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OBSERVATIONS ON THE CONTROL OF SOME INSECTS OF MEDICAL IMPORTANCE IN GREECE

Riassunto. — 1. - In Grecia, *Anopheles sacharovi* è attualmente resistente al DDT. I due fatti seguenti dettano tale conclusione: *a*) un gran numero di Anopheli di questa specie si posano su delle superfici trattate con DDT e dimorano per lungo tempo in contatto con esse senza manifestare alcun segno di irritazione od altri sintomi d'intossicazione; *b*) si osserva una ristretta mortalità tanto fra le Anopheli ora menzionate quanto fra controlli sperimentalmente esposti a forti dosi di DDT.

Questi cambiamenti di comportamento delle *A. Sacharovi* DDT-resistenti sono chiaramente messi in evidenza dall'adattamento di questi insetti a ripari esterni diurni di un tipo per il quale essi non manifestavano alcuna predilezione prima dei trattamenti con il DDT. Pare che ora il DDT non irriti l'*A. Sacharovi*; queste Anopheli mostrano una forte resistenza all'azione di questo prodotto e si può di nuovo osservare un gran numero di esse negli immobili trattati durante la stessa giornata.

2. - Alcuni fatti indicano che *A. Sacharovi* è ugualmente resistente al chlordane.

3. - *Aëdes aegypti* — specie che normalmente pullulava su tutto il territorio greco — è oggi quasi completamente sparita. Si pensa che questo risultato sia dovuto ad un'azione indiretta piuttosto che diretta del DDT, il quale è stato intensamente utilizzato in questi ultimi anni sotto forma di polverizzazione a effetto rimanente. Questa specie, che il DDT irrita fortemente, è stata cacciata dagli immobili trattati e, a quanto pare, non ha potuto adattarsi alle condizioni di vita all'esterno.

4. - *Culex molestus* è una specie di DDT-resistente. Abbonda attualmente in numerosi rioni urbani e regionali rurali.

5. - Fin dal 1946 campagne di polverizzazione a mezzo aerei sono state fatte contro le zanzare; finora, non è stato possibile valutare i

risultati ottenuti con questo metodo. Il DDT e il chlordane diffusi sotto forma di nubi dagli aerei, sembrano molto più efficaci che il DDT usato sotto forma di aerosol.

Le polverizzazioni di DDT a mezzo di aerei non hanno permesso di combattere efficacemente in Grecia le zanzare delle saline.

6. - I gambusia hanno contribuito molto a fare sparire il paludismo ma per diverse ragioni la loro parte non è stata sufficientemente stimata.

Converrebbe utilizzare questo pesce per la distruzione delle larve di Anophele nelle risaie che, per via della loro recente estensione, sono diventate un serbatoio importante di insetti vettori.

Siccome il DDT non fornisce i risultati sperati, si potrebbe ugualmente utilizzare il verde di Parigi per distruggere le Anophele delle risaie.

7. - Le mosche domestiche sono resistenti sia al DDT, sia al chlordane. Non si sono provati altri insetticidi clorati nelle condizioni che si riscontrano sul terreno, ma, per alcune è possibile che si possa beneficiare di un breve periodo durante il quale i prodotti potranno agire. In attesa della messa a punto di un nuovo insetticida efficace contro le mosche domestiche, ci si dovrebbe sforzare di migliorare le condizioni di salubrità con metodi semplici tenendo conto della situazione esistente in ogni paese o collettività.

8. - I flebotomi si sono finora mostrati molto sensibili al DDT. In Grecia la densità delle diverse specie è molto diminuita come anche la frequenza di malattie da essi trasmesse.

9. - In Grecia, *Pulex irritans* è oggi una specie DDT-resistente. Nessuna osservazione precisa permette di affermare che altre specie di pulci siano ugualmente resistenti.

Finora, le cimici di letto sono state combattute con successo con il DDT.

Résumé. — 1. En Grèce, *Anopheles sacharovi* est actuellement résistant au DDT. Cette conclusion ressort des deux faits suivants: *a*) un grand nombre d'anophèles de cette espèce se posent sur des surfaces traitées par le DDT et demeurent longtemps en contact avec elles sans manifester de signe d'irritation ou d'autres symptômes d'intoxication; *b*) on observe une mortalité restreinte aussi bien parmi les anophèles dont il vient d'être fait mention que parmi d'autres spécimens expérimentalement exposés à de fortes doses de DDT.

Les changements de comportement des *A. sacharovi* DDT-résistants sont nettement mis en évidence par l'adaptation de ces insectes à des abris extérieurs diurnes d'un type pour lequel ils ne manifestaient aucune prédilection avant les traitements par le DDT. Il apparaît maintenant que le DDT ne les irrite guère; ces anophèles témoignent d'une forte résistance à l'action de ce produit et l'on peut de nouveau observer un grand nombre d'entre eux dans les bâtiments traités au cours de la journée.

2. Certains faits indiquent que *A. sacharovi* est également résistant à l'égard du chlordane.

2. *Aëdes aegypti* — espèce qui, normalement, pullulait sur tout le territoire grec — a aujourd'hui presque entièrement disparu. Il y a lieu de penser que ce résultat est dû à une action indirecte plutôt que directe du DDT, qui a été intensément utilisé ces dernières années sous forme de pulvérisations à effet rémanent. Cette espèce, que le DDT irrite fortement, s'est trouvée chassée des bâtiments traités et, semble-t-il, n'a pas su s'adapter aux conditions de vie à l'extérieur.

4. *Culex molestus* est une espèce DDT-résistante. Elle abonde actuellement dans de nombreux districts aussi bien urbains que ruraux.

5. Depuis 1946, des campagnes de pulvérisations par avion ont été adoptées contre les moustiques. Jusqu'ici, il n'a pas été possible d'évaluer les résultats obtenus par cette méthode. Le DDT et le chlordane diffusés sous forme de brouillards lancés par les avions semblent beaucoup plus efficaces que le DDT employé sous forme d'aérosol.

