	number caged	survivors			
				after 24 hours		after 48 hours	
				number	%	number	%
6-15 July	1-10	10	1,835	1,444	78.7	1,202	65.5
16-25 July	11-20	10	1,527	1,124	73.6	888	58.2
26 July-4 August	21-30	10	1,743	1,197	68.7	1,013	58.1
5-14 August	31-40	9	1,471	1,107	75.3	1,015	69.0
15-24 August	41-50	10	1,763	1,540	87.4	1,439	81.6
25 August-3 September	51-60	10	825	672	81.5	578	70.1
4-13 September	61-70	9	1,134	1,014	89.4	954	84.1
14-23 September	71-80	10	1,558	1,483	95.2	1,425	91.5
24 September-3 October	81-90	9	302	276	91.4	257	85.1

transport difficulties, the improvised nature of the installations, and the lack of various facilities had some effect on the progress of the work and, to some extent, prevented its integration into the originally planned programme. Additional difficulties arose from the action of a service of the Ministry of Agriculture which, in compliance with outside pressure, resumed, at various intervals during the year, DDT air-spraying of the rice-fields in the Skála district. However, our observations do not seem to have been seriously affected by this activity.

Village of Asterion. During the period 2-4 July 1951, all houses and stables in the village were sprayed with DDT emulsion, as usual. A dose of 2.0 g of active ingredient per square metre of surface was used. Two stables were left unsprayed as control stations. The village had thus been sprayed for the seventh time since 1946.

1. Two days after spraying, catches made within the sprayed quarters proved positive for *A. sacharovi*, and in the ten stations searched, 110 specimens of this species were found. Then followed a progressive rise of anopheline density in the sprayed catching-stations; about a month after

AND UNSPRAYED QUARTERS IN THE VILLAGE OF ASTERION

		<i>A. sacharovi</i> caught in unsprayed quarters							
after 72 hours		number of tests	number caged	survivors					
number	%			after 24 hours		after 48 hours		after 72 hours	
				number	%	number	%	number	%
968	52.8	10	1,516	1,261	83.2	1,000	66.0	768	50.7
654	42.8	10	1,492	1,426	95.6	1,192	79.9	981	65.8
870	49.9	10	1,606	1,540	95.9	1,442	89.8	1,262	78.6
921	62.6	8	1,080	1,059	98.1	1,018	94.3	955	88.4
1,255	71.2	9	884	857	96.9	780	88.2	746	84.4
432	52.4	10	851	824	96.8	745	87.5	592	69.6
883	77.8	9	646	626	96.9	581	89.9	531	82.2
1,332	85.5	10	661	644	97.4	602	91.1	554	83.8
230	76.2	9	441	421	95.5	402	91.2	376	85.3

the spraying, anopheline density in the sprayed catching-stations approached the level of density observed in the unsprayed ones (1,200-1,500 anophelines per 10 stations). About the middle of September, the natural seasonal decline of anopheline density was noted in both the sprayed and unsprayed stations.

2. During the catches made every morning in the sprayed quarters of the village for the collection of entomological material to be used for carrying out the proposed tests, some dead specimens of *A. sacharovi* were found on the floors and furniture. Of the living mosquitos, some presented advanced toxic symptoms, moving continually from place to place, while others (20%-30%) were seen calmly resting on the sprayed surfaces with no sign whatever of poisoning.

3. The *A. sacharovi* caught^b in sucking-tubes during the daily search in sprayed and unsprayed quarters were transferred to large, wire cages, within which a high relative humidity was maintained by placing wet

^b "Catching" included all mosquitos found, irrespective of whether or not they showed signs of poisoning.

