

WORLD HEALTH
ORGANIZATION

a 60287

ORGANISATION MONDIALE
DE LA SANTE

WHO/Mal/80 ✓

22 January 1953

ORIGINAL: ENGLISH

The Secretary of the Expert Committee on Malaria
has the honour to communicate hereunder
the following note:

DEVELOPMENT OF RESISTANCE TO DDT BY ANOPHELES SACHAROVI IN GREECE

by

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House spraying with DDT, as shown from its first experimental applications 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 repeatedly appeared in the case of other domestic pests (Culex molestus, fleas, bed-bugs, cockroaches, etc.). However, until the end of the 1950 malaria season there was not the slightest indication of the presence 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 the senior writer in a brief report submitted by him in October 1951 to the Malaria Section of the World Health Organization.⁷ The present paper includes more recent observations made from July to September 1952 in certain areas, with particular reference to Skála, Lakonia, in the Peloponnese.

In various villages sprayed with DDT during 1952, a weakening of DDT efficacy on anophelines was observed. In the villages of the Mórnos area of Návaktos, where systematic daily catches were made, it was observed that catches within treated quarters remained negative for 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 in the same villages before spraying, or the density observed during the same period in unsprayed villages of the area.

Observations in Skála, Lakonia (Peloponnese)

The Skála area is located near the outlet of the river Evrótas and is approximately 45 kilometres (28 miles) from Sparta. It includes 13 villages with a total population of 7,182 (see fig. 1). The population is mainly engaged in rice cultivation, which has assumed great importance in that area during recent years, and in the cultivation of cotton.

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

After the application of DDT 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

Early in 1951, difficulties involved in connexion with the procurement of the necessary quantity of insecticides made a reduction in the extended spraying-programme previously applied in Greece advisable. It was thus decided that two districts - 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 area at first. A little later - about the middle of the 1951 malaria season - the high anopheline density observed in that area resulting from the too highly developed rice-cultivation suggested that house spraying should be resumed. Nevertheless, it was observed that, in spite of the spraying, the anopheline density in treated premises continued to be unusually high.

The Skála area 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 - one in the village of Asterion and the second in the village of Souli. Of all the villages in the area only in Asterion was a systematic DDT house-spraying programme 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 supervision of the work done in these two stations was made successively by the writers. It should be noted here that transport difficulties, the improvised nature of the installations, and the lack of various

^(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.

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 area. However, our observations do not seem to have been seriously affected by this activity.

Observations in the village of Asterion

During the period 2 - 4 July 1951, the houses in the village were sprayed with DDT emulsion. A dose of 2.0 g active ingredient per square metre of surface was used. Two premises 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 the spraying, anopheline density in the catching centres approached the level of density observed in the unsprayed catching-stations (1,200 - 1,500 anopheline 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 into large wire cages, within which a high

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

relative humidity was maintained by placing wet pieces of cloth on the cage walls. The time mosquitos stayed within these cages was 72 hours. At the end of each 24-hour period the dead specimens found in the cages were withdrawn and the number recorded on a card. The following observations are 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 figs. 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 figs. 2, 3, and 4).

Of the 12,158 A. sacharovi caught in sprayed quarters in 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%) 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 of 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 the A. sacharovi caught in sprayed quarters of Asterion village were placed in clean cages. After 72 hours, the surviving mosquitos were transferred, at intervals, to one or the other of two special contact-boxes. (c) One of these boxes had been sprayed with DDT emulsion at the rate of 2 g active ingredient per square metre of surface on 1 August 1952; the other was left unsprayed and was used as a

(c) These special contact-boxes were first used by Hadjinicolaou for his experiments in the control of Dacus fly. These boxes are made of 5-mm-thick wood, 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 has expired, 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.

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, 21.3% survived after 48 hours, and 18.3% survived after 72 hours. Of the 808 control mosquitos, 81.5% survived after 24 hours, 63.9% 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 area, 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 33.2% after 72 hours. Of the 199 control mosquitos, 91.9% survived after 24 hours, 75.4% after 48 hours, and 68.3% after 72 hours. For the reason stated earlier, these mosquitos were not fed after their hatching.