Les pulvérisations de DDT par avion n'ont pas permis de combattre efficacement, en Grèce, les moustiques des marais salants.

6. Les gambusias ont beaucoup contribué à faire disparaître le paludisme, mais, pour diverses raisons, leur rôle n'a pas été évalué convenablement. Il conviendrait d'utiliser ce poisson pour la destruction des larves d'anophèles dans les rizières, qui, par suite de leur récente extension, deviennent un important réservoir d'insectes vecteurs.

Comme le DDT ne donne pas les résultats souhaités, on pourrait également utiliser le vert de Paris pour détruire les anophèles dans les rizières.

7. Les mouches domestiques sont résistantes tant à l'égard du DDT que du chlordane. On n'a pas essayé d'autres insecticides chlorés dans les conditions qui se recontrent sur le terrain, mais, pour certains d'entre eux, il est possible que l'on puisse bénéficier d'une brève pé-

riode pendant laquelle les produits peuvent agir. En attendant la mise au point d'un nouvel insecticide efficace contre les mouches domestiques, on devrait s'efforcer d'améliorer les conditions de salubrité par des méthodes simples, compte tenu de la situation existant dans chaque pays ou collectivité.

8. Les *phlébotomes* se sont montrés, jusqu'ici, très sensibles au DDT. En Grèce, la densité des diverses espèces a beaucoup diminué, tout comme la fréquence des maladies qu'elles transmettent.

9. En Grèce, *Pulex irritans* est, aujourd'hui, une espèce DDT-résistante. Aucune observation précise ne permet d'affirmer que d'autres espèces de puces soient également résistantes.

Jusqu'à présent, les punaises de lit ont été combattues avec succès par le DDT.

Summary. — 1. *Anopheles sacharovi* in Greece is now resistant to DDT. This is borne out by *a*) the fact that great numbers of specimens of this species alight on DDT-treated surface and remain in contact with them for long, without demonstrating any signs of irritation or other toxic symptoms, and *b*) the limited mortality observed among such specimens or others experimentally exposed to heavy dosage of DDT.

Behavioristic change of DDT-resistant *sacharovi* were clearly demonstrated as the insects adapted themselves to outdoor daytime shelters of a type not known to be preferred by them in pre-DDT times. As things have developed now, *An. sacharovi* is not much irritated by DDT; it can withstand it to a great degree and can be seen again in large numbers within sprayed buidings during the day.

2. There is some evidence that *sacharovi* is also resistant to chlordane.

3. *Aedes aegypti*, a species normally very abundant and widespread in Greece, has now been reduced to near the vanishing point. It is felt that this has been brought about not so much by direct as by indirect action of DDT, which has been used extensively as a residual spray during the last few years. The species, being greatly irritated by DDT, is being forced out of sprayed buildings and apparently has not been able to adapt itself to out-door conditions.

4. *Culex molestus* is a DDT-resistant species and is now very abundant in many urban as well as rural areas.

5. Airspray programmes against mosquitoes have been carried on since 1946. No evaluation of this method has been possible to date. DDT and chlordane airsprays in mist form seem to be much more effective than DDT in aerosol form. No effective control of Greek salt marsh mosquitoes has been possible by DDT airsprays.

6. Gambusia have contributed considerably to malaria control, but for various reason their rôle has not been evaluated. It is suggested that this fish should be used in the control of anopheline larvae in rice fields, which, owing to their recent expansion, are becoming a source of immense anopheline production.

Since DDT is not giving the desired results, Paris green could also be used in the control of anophelines in rice fields.

7. Houseflies are resistant both to DDT and chlordane. Other chlorinated insecticides have not been tried under field conditions, but there is a possibility of short period control with some of them. Until a new effective insecticide against houseflies is developed, efforts should be directed to the improvement of environmental sanitation by applying simple practices, as local conditions in the different countries and communities would permit.

8. *Phlebotomus* are, so far, very susceptible to DDT. The density of the various Greek species has been greatly reduced, as have also been the phlebotomus-borne diseases.

9. *Pulex irritans* is now a DDT-resistant species in Greece. There is no concrete evidence as to other species of fleas being resistant.

Bedbugs have so far been successfully controlled with DDT.

Zusammenfassung. — 1) *Anopheles sacharovi* ist zur Zeit in Griechenland DDT-resistent. Dieser Beschluss entstammt aus den zwei folgenden Beobachtungen:

a) eine grosse Anzahl Anophelen dieser Art legen sich auf Oberflächenieder, die mit DDT behandelt wurden, verbleiben länger Zeit darauf und zeigen weder den mindesten Zeichen von Unbehagen noch andere Vergiftungssymptome;

b) man hat eine beschränkte Sterblichkeit unter den so behandelten Anophelen beobachtet, sowie bei anderen *A. sacharovi* die experimentell an starken DDT-Dosen unterworfen wurden.

Das veränderte Verhalten des DDT-resistenten A. *sacharovi* ist offenbar durch die Anpassung dieser Insekten an äusserliche Tagsunter-

kunfte, die vor der DDT-Behandlung gar nicht bevorzugt waren, klargelegt. Es scheint jetzt dass, DDT gar nicht. *A. sacharovi* angreift; denn diese Anophelen bezeugen eine grosse Resistenz zur Wirkung dieses Stoffes und von neuem kann man eine grosse Anzahl dieser Mücken in den Gebäuden beobachten, die am selben Tag bespritzt wurden.

2) Einige Tatsachen weisen eine gleiche Resistenz gegen Chlordane seitens *A. sacharovi* auf.

3) *Aedes aegypti* — Diese Art, die sonst das ganze griechische Land überschwärmte, ist nun fast gänzlich verschwunden. Man könnte annehmen dass, dieses Ergebnis vielmehr zu einer indirekten als einer direkten Wirkung des DDT zuzuschreiben wäre. DDT wurde in den letzten Jahren unter Form von Bestreuung mit residual Wirkung sehr intensiv verwendet. Diese Art, die durch DDT sehr stark gereitzt wird, ist deswegen aus den behandelten Gebäuden ausgetrieben worden und hat sich scheinbar den äusseren Zustände nicht angewöhnen können.