TABLE III. SURVIVAL-RATES OF *A. SACHAROVII* CAUGHT

Observation period (1952)	Interval between spraying of contact- box and observation (days)	Number of tests	<i>A. sacharovi</i> from DDT-		
			number caged	after 24 hours	
				number	%
2-8 August	1-7	7	452	97	21.5
21 August-3 September	20-33	14	742	242	32.6
2 August-3 September	1-33	21	1,194	339	28.4

pieces of cloth on the cage walls. The mosquitos stayed within these cages for 72 hours. At the end of each 24-hour period, the dead specimens found in the cages were withdrawn and the number was recorded on a card. The following observations were made on the results of this test, which lasted from 6 July to 3 October 1952 (see table II) :

(a) After the first 10-day observation period it was noted that a large number of the mosquitos caught in sprayed quarters continued to live longer than 24 hours. The survival-rate was still considerable after a third 24-hour period, and showed an obvious upward tendency as the date of spraying became more and more remote (see fig. 2, 3, and 4).

(b) The same remarks apply to the mosquitos caught in unsprayed quarters except that, in their case, the survival-rate was on the whole higher (see fig. 2, 3, and 4).

Of the 12,158 *A. sacharovi* caught in sprayed quarters in the village of Asterion, 9,857 (81.1%) survived after the first 24-hour period, 8,771 (72.1%) after the second 24-hour period, and 7,545 (62.1%) after the third 24-hour period.

4. A small container of water was placed in the cages into which *A. sacharovi* caught in sprayed places in the village had been introduced. The laying of eggs and the hatching of larvae from them was frequently observed, but no follow-up was possible owing to lack of means and shortage of time.

5. A number of *A. sacharovi* caught in sprayed quarters in the village of Asterion were placed in clean cages. After 72 hours, the surviving mosquitos were transferred, at intervals, to one or other of two special contact-boxes.^c One of these boxes had been sprayed on 1 August 1952

^c These special contact-boxes were first used by Hadjinicolaou for his experiments in the control of the *Dacus* fly.⁴ They are made of wood 5-mm thick, and consist of six separate sides hinged together so that the box can be opened and closed very easily. Mosquitos introduced into this box come into contact with the sprayed interior surfaces. When the contact time is complete, the closed box is placed in a clean cage which has an opening large enough to allow the passage of the hand placing the contact-box in the cage. The contact-box is then opened inside the cage, the mosquitos enter it, and the contact-box is withdrawn.

IN SPRAYED QUARTERS IN THE VILLAGE OF ASTERION

sprayed contact-box				<i>A. sacharovi</i> from unsprayed control-box							
survivors				number caged	survivors						
after 48 hours		after 72 hours			after 24 hours		after 48 hours		after 72 hours		
number	%	number	%		number	%	number	%	number	%	
74	16.4	66	14.6	355	301	84.8	240	67.6	179	50.4	
181	24.4	153	20.6	453	358	79.0	277	61.1	260	57.4	
255	21.4	219	18.3	808	659	81.6	517	64.0	439	54.3	

with DDT emulsion at the rate of 2 g of active ingredient per square metre of surface ; the other was left unsprayed and was used as a control. The mosquitos were left in the boxes for 30 minutes and were then placed in two large, clean cages.

It will be seen from table III, which summarizes the results of this test, that, of the 1,194 mosquitos which had had renewed contact with the insecticide, 28.4% survived after 24 hours, 21.4% after 48 hours, and 18.3% after 72 hours. Of the 808 control mosquitos, 81.6% survived after 24 hours, 64.0% after 48 hours, and 54.3% after 72 hours. It should be noted that the mosquitos surviving this test remained without food for six 24-hour periods owing to the impossibility of feeding them.

6. A number of *A. sacharovi*, which had been hatched in the laboratory from larvae collected from breeding-places in the Skála district, were placed either in the contact-box sprayed on 1 August or in the unsprayed control-box. The mosquitos were left in the boxes for 30 minutes and were then transferred to large, clean cages. As shown in table IV, of the 231 mosquitos that came into contact with the insecticide, 52.4% survived after 24 hours, 41.1% after 48 hours, and 32.0% after 72 hours. Of the 199 control mosquitos, 92.0% survived after 24 hours, 75.4% after 48 hours, and 68.3% after 72 hours. For the reason previously stated, these mosquitos were not fed after hatching.

Village of Souli. This village had been sprayed annually since 1946, but was left unsprayed during 1952.