Observations in the 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:

- (a) DDT solution in kerosene, at the rate of 2 g active ingredient per square metre of surface;
- (b) DDT emulsion concentrate containing 25% DDT, at the rate of 2 g active ingredient per square metre of surface;
- (c) Gammexane L.G. 140 (10%), at the rate of 250 mg active ingredient per sq. metre.

- (d) Chlordane 74%, at the rate of 150 mg per square metre of surface;
- (e) Dieldrin 24%, at the rate of 250 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 described above, an unsprayed box was used as control. The results of each test were recorded on a special card.

Table V summarizes by 10-day periods the results of the test, which lasted from 1 July to 10 October 1952. The following conclusions are drawn:

- (a) All the mosquitos placed in the boxes sprayed with Chlordane and Dieldrin died 24 hours after being transferred to the cages.
- (b) During the first 10-day period of the observations almost all of the mosquitos that had been placed in the box sprayed with DDT solution 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 box sprayed with DDT emulsion survived during the first 10-day observation period. The results of each succeeding 10-day period showed that the percentage of these mosquitos surviving had an obvious upward tendency which increased steadily as the date of spraying 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 had a higher survival-rate than the mosquitos which had contact with either DDT solution or DDT emulsion and, from the fourth 10-day period, this percentage approached the survival-rate 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 this year in the Mornos area (Návpaktos) 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 area, (Lakonia), it appears that the effect of DDT on the malaria vector of that area has considerably reduced.

The catching of a number of A. sacharovi within quarters sprayed with DDT two days after the spraying of Asterion village, 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 of Asterion village, as well as the results of other tests made in the Asterion laboratory, and in the village of Souli (which remained unsprayed during 1952) leave no doubt that the local malaria-vector has developed a considerable degree of resistance to DDT, and it seems even in a higher degree, 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 - which approached the rate 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 mainly attributed to the systematically applied house-spraying in that area since 1946. However, the possibility cannot be excluded that antilarval airspraying applied concurrently with the house-spraying has also contributed to this effect. Airspraying, although irregularly applied in the area during the previous five-year period, probably accelerated in some measure the development of this phenomenon.

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TABLE I. SPLEEN- AND PARASITE-RATES AMONG SCHOOLCHILDREN IN SKÁLA, 1933-1938
TABLEAU I. INDICE SPLENIQUE ET INDICE PARASITAIRE CHEZ LES ÉCOLIERS DE SKÁLA, 1933-1938

Year Année	Spleen survey Détermination de l'indice splénique		Parasite survey Détermination de l'indice parasitaire	
	Number examined Nombre d'écoliers examinés	Rate Indice (%)	Number examined Nombre d'écoliers examinés	Rate Indice (%)
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

TABLE II. SURVIVAL-RATES OF A. SACHAROVII CAUGHT IN SPRAYED AND UNSPRAYED QUARTERS OF ASTERION
 TABLEAU II. TAUX DE SURVIE DES A. SACHAROVII CAPTURES DANS DES SECTEURS TRAITES ET DES SECTEURS NON TRAITES D'ASTERION