4) *Culex molestus* ist eine DDT-resistent Art. Sie ist jetzt reichlich in zahlreichen städtlichen und ländlichen Gegenden vorhanden.

5) Seit 1946, ist gegen die Mücken eine Bestäubungsplan mit Flugzeuge unternommen worden. Seit dem ist es nicht möglich gewesen, die Ergebnisse dieser Methoden zu schätzen. DDT und Chlordane, vom Flugzeug aus bespritzt, scheinen als Nebel und nicht als Aerosol wirksamer zu sein.

Die DDT-Bestäubung vom Flugzeug aus aber hat in Griechenland keinen wirksamen Erfolg gegen Stechmücken der Salzsümpfe erwiesen.

6) Die *gambusiae* haben zuviel bei der Vernichtung von Malaria mitgewirkt; aus verschiedenen Gründen jedoch ist ihre Rolle nicht geschätzt worden. Es würde vorteilhafter sein, dieser Fisch gegen Anopheleslarven in den Reisfeldern die, infolge ihrer neulichen Ausdehnung, wichtige Brutstellen von Insekt-Träger werden, zu verwenden.

Da DDT nicht die erwünschten Ergebnisse erwiesen hat, könnte mann ebenfalls Schweinfurtergrün brauchen um die Anopheles in den Reisfeldern zu vernichtern.

7) Die Hausfliegen sind sowohl gegen DDT als auch gegen Chlordane resistent. Unter den auf dem Felde vorkommenden Verhältnisse hat man keine andere chlorinierte Insekticiden versucht, doch ist es wohl möglich dass es unter ihnen einige Stoffe gibt die, solange sie noch wirkend sind, für einer kurzen Frist mit Vorteil ausgenutzt werden können. Während man auf der Vervollkommnung eines neuen, wirk-

samen Insekticiden gegen Hausfliegen abwartet, müsste man sich bemühen die Gesundheitszustände durch einfachen Methoden zu verbessern, unter Berücksichtigung der vorhandenen Lage jedes Landes oder jeder Gemeinde.

8) *Phlebotomus* hat sich bis jetzt sehr DDT-empfindlich gezeigt. In Griechenland ist die Dichtigkeit der verschiedenen Arten sehr verringert und zwar eben wie die Häufigkeit der von ihnen übertragenen Krankheiten.

9) *Pulex irritans* ist jetzt in Griechenland DDT- resistent. Es gibt keine genaue Beobachtung die uns zu behaupten gestattet, dass andere Floharten ebenfalls resistent sind.

Bis jetzt, hat die DDT-Bekämpfung gegen Wanzen einen guten Erfolg gehabt.

INTRODUCTION

Prior to the use of DDT against anopheline mosquitoes, malaria control in Greece was based chiefly on various methods (mechanical, chemical, biological and therapeutical) aiming at the suppression of anopheline development and the distribution of anti-malaria drugs to the population. (1)

Application of such methods, singly or in combination, resulted in a fairly good protection from the disease. During the period 1930-1944, larviciding with Paris green in dust or spray form was the principal chemical method used, especially in protecting certain urban areas from malaria.

The malaria control picture has completely changed since the advent of DDT. It was observed from the beginning of its use that not only anophelines but practically all other domestic insect pests were fully controlled. An unprecedented optimism affected everywhere, not only the general public but scientific men as well.

Things, however, did not stay this way for long. In 1947, just about a year after an extensive DDT residual spray programme was carried out, *Musca domestica* proved DDT-resistant, simultaneously in Greece and Italy and later in other countries. As time went on, other insects of medical importance were added to the list of DDT-resistant species. Now that some *Culicidae* also appear on the list, there is a feeling of anxiety, growing more and more intense.

As far as Greece is concerned, much of the information available has already been published, or in some way communicated to colleagues abroad and perhaps there is little to add at this moment. However, the invitation to attend this Symposium has been accepted mainly to communicate my own observations and opinions as well as those of a small group of Greek workers who have been working together for a number of years on malaria and insect control at the Athens School of Hygiene, and an attempt is made here to review the situation in Greece regarding insect control by chemical and other methods, with special reference to the reaction of some insects to the chlorinated hydrocarbon insecticides and bearing in mind certain problems peculiar to Greece.

A) MOSQUITOES.

I. - Chemical methods of control

(a) *Residual DDT sprays against adult anophelines.*

DDT was extensively used in Greece, chiefly in the form of residual spray applications. It was also used as a larvicide, either from the air or from the ground. Our first observations following its use as a residual spray was that anophelines could not be found in any of the sprayed buildings. This was further substantiated from various field reports for a considerable number of years. According to these reports, the malaria vector species still could not be found in DDT sprayed houses and outbuildings.

As a result of DDT application, malaria incidence was brought down to a negligible point ([2]).

In laboratory tests we noticed that the anophelines were irritated to a very high degree shortly after coming into contact with DDT treated surfaces. The irritated mosquitoes would be seen congregating to the lighted side of the cages, and their effort to escape was evident.

Under field conditions, something similar must have happened. The fact that, for a number of years, since the initial application of DDT on a large scale, no anophelines were found in residually sprayed shelters, did not necessarily mean that all were killed through the action of DDT.

In practice, we have repeatedly observed dead anophelines in sprayed buildings, and there is no doubt that the bulk of the others which were driven out on account of the irritability caused by DDT ultimately met with death. We do not know, however, if and to what extent recovery from DDT intoxication took place. We always

wondered whether anophelines irritated or otherwise recovering from DDT poisoning would avoid re-entering DDT treated buildings and would perhaps change preferences for daytime shelters.

Unfortunately we are not in a position to present objective evidence regarding this phenomenon, but we have a feeling, based on observations, that such change in habits has occurred among our malaria vector anophelines.