1. On 27 June 1952, five contact-boxes were sprayed in the Souli laboratory each with one of the following insecticides :

- DDT solution in kerosene, at the rate of 2 g active ingredient per square metre of surface ;
- DDT emulsion concentrate containing 25% DDT, at the rate of 2 g of active ingredient per square metre of surface ;
- Gammexane L.G. 140 (10%), at the rate of 150 mg of active ingredient per square metre of surface ;

TABLE IV. SURVIVAL-RATES OF *A. SACHAROVI*

Observation period (1952)	Interval between spraying of contact-box and observation (days)	Number of tests	<i>A. sacharovi</i> from DDT-		
			number caged	after 24 hours	
				number	%
24 August	23	1	53	37	69.8
25 August	24	1	96	62	64.6
26 August	25	1	60	14	23.3
30 August	29	1	22	8	36.4
24-30 August	23-29	4	231	121	52.4

(d) chlordane (74%), at the rate of 150 mg per square metre of surface;

(e) dieldrin (24%), at the rate of 200 mg per square metre of surface.

The daily catches of *A. sacharovi* were placed in the contact-boxes. After 30 minutes the mosquitos were transferred to large, clean cages. Twenty-four hours later, the dead specimens found in each cage were counted. As in the tests previously described, an unsprayed box was

TABLE V. SURVIVAL-RATES OF *A. SACHAROVI* CAUGHT IN THE VILLAGE

Observation period (1952)	Interval between spraying of contact- boxes and observation (days)	<i>A. sacharovi</i>									
		contact-box sprayed with DDT solution in kerosene				contact-box sprayed with DDT emulsion concentrate (25% DDT)				contact-box Gammexane	
		number of tests	number caged	survivors		number of tests	number caged	survivors		number of tests	number caged
				number	%			number	%		
28 June-7 July	1-10	10	997	2	0.2	9	921	173	18.8	10	1,027
8-17 July	11-20	10	1,003	116	11.6	10	1,069	367	34.3	10	1,078
18-27 July	21-30	9	876	274	31.3	9	825	425	51.5	9	919
28 July-6 August	31-40	10	1,150	505	43.9	10	1,041	556	53.4	10	1,097
7-16 August	41-50	8	752	317	42.2	9	827	395	47.8	8	825
17-26 August	51-60	9	935	471	50.4	9	1,104	622	56.3	8	856
27 August- 5 September	61-70	10*	1,059	557	52.6	9	956	599	62.7	10	1,066
6-15 September	71-80					6	583	330	56.6	6	688
16-25 September	81-90					8	755	520	68.9	8	874
26 September- 5 October	91-100					7	545	377	69.2	7	548

* The test was interrupted at this point.

HATCHED IN THE LABORATORY AT ASTERION

sprayed contact-box				<i>A. sacharovi</i> from unsprayed control-box								
survivors				number caged	survivors							
after 48 hours		after 72 hours			after 24 hours		after 48 hours		after 72 hours			
number	%	number	%		number	%	number	%	number	%		
29	54.7	18	34.0	41	41	100.0	36	87.8	31	75.6		
49	51.0	42	43.8	69	64	92.8	51	73.9	47	68.1		
11	18.3	10	16.7	71	64	90.1	50	70.4	46	64.8		
6	27.3	4	18.2	18	14	77.8	13	72.2	12	66.7		
95	41.1	74	32.0	199	183	92.0	150	75.4	136	68.3		

used as control. The results of each test were recorded on a special card. Table V summarizes by ten-day periods the results of the test, which lasted from 28 June to 5 October 1952. The following conclusions are drawn :

- (a) All the mosquitos placed in the contact-boxes sprayed with chlordane and dieldrin died 24 hours after being transferred to the cages.