Observation period Période d'observation	Interval between spraying and observation (days) Intervalle entre le traitement et les observations	A. sacharovi caught in sprayed quarters A. sacharovi capturés dans les secteurs traités						A. sacharovi caught in unsprayed quarters A. Sacharovi capturés dans les secteurs non-traités									
		Number of tests Nombre d'expériences	Number caged Nombre d'anophèles capturés	Survivors Survivants		Number of tests Nombre d'expériences	Number caged Nombre d'anophèles capturés	Survivors Survivants		Number of tests Nombre d'expériences	Number caged Nombre d'anophèles capturés	Survivors Survivants					
				after 24 hours après 24 heures	after 48 hours après 48 heures			after 72 hours après 72 heures	after 24 hours après 24 heures			after 48 hours après 48 heures	after 72 hours après 72 heures				
				Number Nombre	%			Number Nombre	%			Number Nombre	%	Number Nombre	%		
6-15 July 6-15 juillet	10	10	1,835	1,444	78.7	1,202	65.5	968	52.7	10	1,516	1,261	83.2	1,000	65.9	768	50.6
16-25 July 16-25 juillet	11-20	10	1,527	1,124	73.6	888	58.1	654	42.8	10	1,492	1,426	95.6	1,192	79.9	981	65.7
26 July - 4 August 26 juillet - 4 août	21-30	10	1,743	1,197	68.7	1,013	58.1	870	49.9	10	1,606	1,540	95.9	1,442	89.8	1,262	78.6
5-14 August 5-14 août	31-40	9	1,471	1,107	75.2	1,015	69.0	921	62.6	8	1,080	1,059	98.0	1,018	94.3	955	88.4
15-24 August 15-24 août	41-50	10	1,763	1,540	87.3	1,439	81.6	1,255	71.2	9	884	857	96.9	780	88.2	745	84.4
25 August - 3 September 25 août - 3 septembre	51-60	10	825	672	81.4	578	70.1	432	52.4	10	851	824	96.8	745	87.5	592	69.6
4-13 September 4-13 septembre	61-70	9	1,134	1,114	89.4	954	84.1	883	77.7	9	646	626	96.9	581	89.9	531	82.2
14-23 September 14-23 septembre	71-80	10	1,558	1,483	95.2	1,425	91.4	1,301	85.2	10	661	644	97.4	602	91.1	554	83.8
24 September - 3 October 24 septembre - 3 octobre	81-90	9	302	275	91.4	257	85.1	230	76.1	9	441	421	95.5	402	91.1	376	85.3

TABLE III. SURVIVAL-RATES OF A. SACHAROVII CAUGHT IN SPRAYED QUARTERS OF ASTERION
TABLEAU III. TAUX DE SURVIE DES A. SACHAROVII CAPTURÉS DANS DES SÉCTEURS TRAITÉS D'ASTERION

Observation period	Interval between spraying of contact box and observation (days)	Number of tests	Number caged	Survivors						Number caged	Survivors								
				A. sacharovi having renewed contact with the insecticide			A. sacharovi without renewed contact with the insecticide				A. sacharovi ayant été remis en contact avec l'insecticide			A. sacharovi n'ayant pas été remis en contact avec l'insecticide					
				No. after 24 hours	%	Non-bre	No. after 48 hours	%	Non-bre		No. after 72 hours	%	Non-bre	No. after 24 hours	%	Non-bre	No. after 48 hours	%	Non-bre
2 - 8 August 2 - 8 août	1-7	7	452	97	21.5	74	17.4	66	14.6	355	301	84.5	240	67.6	179	60.4			
21 August - 3 September	22-33	14	742	242	32.6	181	24.4	153	20.6	453	358	79.0	277	61.1	260	57.4			
2 August - 3 September 2 août - 3 septembre	1-33	21	1,194	339	28.4	255	21.3	219	18.3	808	659	81.5	517	63.9	439	54.3			

TABLE IV. SURVIVAL-RATES OF A. SACHAROVII HATCHED IN THE ASTERION LABORATORY
TABLEAU IV. TAUX DE SURVIE DES A. SACHAROVII ECLOS DANS LE LABORATOIRE D'ASTERION