In our former experience, prior to the use of DDT *An. sacharovi* (*elutus*), *An. superpictus* and *An. maculipennis* were not only found indoors during the day but also in some numbers in suitable outdoor daytime shelters which would fulfil their requirements as to the various microclimatic factors.

Shannon ([3]) working in Greece on the reaction of anophelines to certain microclimatic factors, states that « a light intensity of 1 to 5 foot candle power is suitable for them; greater or less than this is avoided », and that «from the three malaria vector species, *An. superpictus* is somewhat more tolerant of light and higher temperature than the other two, while *maculipennis* is the least tolerant ». In fact, all three species were found in relatively dark shelters out of doors, usually in caves, the interior of large tree holes and, occasionally, in somewhat exposed animal sheds, under road bridges, etc.

As the residual DDT programme was expanding in the country, reports were progressively coming in that, where habitual daytime shelters were lacking, more and more anophelines were seen in types of shelters not previously known to be preferred by them.

Thus a great number of *sacharovi* were reported from various areas as spending the day under road bridges near inhabited places. The writer, in 1949, during a field trip to Epirus, in an area near the Albanian frontier, did not find any *sacharovi* in treated bedrooms or stables of a number of villages, but observed considerable numbers under numerous small road bridges. In 1950, *sacharovi* were collected during the daytime from trunks and leaves of trees in an orange grove in Southern Peloponnessus. Regarding *maculipennis*, Belios, in a personal communication, writes: « In the area of Stymphalia lake — Peloponnessus, 550 m elevation — the following observations were made in August 1951 on the resting habits of *An. maculipennis*. Large numbers of adults of this species, which is known for its little tolerance of light, were found resting in rather sparse bushes and under two well lighted bridges (diffused light of from 15 to 40 foot candle power). A total of 350 *An. maculipennis* were collected from under these bridges. Many of these specimens had blood in their stomachs or had mature ovaries.

Nearby houses treated with DDT the previous year were practically anopheline-free. A few, very suitable stables did yield some specimens of the same species, but these structures had not been treated with DDT for two consecutive years. The local population reported a high degree of mosquito biting during the night, apparently from this species, since the culicine density in the area was very low. It should be concluded that irritation caused by DDT was responsible for the unusual resting habits of this species ».

Regardles of the theory of anophelines changing habits being sound or not, the fact remains that, due to the irritant effect of DDT, the mosquitoes could not stay long enough on treated surfaces and as a rule they were never found on such.

This irritability greatly diminished during the last two or three years, particularly as regards *An. sacharovi*. Since 1950 we have had several reports of all three species being caught in stables or bedrooms DDT residually sprayed not very long before. This is especially true of *An. sacharovi*.

In August 1951, in Lamia district (Central Greece), a total of 120 male and 1776 female *An. sacharovi* and a total of 12 *An. maculipennis* (6 male and 6 female) were caught in 30 catching stations residually sprayed 3 months previously (about the middle of May).

In June 1951, the inhabitants of the region Skala-Laconia (Southern Peloponnessus) complained of the presence of innumerable mosquitoes inside and outside their homes. The writer visited the place to investigate the matter and found heavy breeding of *An. sacharovi* in the rice fields area. In bedrooms and stables many adults of this species were seen resting on DDT treated walls. Some of them showed signs of DDT poisoning, but many were seen resting comfortably, not demonstrating any such symptoms. An immediate DDT spraying of all buildings in some of the villages of this area was decided and carried out under strict supervision.

The following table presents the results of this work in one of the sprayed villages which may be considered as representative of the area. The collection of mosquitoes continued for one month after spraying.

In 1952, Livadas and Georgupoulos studied the phenomenon and carried out a series of experiments which I will outline here. All buildings of a certain village in Skata area were residually sprayed with DDT emulsion on 2-4 July, with an estimated deposit of 2 g per sq m of active ingredient. Two catching stations were left unsprayed. This was the seventh consecutive spraying of this village with DDT since

Numbers of *An. sacharovi* caught from sprayed buildings of the village of Elos in the district of Laconia

Date of spraying	*Estimated DDT active ingredient deposit per sq. m.*	*Dates of mosquito catchings*	*No. of catching stations*	*No. of males*	*No. of females*	*Total number*	*Number of anophelines per catching station*
27 June 1951	2 g	1.7.51	14	5	174	179	12.8
		10.7.51	10	15	181	196	19.6
		15.7.51	6	28	744	772	129.3
		21.7.51	8	116	1,330	1,446	180.0
		26.7.51	8	177	1,381	1,558	194.9
		30.7.51	8	80	641	726	90.7

1946. Two days after spraying, an average of all *sacharovi* per station were caught in 10 catching stations. These numbers were considerably increased as the « age of the residue » was advancing, reaching eventually a figure of 120-150 per catching station, which equalled the catches from the unsprayed stations. In order to get information on the survival rate of these mosquitoes, the following experiments were made with material collected from this area:

(1) During the period from 5 July to 3 October, an average of 135 specimens were collected per day from sprayed stations and put into clean recovery cages for determining their survival rate. The same was done with species collected from the untreated stations. The 24 hour survival rate was found to be 81% out of a total of 12,158 specimens, as compared to 94% of the check (total of 9,177 specimens).

(2) Specimens collected from the same stations were exposed in special « contact cages » to a deposit of technical DDT of 2 g per sq. m for 30 minutes, and then transferred to clean cages. Out of a total number of 1.194 mosquitoes, 48% survived in 24 hours, as compared to 81.5% of the check (a total of 808 specimens). By this procedure approximately the same rate of survival was obtained from another village of the same district which was also sprayed with DDT for 6 consecutive years, but not during 1952.

3) A number of adults, emerged from pupae collected from the area, were exposed to DDT (same procedure as above) for 30 minutes and then transferred to clean cages. In 24 hours a survival rate of 231 mosquitoes was observed, as compared to 91.9% out of a total of 199 specimens of the check.