OF SOULI, AFTER CONTACT WITH VARIOUS INSECTICIDES

from													
sprayed with L.G. 140 (10%)		contact-box sprayed with chlordane (74%)				contact-box sprayed with dieldrin (24%)				unsprayed control-box			
survivors		number of tests	number caged	survivors		number of tests	number caged	survivors		number of tests	number caged	survivors	
number	%			number	%			number	%			number	%
111	10.8	10	1,055	0	0.0	10	1,010	0	0.0	10	1,288	1,241	96.3
522	48.4	10	1,074	0	0.0	10	1,036	0	0.0	9	950	871	91.7
664	72.3	9	885	0	0.0	9	853	0	0.0	10	1,221	1,168	95.7
952	86.8	10*	1,057	0	0.0	10	1,111	0	0.0	10	1,369	1,337	97.7
713	86.4					9	945	0	0.0	9	1,041	998	95.9
757	88.4					9	974	0	0.0	10	1,264	1,225	96.9
956	89.7					10*	1,074	0	0.0	10	1,166	1,119	96.0
634	92.2									10	1,241	1,195	96.3
791	90.5									9	970	943	97.2
484	88.3									10	1,016	883	86.9

(b) During the first ten-day observation period, almost all the mosquitos that had been placed in the contact-box sprayed with DDT solution in kerosene died after 24 hours. However, as the experiment proceeded, the number of mosquitos surviving more than 24 hours increased considerably.

(c) A considerable number of the mosquitos which had been placed in the contact-box sprayed with DDT emulsion concentrate survived during the first ten-day observation period. The percentage of survivors after each succeeding ten-day period showed a tendency to increase, which developed into a steady increase as the date of spraying of the contact-boxes became more and more remote. During the entire observation period this percentage was steadily higher than that observed in the mosquitos which had had contact with DDT solution.

(d) The mosquitos that had been placed in the box sprayed with Gammexane showed a higher survival-rate than the mosquitos which had had contact with either DDT solution or DDT emulsion concentrate and, from the fourth ten-day period, this survival-rate approached that observed in the mosquitos of the control box (see fig. 5). It should be noted that the area in which these observations were made had never been sprayed with Gammexane.

2. For the sake of brevity, male and female mosquitos are not shown separately in the tables. It is noteworthy, however, that in all the tests the effect of the insecticides on male mosquitos was decidedly greater than on the female mosquitos.

Conclusions

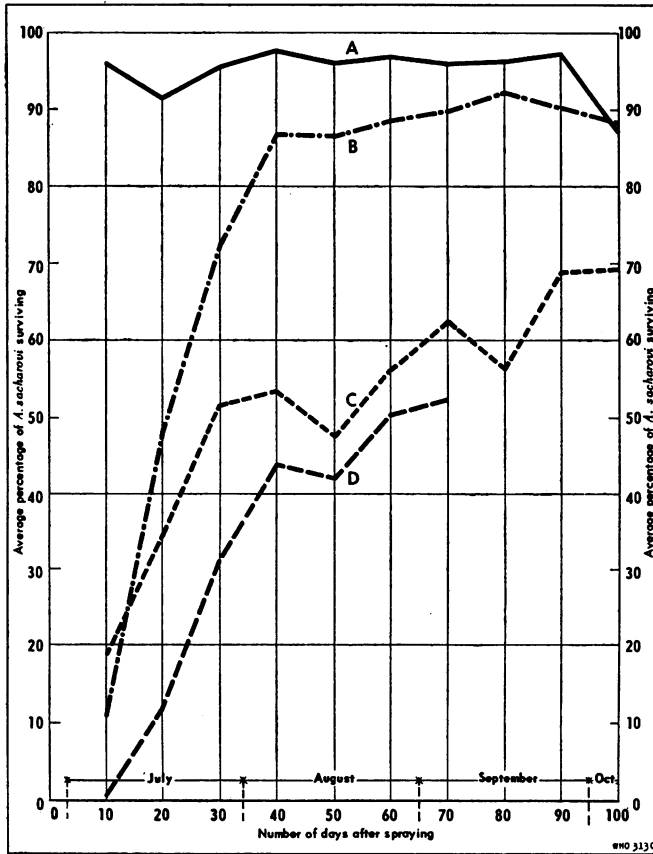
The observations made in 1952 in the Mórnos district (Navpaktos) indicate that the effect of DDT on the local malaria-vector, although presenting signs of weakening, is, however, still sufficiently strong. On the other hand, from the observations made in the Skála district (Lakonia), it appears that the effect of DDT on the malaria-vector of that area is considerably reduced.