Observation Period Date d'observation	Interval between spraying of contact box and observation (days) Intervalle entre le traitement de la cage d'intoxication et les observations	Number of tests Nombre d'expériences	A. sacharovi having contact with the insecticide A. sacharovi ayant été en contact avec l'insecticide				A. sacharovi without contact with the insecticide A. sacharovi n'ayant pas été en contact avec l'insecticide								
			Number caged Nombre d'anophèles capturés		Survivors Survivants		Number caged Nombre d'anophèles capturés		Survivors Survivants						
			after 24 hours après 24 heures	after 48 hours après 48 heures	after 72 hours après 72 heures	Number Nombre	%	after 24 hours après 24 heures	after 48 hours après 48 heures	after 72 hours après 72 heures	Number Nombre	%			
24 August 24 août	23	1	53	37	69.8	29	54.7	18	33.9	41	100.0	36	87.8	31	75.6
25 August 25 août	24	1	96	62	64.6	49	51.0	42	43.7	69	92.7	51	73.9	47	68.1
26 August 26 août	25	1	60	14	23.3	11	18.3	10	16.6	71	90.1	50	70.4	46	64.8
30 August 30 août	29	1	22	8	36.4	6	27.3	4	18.2	18	77.8	13	72.2	12	66.7
21 - 30 August 21 - 30 août	23 - 29	4	231	121	52.4	95	41.1	74	33.2	199	91.9	150	75.4	136	68.3

TABLE V. A. SAGHAROTI CAUGHT IN SOULI. SURVIVAL-RATES AFTER CONTACT WITH DIFFERENT INSECTICIDES
TABLEAU V. A. SAGHAROTI CAPTURES A SOULI : TAUX DE SURVIE APRES CONTACT AVEC DIFFERENTS INSECTICIDES

Période d'observation	Interval entre le traitement de la cage d'intoxication et les observations	Box sprayed with DDT solution in kerosene			Box sprayed with DDT emulsion concentrate (25% DDT)			Box sprayed with Gammaxane E.G. 140 (1.0%)			Box sprayed with Chlorthane (à 74%)			Box sprayed with Dieldrin 24%			Unsprayed box								
		Number of tests	Number of caged survivors	% of survivors	Number of tests	Number of caged survivors	% of survivors	Number of tests	Number of caged survivors	% of survivors	Number of tests	Number of caged survivors	% of survivors	Number of tests	Number of caged survivors	% of survivors	Number of tests	Number of caged survivors	% of survivors						
28.VI - 7.VII	-10	10	997	2	0.2	9	921	173	18.8	10	1,027	111	10.8	10	1,055	0	0.0	10	1,010	0	0.0	10	1,288	1,241	96.3
8.VII-17.VII	11-20	10	1,003	116	11.6	10	1,069	367	34.3	10	1,078	522	48.4	10	1,074	0	0.0	10	1,036	0	0.0	9	950	871	91.7
18.VII-27.VII	21-30	9	876	274	31.3	9	825	425	51.5	9	919	664	72.2	9	885	0	0.0	9	853	0	0.0	10	1,221	1,168	95.6
28.VII-6.VIII	31-40	10	1,150	505	43.9	10	1,041	556	53.4	10	1,097	952	90.0	10	1,057	0	0.0*	10	1,111	0	0.0	10	1,369	1,337	97.7
7.VIII-16.VIII	41-50	8	752	317	42.1	9	827	395	47.8	8	825	713	86.4	9	945	0	0.0	9	945	0	0.0	9	1,041	998	95.9
17.VIII-26.VIII	51-60	9	935	471	50.4	9	1,104	622	56.4	8	856	757	88.4	9	974	0	0.0	10	974	0	0.0	10	1,264	1,225	96.9
27.VIII-5.IX	61-70	10	1,059	557	52.6*	9	956	599	62.6	10	1,066	956	89.7	10	1,074	0	0.0*	10	1,074	0	0.0*	10	1,166	1,119	95.9
6.IX-15.IX	71-80					6	583	330	56.6	6	688	634	92.1					10	1,241	0	0.0	10	1,241	1,195	96.3
16.IX-25.IX	81-90					8	755	520	68.9	8	874	791	90.5					9	970	0	0.0	9	970	943	97.2
26.IX-5.X	91-100					7	545	377	69.2	7	548	484	88.3					10	1,016	0	0.0	10	1,016	883	86.9

*The test was interrupted at this point
Les expériences ont cessé après cette date