The figures in this last experiment are very small indeed, but it is hoped that, during this season, more relevant data will be available and communicated in due time.

On the basis of the above observations, the authors conclude that the local vector, *An. sacharovi*, shows a considerable degree of resistance to DDT.

(b) *Residual sprays of chlorinated hydrocarbons other than DDT against mosquitoes.*

Chlordane, Dieldrin and BHC were tested at various times against adult *Culex molestus* and *An. sacharovi*, under laboratory conditions, the two first giving excellent results.

Chlordane was used for the first time under field conditions in 1949 as a residual spray against houseflies of the region of Sounion-Laurion-Keratea. It was stated then that not only houseflies but mosquitoes as well were controlled with it. In 1952, it was used as a residual spray against anophelines under field conditions and on a comparatively significant scale (5). This was done in a village of the district of Skala-Laconia, where rice fields are rather extensive and the anopheline population has been very dense of late. This village had been residually sprayed with DDT for 5 consecutive years (1946-1950). In 1951, no spraying at all was done. In early July 1952, the anopheline density in the village was exceptionally high. On 12 July 412 male and 4,542 female *An. sacharovi* were collected from 4 catching stations in the village, or an average of 1,238 males and females per catching station. All stables and houses of the village were subsequently sprayed between 14 and 17 July, with 74% Chlordane emulsifiable concentrate, with an estimated deposit of 1.5 g per sq m of active ingredient.

The following table shows the numbers of *An. sacharovi* caught in the sprayed buildings, during the period from 22 July to 9 September, as well as from another village 7 kilometres distant which was left unsprayed to serve as a check.

Numbers of *An. sacharovi* collected from sprayed and unsprayed villages.

Date of spraying	*Dates of collection*	*Sprayed village*				*Check*		
		No. of catching stations	*Males*	*Females*	*Total*	*No. of catching stations*	*Males*	*Females*
14–17 July 1952	22–31 July	6	141	944	1,085	5	1,318	1,399
	1–10 Aug.	6	192	1,246	1,438	5	1,187	1,221
	11–20 Aug.	6	77	685	762	5	1,143	1,155
	21–30 Aug.	6	70	866	936	5	1,100	1,129
	31 Aug. – 9 Sept.	6	27	526	553	5	1,200	1,230

From previous laboratory tests carried out at the entomological laboratory of the Athens School of Hygiene, it was known that chlordane, under field conditions, becomes inactivated in a comparatively short period, particularly when compared to dieldrin ([6]); it is worth while to note, however, that in this particular instance this insecticide did not work even during the first week after its application.

This was further demonstrated when the mosquitoes collected from the residually sprayed buildings, after being transferred and kept in clean cages for 24 hours, showed an average survival rate of 76.4%, as compared to 96.3% of the check.

The high percentage of survival observed among chlordane exposed individuals of *An. sacharovi* indicates that this species, at least in this area where DDT had been used for a considerable number of years, is also resistant to chlordane.

c) *Residual DDT sprays and* Aedes aegypti.

Aedes aegypti, a stenogamous species, is usually reared in all entomological laboratories and a great deal of work has been done by various workers on the reaction of this insect to the chlorinated hydrocarbon insecticides. Hadjinicolaou and Busvine ([7]), working in London in the spring of 1946, reported that *Aedes aegypti*, on coming in contact with DDT, would become greatly irritated and that « after an exposure of one or two hours to DDT treated cages, all the insects would show the characteristic signs of DDT poisoning. If they were then removed to a clean cage, a proportion of them would usually recover by next day, and if offered a blood meal, some would feed and lay viable eggs. Thus mosquitoes can recover from mild DDT intoxication ».

Kennedy ([8]), working about the same time on *Aedes aegypti* and *Anopheles maculipennis atroparvus*, made similar observations and reported that « it was repeatedly observed that, contrary to widespread belief, mosquitoes can recover after showing all the preliminary symptoms of DDT poisoning ».

It was then that the first suspicion of insect resistance to DDT was formed in our minds. Personally, I had the impression that it would not take very long before *Aedes aegypti* would demonstrate resistance to DDT. Things, however, turned out quite differently so far.

Aedes aegypti was a widespread species in Greece, breeding in abundance chiefly in clear drinking water, which, as a rule, was stored in various types of receptacles all over the country. The pandemic of dengue fever in 1928 is well known and it testifies to the distribution and density of the species. Since the generalized use of DDT inside

houses and other buildings as a residual spray, this species practically disappeared. The city of Athens has never been sprayed in toto, but most of the buildings have somehow been sprayed by their owners, and this explains why *Aedes aegypti* has disappeared also from the city.

We do not have enough evidence to convince ourselves that this species has been eradicated, but on repeated and organized special searches, we were unable to detect any specimens, either indoors or out of doors, in areas where DDT was applied as a residual spray.

Aedes aegypti is an endophilous and endophagous species and as far as I know is the only Greek mosquito which definitely fits in Senior-White's category I-III (9).

This species which, indeed, under Greek conditions, is primarily domestic and anthropophilic, apparently did not manage to adapt itself out of doors, unlike *An. sacharovi*, which, as stated previously, managed to do so. The latter is beginning to re-enter and remain in sprayed buildings, now that the irritant effect of DDT is not so pronounced.

(d) *DDT and Culex.*

In pre-DDT days the control of the so-called nuisance mosquitoes in different municipalities of the country consisted in detecting and oiling all culicine breeding places in the town. Since 1946, when DDT became available in Greece, this treatment was substituted by either emulsions or oil solutions of this insecticide.

In 1948, a mixed population of *Culex* larvae was collected in the Athens area and transported to the laboratory. The emerging adults were exposed for 30 minutes to DDT treated surfaces (small folding cages) with an estimated deposit of 2 g per sq. m. A high percentage of survival (50% as compared to 70% of the check) was noted from the very beginning.