The catching of a number of *A. sacharovi* within quarters sprayed with DDT in the village of Asterion two days after the spraying, the progressive increase in the numbers found during the following days, and the absence of toxic signs in a considerable number of these mosquitos are all in complete contrast with observations made in Greece during the years 1946-50. The high survival-rate observed among the mosquitos captured within sprayed quarters in Asterion, as well as the results of other tests made in the Asterion laboratory and in the village of Souli (which remained unsprayed during 1952), leaves no doubt that the local malaria-vector has developed a considerable degree of resistance to DDT and, it seems, an

even higher degree of resistance to Gammexane, although this insecticide had never been used in the area.

The high survival-rate observed during the last phases of the test among mosquitos that had had contact with Gammexane—a rate which approached

FIG. 5. PERCENTAGE OF *A. SACHAROVII* CAUGHT IN THE VILLAGE OF SOULI SURVIVING AFTER CONTACT WITH VARIOUS INSECTICIDES



A = unsprayed control box

B = contact-box sprayed with Gammexane L.G. 140 (10%) : 150 mg per m³

C = contact-box sprayed with DDT emulsion concentrate (25% DDT) : 2 g per m³

D = contact-box sprayed with DDT solution in kerosene : 2 g per m³

With the contact-box sprayed with 74% chlordane (150 mg per m³) and with the contact-box sprayed with 24% dieldrin (200 mg per m³) no survivors were observed (see table V).

that observed among the mosquitos in the control cage—is possibly associated with the shorter residual action of this insecticide.

The resistance developed by *A. sacharovi* in Skála should be attributed mainly to the systematically applied house-spraying in that district

since 1946. However, the possibility cannot be excluded that antilarval air-spraying applied concurrently with the house-spraying has also contributed to this effect. Air-spraying, although irregularly applied in the area during the previous five-year period, probably accelerated in some measure the development of this phenomenon.

RÉSUMÉ

Les pulvérisations de DDT dans les maisons, effectuées dans le cadre du programme de lutte antipaludique en Grèce, de 1946 à 1950, ont eu d'excellents résultats et se sont montrées très efficaces vis-à-vis des deux vecteurs locaux (*Anopheles sacharovi* et *A. superpictus*) et d'autres insectes domestiques. Cependant, depuis 1947, la mouche domestique (*Musca domestica*) a réapparu en grand nombre; au cours des années suivantes, d'autres insectes, tels que *Culex molestus*, des puces, des punaises et des cafards ont présenté une résistance au DDT analogue à celle des mouches. En 1951, pour la première fois, on constata que le DDT semblait perdre de son efficacité vis-à-vis de *A. sacharovi*.

Afin d'étudier ce problème, les auteurs ont effectué une série d'observations et d'expériences, de juillet à octobre 1952. Dans les districts de Mórnos (Navpaktos) et de Skála (Laconie, Péloponnèse), où la résistance des insectes s'était manifestée, ils prélevèrent des moustiques, les mirent en contact avec les insecticides dans des cages d'élevage, puis les transfèrent dans d'autres cages et notèrent la période de survie, qui fut ensuite comparée à celle de moustiques témoins.

Les résultats de ces expériences ont montré que, dans le district de Mórnos, l'effet du DDT sur *A. sacharovi*, bien que présentant des signes d'atténuation, reste suffisant. Dans le district de Skála, par contre, le vecteur a acquis une résistance considérable au DDT et plus grande encore, semble-t-il, au Gammexane, quoique ce dernier insecticide n'ait pas été utilisé pour les pulvérisations domestiques dans cette région. De l'avis des auteurs, l'acquisition de cette résistance, dans le district de Skála, doit être attribuée principalement aux pulvérisations d'insecticides dans les maisons depuis 1946. Il est possible que l'utilisation d'aérosols pour la lutte antilarvaire, bien qu'irrégulière dans cette région, ait contribué aussi à intensifier ou à accélérer l'acquisition de cette résistance.

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