FIG. 1

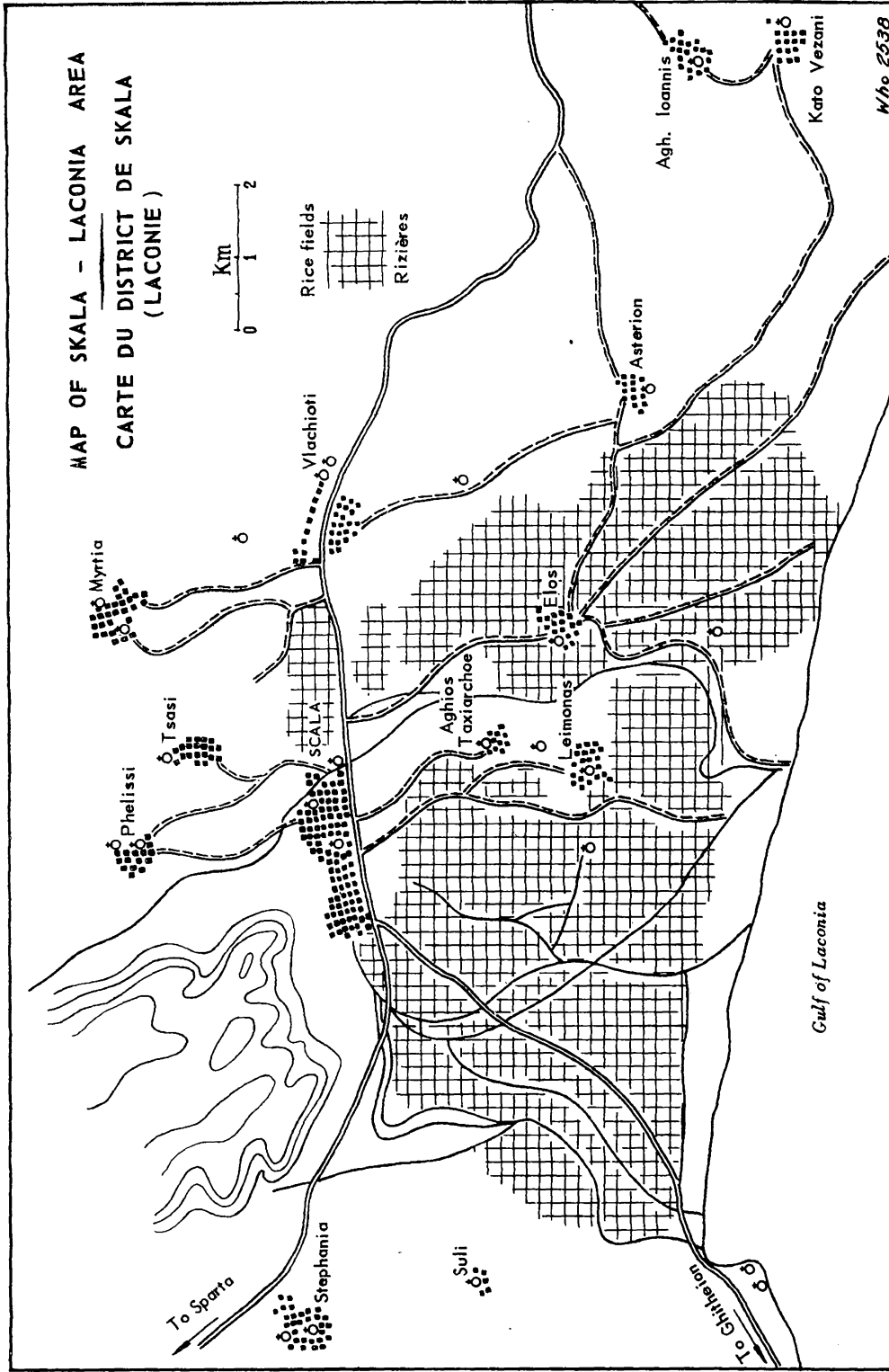


FIG. 2

Asterion 1st 24 hours period
Astériorion : Première