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OBSERVATIONS ON THE EFFECT OF NUMBERS AND AGE ON THE SUSCEPTIBILITY OF MOSQUITOS TO DDT

by

G. Raffaele,[1] A. Coluzzi,[1] & J. de Zulueta,[2]

The influence on the results of susceptibility tests of factors such as the age of mosquitos, their feeding conditions and the season of the year is well recognized (Busvine, 1956a). The way the mosquitos are handled by each investigator must also influence the results of the tests. Recent work by Hadaway & Barlow (1956) with Anopheles stephensi and Aëdes aegypti has shown clearly how with both species and using the topical application method the DDT median lethal concentration is double 24 hours after a blood meal and returns to normal level 48 hours after feeding. Mosquito age did not seem to affect the results of these tests but it should be pointed out that the maximum age of the mosquitos used in them was only ten days. The importance of season has been shown recently by Zulueta et al. (1957) who observed, when making a susceptibility survey of anophelines in Iran, that there was an increase of about three times in the DDT LC_{50} of A. maculipennis at the beginning of hibernation when females of this species had already developed their fat body.

The observations summarized in this note stress the importance of mosquito age and mosquito numbers in the results of susceptibility tests. Observations made first with A. atroparvus and then with A. stephensi indicated that mosquitos a few days old are much less susceptible to DDT than mosquitos over 20 days old. Another series of observations made with A. atroparvus showed that this species could withstand higher amounts of DDT when the mosquitos were tested singly than

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[1] Istituto di Malariologia "Ettore Marchiafava", Rome

[2] Malaria Section, WHO, Geneva

when several mosquitos were used in each exposure vial. Thus, a factor inherent to the condition of the mosquitos and a factor due to the way in which they were handled by the investigator proved to have a considerable bearing on the results of the tests.

The susceptibility tests presented in this note and summarized in the three adjoining tables were made following two different methods. One is the ordinary Busvine & Nash method (1953, 1954) and the other is the method used by two of us and recently described in detail (Raffaele & Coluzzi, 1956). It may be sufficient to mention here that in this method mosquitos are captured one by one with small 2" x 1" vials, kept singly for one hour in 3" x 1" exposure chambers lined with filter paper impregnated with insecticide and finally released into 2" x 1" vials provided with a piece of moist blotting paper and stopped with a cork, which are used to keep the mosquitos during the 24 hours following exposure. This method reduces to a minimum the traumatism due to the handling of the mosquitos and has the additional advantage of keeping the mosquitos under uniform humidity conditions during the 24 hours following exposure. The complete lack of mortality in the controls both in the laboratory and in the field using a wide range of species (Raffaele & Coluzzi, 1956) shows how little traumatism the mosquitos suffer with this method.

In the experiments described in this paper, made following this method, the filter papers were impregnated either with a 2% DDT solution in xylene (as in the tests summarized in Table I) or with various DDT solutions in Risella oil (as in the tests referred to in Table II), the impregnation then being made following Busvine's (1954) technique.

In other experiments the Busvine method was followed throughout the tests (as in the tests presented in Table III). In all the experiments in which filter papers were treated with DDT solutions in Risella oil, the filter papers used for lining the exposure chambers were treated with the insecticide solutions the day before commencing the test. The treated filter papers were discharged after three days during which the maximum number of tests carried out was five. In the tests made following the ordinary Busvine method, mosquitos were kept after exposure in 4" x 3" carton cups which were discharged after being used a few times.

The cups were covered with a piece of mosquito netting fixed with a rubber band. A hole was made in this cover so that the mosquitos could be released from the exposure chambers into the carton cups; the hole was stoppered after the transfer of the mosquitos with a piece of cotton wool with glucose solution. The use of these carton cups had been recommended to us by G. Davidson (personal communication, 1957) and we found them more suitable than the ordinary muslin cages commonly used for keeping the mosquitos during the 24 hours following exposure. Since emphasis is placed in this note on the effect of numbers, we should point out here that no difference was observed in the results of the tests whether five, ten or twenty mosquitos were kept in the carton cups during the 24 hours following exposure. The important fact we found was the number of mosquitos kept in the exposure vials.

Mosquitos used in the tests were always blood fed and a record was made of the mosquito age which, as will be shown later, proved to have considerable effect on the results of the tests. All the work discussed in this note was carried out at the insectary of the Istituto di Malariologia where a temperature of about 27°C and a relative humidity of approximately 80% are maintained. Artificial light was used during exposure time but the mosquitos were kept in complete darkness during most of the following 24 hours.

Three colonies of mosquitos were used in these experiments. The A. atroparvus colony is an English strain colonized in 1933 in Mr P.G. Shute's laboratory where it has been maintained without interruption since then. The A. stephensi colony is of Indian origin; it was established in Mr Shute's laboratory and, like the atroparvus colony, was brought over to Rome in recent years. The A. aegypti colony is a French strain colonized many years ago in the laboratory of the late Professor E. Brumpt.