The recovering mosquitoes were kept for some time in clean cages, and one day it was unexpectedly noticed that some of these mosquitoes laid egg rafts in a bowl where water-saturated cotton was kept. More egg rafts were likewise collected in the following days. None of these mosquitoes that laid eggs had taken any blood meal. All the eggs hatched and developed into healthy adults, which in turn mated in captivity and further laid eggs in abundance.

This species of stenogamous *Culex* is still mantained in our laboratory.

Although this autogenous *Culex molestus* had at some time been reported as occurring in Greece, this was my first acquaintance with it. The DDT resistant species is now very abundant in the Athens area, as in many rural areas.

(e) *DDT and Chlordane applied from the air against anopheline and culicine larvae.*

Parallel to the vast DDT residual spray programme, initiated in 1946, an airspray programme against aquatic stages of mosquitoes was concurrently carried out in certain areas. Some of the big swamps which could not easily be treated from the ground, as well as some rice fields, were sprayed with a 20% DDT formulation (Thermal aerosol), with the theoretical dosage of 1 American gallon to 17 acres, which is equivalent to 12 g of active ingredient per stremma (1,000 sq. m).

Roughly an area of 20,000 hectares was sprayed by air during the 1952 season.

Exactly how much DDT airspray has helped in the control of malaria in Greece is very difficult to say. No evaluation of this method has been possible.

Routine entomological survey carried out at various times did not reveal any clearcut evidence.

J. Costopulos, sanitary engineer in charge of the airspray programme, in a personal communication states that, during 1952, due to the fact that DDT thermal aerosol airspray had not proved very effective against the aquatic stages of mosquitoes, a change in the method of application of the insecticide from the air was decided upon and carried out experimentally. This change consisted in applying the insecticide in mist form instead of thermal aerosol.

Besides DDT, Chlordane, 1 part by volume of a 74% formulation, plus 4 parts of Diesel oil, was also applied from the air in mist form.

The following are Castopulos' conclusions from his preliminary tests:

1. There is much better penetration of the insecticide in the form of mist than in the form of thermal aerosol.

2. Both DDT and Chlordane, applied from the air in mist form, are very effective against mosquito larvae.

3. Pupae appear to be somewhat resistant to both DDT and Chlordane.

4. The residual effect of both insecticides, particularly during

summer time, is very small (1-2 days) and therefore frequent resprayings are necessary.

5. No injury to aquatic animal or plant life is observed with insecticides in either form.

The airspray programme is aiming not only at the control of anophelines, but at the control of culicines as well, some of which are a real nuisance to the people, particularly in certain farming areas and bathing resorts. The spraying programme is, therefore, partly sponsored by the Ministry of Agriculture and the enterprises concerned.

Salt marsh mosquitoes, which are extremely abundant in certain coastal areas of the country, are the main source of trouble. The commonest species involved are *Aedes caspius*, *A. detritus*, and *A. dorsalis*. *Aedes mariae* is also troublesome, but breeds in sea water (rock pools) and is not very abundant.

The DDT airspray programme has been a complete failure as far as these mosquitoes are concerned. It may be that our species are also DDT resistant as other species of salt marsh mosquitoes reported by various workers in the US and elsewhere. On the other hand, airspraying of breeding places is practically impossible, as these species breed in very small water collections, very often in water accumulating in animal footprints and even in places just saturated with water, and it is a very difficult matter for the pilot of the aircraft to detect any such breeding places.

It is therefore not surprising that swarms of these mosquitoes invade villages and even the centres of big towns. As a rule they do not enter houses but bite in the open, particularly during the first one or two hours after sunset.

II. - Biological control.

(a) *Introduction and distribution of top-minnows.*

Gambusia were introduced into Greece in 1928 The real work for its distribution was initiated by Balfour in 1936 and was continued and expanded by the Malaria Division until the year 1941. The extent of their distribution was limited by the very nature of the mosquito breeding places. The fish established itself very well in all permanent marshes, ponds and other bodies of stagnant or slowly flowing water. Efforts to establish the fish in such bodies of water as fast flowing streams, rivers, small bodies of water which would become dry rather quickly, or in thickly vegetated ones, naturally failed.

The habitual breeding places of *A. sacharovi* and, to a lesser extent, those of *An. maculipennis*, were, in most cases, stocked with it. The habitual breeding places of *superpictus* were of such nature that the fish never succeded in establishing itself.

The effect of gambusia on anopheline control has been, to say the least, pronounced and noticeable, particularly in places where the fish had time enough to establish itself. Unfortunately there are no adequate data to show the exact extent of their contribution to malaria control. The spectacular effect of DDT has, in the meantime, obscured their role, whatever this might be. However, we do have some evidence from some areas that might be suggestive of their usefulness.

In the Thermopylae marshes of the lower Sperchios valley, prior to the distribution of gambusia in the area, the anopheline density was so high that one could dip in any spot and collect hundreds of larvae in one dip. In 1939 one could dip fish but no larvae in these same breeding places. The effect of gambusia was also evident on the adult anophelines. In the village Aghia Trias, in the lower Sperchios valley, a field laboratory was established during the summer of 1936. Total collections of mosquitoes from one particular stable were made daily, from 15 June until September. The numbers of *sacharovi* varied from one to two thousand per day. In 1937, the numbers from the same stable were noticeably less, and in 1938, 1939 and 1940, occasional total collections amounted to tens instead of hundreds or even thousands of *sacharovi*.

It must be noted that during all these years no other method of anopheline control was practised in the area.

Regarding the effect of gambusia on malaria, Sphangos ([10]) gives the following figures related to spleen and parasite indices of school children of three villages in the area including Aghia Trias. The total population of the three villages was 2,554.