période de 24 heures

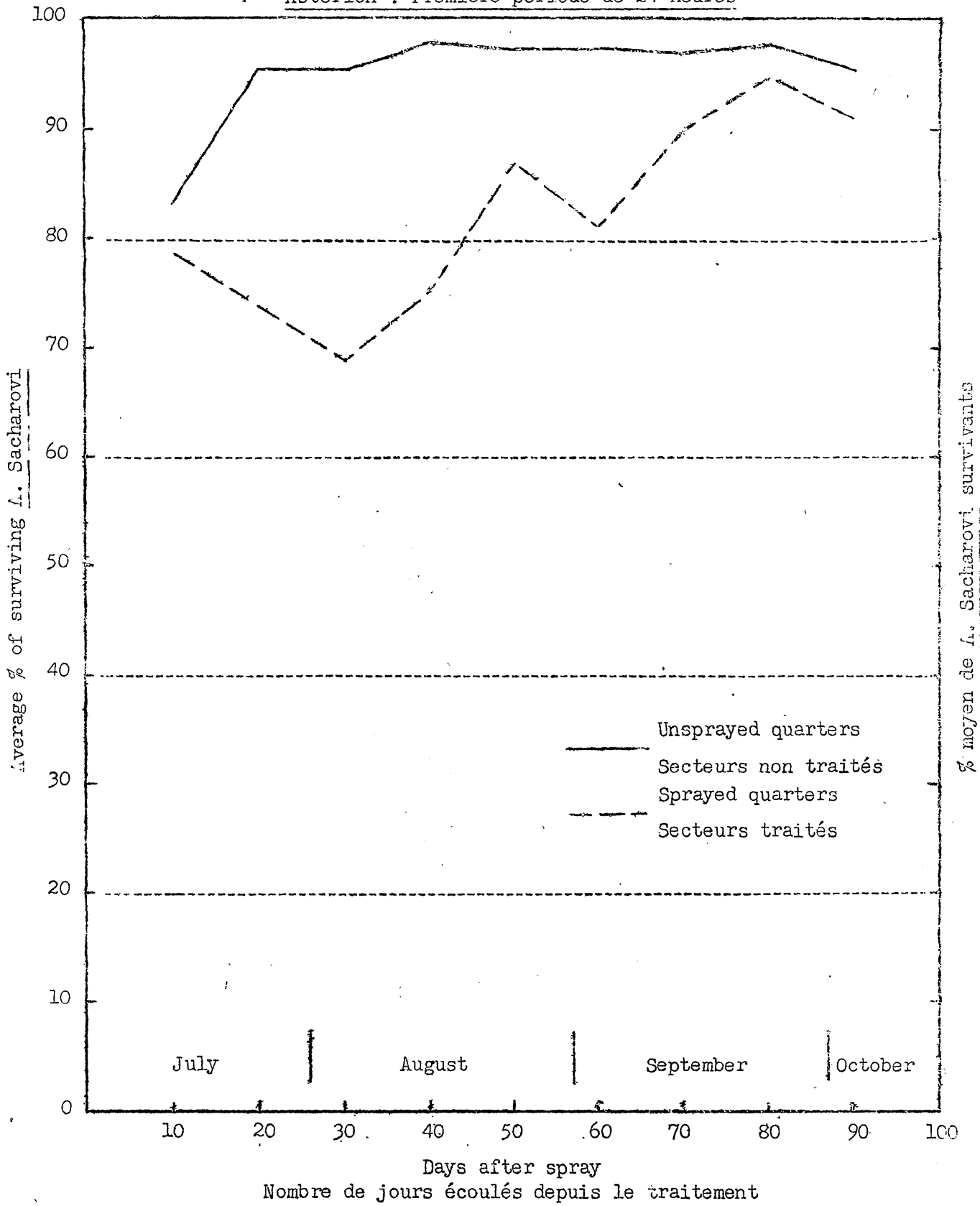


FIG. 3

Asterion 2nd 24 hours period