The results presented in Tables I and II show clearly to what extent the mosquito's age can influence the results of susceptibility tests. In the trials summarized in Table I DDT in xylene had been used but, since this is a volatile solvent, by the time the mosquitos were brought into contact with the insecticide the xylene had evaporated and only a DDT deposit was left in the filter papers. Thus, the effect of age was observed both when dry DDT deposits were used (Table I) and when the toxicant was in an oil solution (Table II).

The results summarized in Table III show the effect of numbers in the results of susceptibility tests. Though the age of the atroparvus mosquitos was not the same in all the tests, it was nevertheless uniform enough for the age factor to be excluded. No significant difference was observed when one mosquito per exposure vial was used than when two were tested. It was only when five mosquitos were used that a significant difference was observed. This increase of mortality following an increase in the number of mosquitos tested may be due to several factors. When five mosquitos are confined into a small 3" x 1" exposure vial they show a greater activity than when kept singly in the exposure vial. The more often a mosquito alights and takes off from an insecticide treated surface the more insecticide is probably picked up, so that by increasing the number of mosquitos confined in an exposure vial and therefore increasing their activity, the amount of insecticide picked up by them may also be increased. Crowding may also affect the mosquitos in a different way. When they begin to feel the toxic effects of the insecticide they lose their co-ordination of movements; they collide against each other and against the walls. They often fall and walk over each other and they must suffer from this a considerable amount of damage. Mosquitos often lose their legs in exposure vials but this seldom happens in the control vials. This, we found, is a common occurrence when several mosquitos per exposure vial are tested but it seldom happens when the mosquitos are kept singly in the exposure vials. It thus appears that when several mosquitos are confined in a small vial and exposed to a toxicant like DDT they are harmed and they show mortalities which in all probability are due not only to the effect of the toxicant but also to the traumatism suffered in the confined space of the exposure vials.

Our interest in bringing these observations up is above all to show to workers in the field that the results of their tests may be influenced by the number of mosquitos used during exposure time. This is a point of practical importance, particularly in areas treated with residual insecticides where anophelines may be difficult to find and where susceptibility tests may be made and in fact have been carried out with one mosquito per exposure vial. Such tests will give lower mortalities and consequently higher LC_{50}'s than if several mosquitos per exposure vial had been used. Age is also likely to influence the results of field trials, particularly when old mosquitos are used, but the field worker will always have difficulty in ascertaining the exact age of the mosquitos he is testing.

Before concluding, we would like to point out that the mortalities observed with the A. atroparvus tested are much below what has been considered normal for anopheline mosquitos (Busvine 1956b, Malaria Section, WHO, 1957). Whether the DDT tolerance observed was inherent to this particular strain or whether it was due to the artificial laboratory environment, it is difficult to say. Davidson (1957) observed an increased DDT tolerance in a laboratory culture of A. stephensi (brought without insecticide pressure) and attributed this fact to the effect of improved techniques in laboratory breeding. We believe that, in the present case, the possibility of some effect due to the artificial laboratory environment where the strain has been kept for more than 20 years cannot be excluded.

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TABLE I

Results of susceptibility tests with three mosquito species[1] showing the effect of age. Tests made with 2% DDT in xylene.

Species	Age (in days)	Number tested	Number dead	Mortality (%)
A. stephensi	< 20	100	90	90
	4-6	100	36	36
A. atroparvus	< 20	393	177	45
	4-6	199	16	8
Aëdes aegypti	< 20	100	46	46
	4-6	100	10	10

[1] Mosquitos caught with a small vial and kept singly throughout the test.

TABLE II

Results of susceptibility tests with A. atroparvus[1] showing the effect of age. Tests made with DDT in Risella Oil.

DDT concentration in the non-volatile oil (%)	Age (in days)	Number tested	Number dead	Mortality (%)
0.5	3-5	-	-	-
	< 20	40	2	5
1	3-5	30	0	0
	< 20	40	3	7.5
2	3-5	30	0	0
	< 20	40	6	15
4	3-5	30	3	10
	< 20	40	11	27.5

[1] Mosquitos caught with a small vial and kept singly throughout the test.

TABLE III

Results of susceptibility tests with A. atroparvus showing the effect of mosquito numbers. Tests made following Busvine's method.

Number of mosquitos per exposure vial	Mosquito age (in days)	DDT concentration in the non-volatile oil (%)	Number tested	Number dead	Mortality (%)
1	5 to 12	0.5	80	7	8.8
"	"	1	80	4	5
"	"	2	80	4	5
"	"	4	80	12	15
"	"	0 (control)	20	1	5
2	4 to 9	0.5	60	3	5
"	"	1	60	2	3.3
"	"	2	60	3	5
"	"	4	40	1	2.5
"	"	0 (control)	-	-	-
5	3 to 8	0.5	100	6	6
"	"	1	140	0	0
"	"	2	140	3	2.1
"	"	4	140	32	22.8
"	"	0 (control)	130	1	0.8