Spleen and Parasite Indices among schoolchildren of 3 villages in the lower Sperchios valley

Year	*Number of schoolchildren examined*	*Spleen index %*	*Parasite index %*
1936	178	70.3	32.6
1937	196	54.7	18.4
1938	206	58.8	7.7
1939	208	36.5	8.2
1941	167	32.9	6.0

One might also mention several other areas in Greece where gambusia proved to be valuable in bringing down the anopheline population. Much work, indeed, has been done on the distribution of this valuable fish, but I do not think that its potentialities have been fully exploited and any effort to achieve this end should be encouraged.

(b) *Rice fields in Greece and anopheline production.*

It is interesting to note that the beginning of resistance of *An. sacharovi* to DDT in Greece coincided with an expansion of rice cultivation during the last three or four years. The following figures, obtained from the Greek Ministry of Agriculture, show the rate of expansion of rice cultivation, in stremmata (1 stremma equals 1,000 sq. m):

Year	*District of Laconia*	*All Greece*
1938	350	36,980
1945	0	11,260
1946	0	25,190
1947	0	25,730
1948	0	36,940
1949	8,000	76,740
1950	10,000	99,100
1951	20,000	193,700
1952	24,000	212,240

This is an enormous expansion. For the whole of Greece, more than five times the area covered in 1938 was cultivated with rice in 1952. A still further expansion of rice cultivation is anticipated, in as much as there are still extensive flat areas of uncultivated alkaline land, where no other crop can be grown successfully. As far as Laconia is concerned, where the phenomenon of resistance was first observed, we have an increase of rice land by 68 times. Each additional stremma of land given to rice culture means another stremma of ideal breeding places of *An. sacharovi* in particular.

The problem is indeed very serious and needs urgent consideration. Any government legislation restricting rice cultivation would be out of the question today, when there is a generally recognized need of exploiting the land to the utmost. On the other hand, present day methods employed for the control of mosquitoes in the rice fields, namely DDT airspray, have not been satisfactory, at least as far as *An. sacharovi* is concerned in this country.

It is not an easy matter to recommend offhand any alternative method of control, but I feel that, until a more effective insecticide is discovered, the return to older methods might be justified, as an emergency. I mean Paris green for the chemical and gambusia for the biological control of mosquito larvae in rice fields. It might be that some more experimenting with both these methods will be needed, but judging from past experience, the prospects of success are good.

Paris green has been used extensively in this country prior to DDT and has given very good results. The complete success of the method depended upon the detection and the careful treatment of all anopheline breeding places, within a radius of three to four kilometres from the outskirts of the town or village which was to be protected from malaria. This was not achieved as a rule, since some of the breeding places would occasionally escape notice and treatment. In the case of rice fields, this is not likely to happen, as these are well defined and easily accessible. They can be treated with Paris green in dust or spray form, from the ground or possibly from the air.

Regarding gambusia, it is believed that a special method should be worked out for stocking the rice fields with them, during the rice growing season. There is no doubt that this would help enormously in bringing down the anopheline population in the rice fields. In Greece, as a rule, this fish is absent from the rice fields. In the area of Scala, Laconia, for instance, water is pumped into the rice fields, either from the river Eurotas or from a number of deep wells, where no gambusia exist. Belios reported in 1939 ([11]) that, while anopheline larvae density in most of the rice fields in the Lamia plain was very high, the larvae were practically absent from a certain rice field, which was watered from a ditch abounding with gambusia. Thousands of dips made in this particular rice field, averaged 1 larva or pupa per forty dips.

In order to have an abundant supply of gambusia, it is evident that natural or artificial fisheries should be established near the rice fields, whence they can be distributed in the rice fields. According to the method of cultvation applied in Greece, the water remains in the fields during the whole growing season and the fish, which multiply very quickly, would have ample time to establish themselves and bring about the desired result. It is understood that the number of natural fisheries should depend on the size of the rice fields in the area.

A programme consisting either in Paris greening the rice fields, or in stocking them with gambusia, or both, might prove effective and practical. The cost of the application in either case would be low and,

what is more important, the associations of rice growers would certainly co-operate in carrying out a programme of this sort.

B) HOUSEFLIES.

The question of housefly resistance to DDT has received, so far, the wider publication.

In Greece, the phenomenon of resistance was observed in the field in 1947. In laboratory experimenting, it was proved then that the failure of DDT to control houseflies was not due to bad quality or a different formulation of it, but was a phenomenon of resistance transmitted from one generation to another. In comparing the reaction of houseflies to DDT and BHC, it was observed that DDT irritated houseflies, whereas BHC did not ([12]).

In 1949, chlordane was substituted for DDT, with the theoretical dosage of 2 g of active ingredient per sq. m, in the control of houseflies in some villages of Attica. In 1950 the town of Laurion (population 7,000), was also residually sprayed with chlordane. Here it gave excellent results for two full seasons and fair results for a third season.

BHC was tried for one season in a settlement neighbouring one of the city's refuse dumps, with poor results.

Other chlorinated hydrocarbon insecticides have not been tried in Greece under field conditions. Although some of these may give, in certain cases, short-time control, there is no doubt that eventually houseflies will prove resistant to them all, as was the case in the US ([13]) and elsewhere.

Regarding the control of housefly larvae with chlorinated hydrocarbon insecticides, some preliminary studies, carried out in Greece, gave very promising results, particularly with dieldrin, but when these were tried on a larger scale, in some locations in New Jersey, they failed to give satisfactory results.

Laboratory studies of adults from these locations, and of larvae reared from these flies, showed them resistant to all chlorinated insecticides tested ([14]).

In view of the failure of chlorinated hydrocarbons to control houseflies, the necessity of basic sanitation becomes again more and more important.

Dissemination of fundamental sanitary knowledge through education cannot be over-emphasized. But the important thing is to educate the people to do simple things which can be done in their own environment

and with their own limited economic means. Otherwise, sanitary preaching would be preaching in an empty desert.

There is no doubt that prevention of fly breeding by sanitary practices, aiming towards the proper disposal of manure, domestic waste, city garbage, etc., would in theory be the ideal method of housefly control. Such sanitary measures have been taken in different countries with varying degrees of success, but the results have been discouraging, particularly where the carrying out of such measures was left to the initiative of, or depended upon the economic means of the average citizen.