Astériorion : Deuxième période de 24 heures

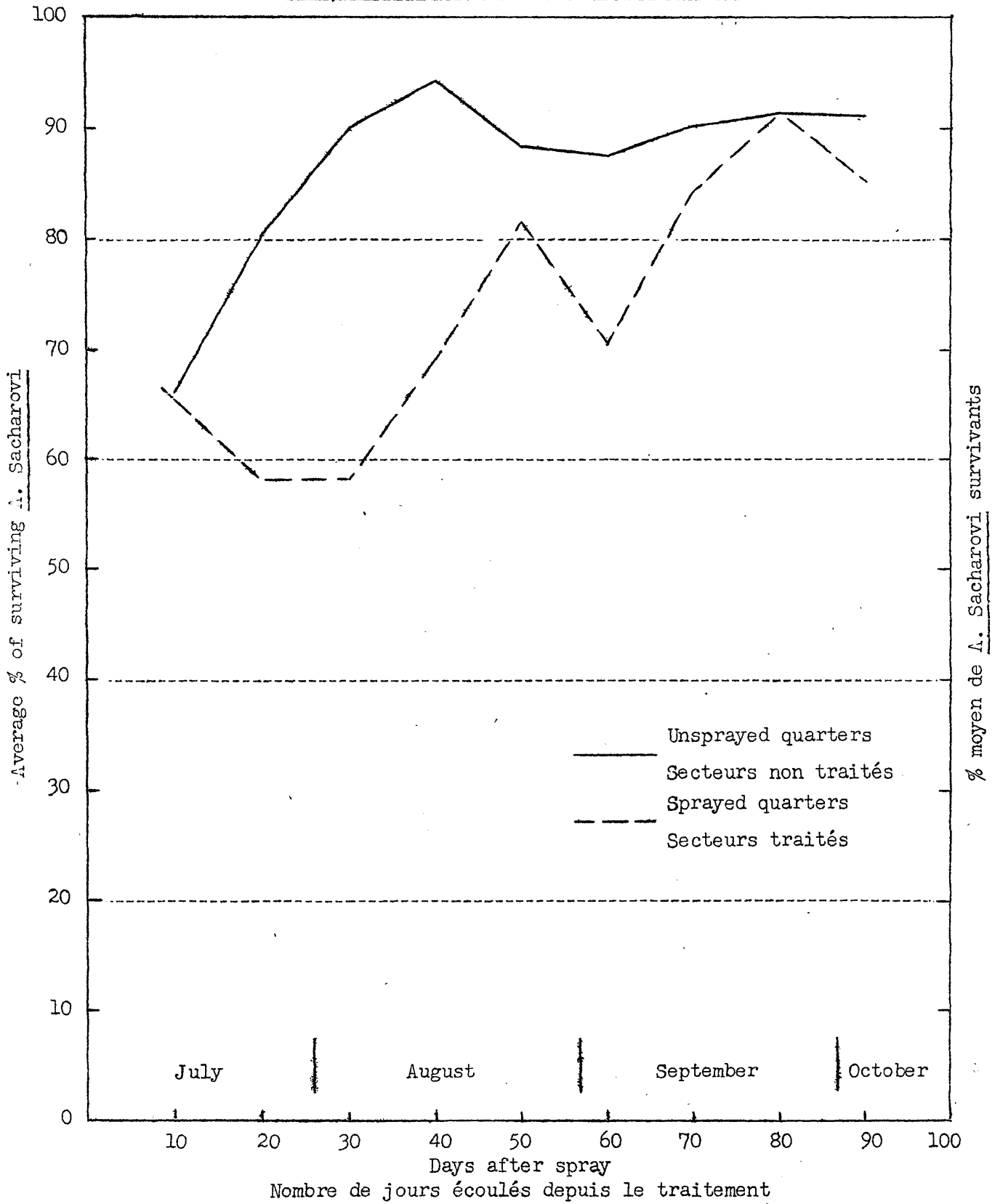


FIG. 4

Asterion 3rd 24 hours period
Astériorion : Troisième période de 24 heures

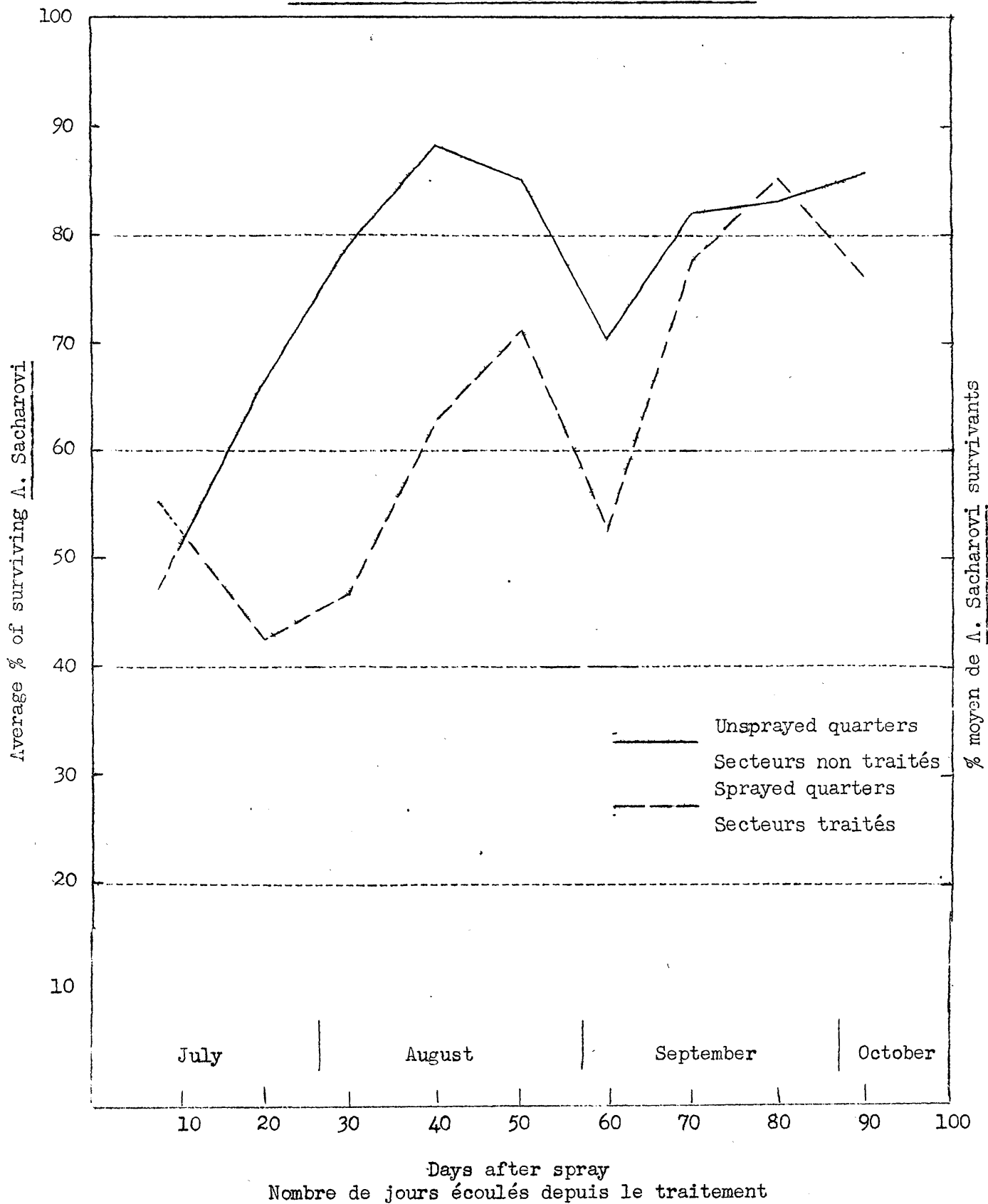


FIG. 5