Where, however, governments or municipalities are involved, the results are at times very satisfactory. This is evident especially in the handling of the problem of city garbage disposal.

In big cities and towns, there are of course innumerable breeding places scattered all over, but city dumps, particularly where these are situated near inhabited areas, are a source of enormous fly production and distribution.

In the city of Athens, for instance, there are numerous garbage dumps at the outskirts of the city, most of them very close to thickly populated areas and some to centres of international communications, such as airports and sea ports.

Through the co-operation of public-health service with the municipality and groups of private individuals, a system of sanitary land filling was recently organized on an experimental basis, and this proved to be quite satisfactory to everyone concerned. The city garbage is transported with city vehicles and dumped into huge excavations in the ground and immediately spread and compacted by a bulldozer. Earth is carried from a distance, spread over the refuse, and again compacted by a bulldozer.

Two or three sanitary fills of this sort operated in the Athens area in 1952, and it is hoped that more will be operating this year.

Houseflies are very scarce in the vicinity of such sanitary fills, whereas in other city dumps, where the refuse lies exposed, the fly and rat population is very great. It is felt that sanitary fills would be more useful and practical in smaller cities or towns

In the uncovered city dumps of the Athens area, the situation is bad indeed. Most of these dumps operate on a contract system between the municipality and a group of private individuals who undertake, for a small amount of pay, to collect city refuse with their own vehicles. They sort out what useful material they find in the refuse and keep herds of pigs which feed on all sorts of organic materal of the refuse.

While these pigs consume most of such material, which otherwise would be ideal fly breeding medium, their excreta contribute at least as much to fly breeding.

In an effort to exterminate the swarms of flies which are characteristic of these uncovered city dumps, the Health Centre of Attica tried in some of them a bait consisting of 2.5 per thousand sodium arsenite plus 15% molasses.

The results of this work were stated as « very good », but there was much protest and opposition on the part of the pig owners, who claimed that their animals were getting poisoned with arsenic.

During 1951 and 1952 some cases of trichinosis were detected among the pigs of the city dumps, and the keeping of such animals under these conditions is going to be prohibited by law.

C) PHLEBOTOMUS.

Eight major species of Phlebotomus are normally abundant and well distributed through most of Greece. *Phlebotomus papatasi* was very abundant and by far the predominant species in the Athens area. As an indication of this I might mention the findings of a special search for phlebotomus carried out during August 1939, in the city of Athens. Out of a total of 1,606 sandflies collected from 17 statons, 532 male and 988 female *papatasi* and 8 male and 21 female *major* were identified.

Since 1946, when DDT was widely used against anophelines, it has been observed that Phlebotomus also were much affected by it.

Marshall Hertig visited Greece in 1948, with the object of evaluating the results of the anti-malaria campaign with DDT residual sprays, in terms of sandfly control. He carried out an extensive investigation in Attica and Crete, which lasted until early October 1948. I had the privilege of co-operating with him during that period, and I followed the matter after he left.

If I were to report on the situation today, I would almost repeat what Hertig concluded in his paper ([15]) in 1948, that is:

(1) that DDT-sprayed buildings are still uniformly negative for Phlebotomus of any species,

(2) that in unsprayed places, the sandfly population is normal in abundance and distribution,

(3) that a marked decline of Kala-azar and oriental sore has been observed since the introduction of DDT in 1946.

Kala-azar cases have, since 1948, decreased further. The Ministry of Hygiene gives the number of all reported cases in the country as 34 in 1949, 22 in 1950, 29 in 1951 and 61 in 1952.

From the district of Canea (Crete), no cases at all were reported for 1949 and 1950, one case was reported for 1951 and 2 cases for 1952.

Regarding oriental sore there are no data available, but this has certainly further decreased. Belios, in a personal communication writes that the « Scar Index » for oriental sore among school children of the village Aggeliana of Rethymno, which was an intense focus of the disease, with thousands of cases in pre-DDT times, was as follows: number of school children examined (aged 6-14)-119. From these, all under 8 years of age had no visible scars at all; forty five per cent of the rest had the scars.

Papatasi fever, which was endemic in Greece and a regular feature with thousands of cases in the city of Athens, is not heard of any more.

In order to get more information on the density and distribution of sandflies, the entomological laboratory has arranged for a special search during the current season in the Athens area, Crete and the Peloponnessus. In the two latter areas no DDT residual spraying has been done for three consecutive years, including 1953. We hope to communicate the results of this search in due time.

D) FLEAS AND BEDBUGS.

Fleas and bedbugs have been successfully controlled with DDT residual sprays. It was only in 1950 that occasional reports from various sections of the country indicated that some fleas were reappearing in houses and stables. In 1951, the writer made a special search for fleas in the Peloponessus and collected large numbers of them from houses and pigsties which had been definitely sprayed with DDT some time previously. All these fleas were identified as *Pulex irritans*; today this species is very common and is found in great density throughout the country.

We have no data about other species of fleas being also resistant to DDT. In January 1950, the fleas from 27 rats collected from a number of warehouses at Piraeus were identified as follows:

Leptopsylla muscoli . . .	2 male, 7 female
Nosopsyllus fasciatus . . .	3 male, 2 female
Xenopsylla cheopis . . .	10 male, 24 female

The warehouses in which the rats were caught were stated as having been sprayed with DDT, but no details were given about it.

Bedbugs have also disappeared from Greece since the introduction of DDT. We do not have so far any confirmed reports of bedbugs becoming DDT resistant.

There are some reports coming in as to the reappearance of bedbugs in houses, but these have not been confirmed and it is almost certain that the cases involved are much exaggerated.

There is, however, one instance when bedbugs appeared in some numbers in buildings definitely sprayed with DDT. This was in a refugee camp at Laurion. In 1952 chlordane was used as a subsitute for DDT, and the results were stated to be very satisfactory.

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