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SEASONAL VARIATIONS IN THE SUSCEPTIBILITY OF *CULEX PIPIENS PIPIENS* L. TO DDT AND DIELDRIN

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Resistance to DDT in mosquitoes was first discovered in the *Culex pipiens* complex (Mosna, 1947; Missiroli, 1947), but although several workers have investigated the susceptibilities of the larvae to various insecticides (Brown, Armstrong and Peterson, 1954; Burbutis and Davis, 1955; Hamon, Grjebine, Coz, Klein and Michel, 1959) very little information exists on the insecticide susceptibility of adult *C. pipiens pipiens* L. Apart from the work of Kuhlow and Garms (1964), there have been no investigations into any possible seasonal variations in the susceptibility of this species, although Lachmajer (1962) considered hibernating adults in Poland to be DDT resistant.

Davidson (1964) concluded that the

normal susceptibility of *C. pipiens* var. *molestus* Forsk. and various strains of *C. p. fatigans* Wied. to DDT and dieldrin was similar, and Busvine (1965) considered that both these forms and *C. p. pipiens* have approximately the same susceptibilities. Furthermore, a comparison of the susceptibility of *C. p.* var. *pallens* Coquillett to DDT and dieldrin (Yasutomi, 1962) with published results for the above three forms, suggests that this species can also be included in this category. It is therefore appropriate to compare the susceptibilities of these species with those found for *C. p. pipiens* in the present investigations.

METHODS. Hibernating adults of *C. p. pipiens* used in the trials were collected monthly from September 1964 to March 1965 from a variety of dark and damp brick-work shelters on Brownsea Island, which is situated in Poole Harbour, Dorset, southern England. Adults were

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TABLE 1.—Numbers tested and percentages of corrected mortalities (in parentheses) of adult *C. p. pipiens* exposed to various dosages of DDT.

| Months | 3 Hour Exposure | | 2 Hour Exposure | | | 1 Hour Exposure | | | LC 50 | |
|------------|-----------------|--------------|-----------------|--------------|--------------|-----------------|-------------|--------|-------|--|
| | 4% | 4% | 2% | 1% | 4% | 2% | 1% | 2 hrs. | 1 hr. | |
| Sept. 1964 | 49 (98) | 71 (63.4) | 73 (0) | .. | 72 (2.8) | .. | .. | .. | .. | |
| Oct. 1964 | 42 (100) | 79 (78.1) | 77 (24.7) | 50 (6.0) | 56 (30.4) | 53 (13.2) | 52 (7.7) | 2.8 | 10.5 | |
| Nov. 1964 | .. | 78 (88.5) | 51 (31.4) | 45 (8.9) | 47 (42.6) | 74 (13.5) | 69 (4.3) | 2.3 | 6.0 | |
| Dec. 1964 | .. | 77 (87.0) | 71 (33.8) | 76 (5.3) | 48 (37.5) | 74 (20.7) | 68 (2.9) | 2.3 | 6.0 | |
| Jan. 1965 | .. | 33 (90.9) | 38 (39.5) | 10 (20.0) | 44 (50.0) | 72 (11.1) | 31 (3.2) | 2.2 | 4.5 | |
| Feb. 1965 | .. | 27 (88.9) | 37 (45.9) | 48 (8.3) | 42 (69.0) | 48 (23.4) | 41 (0) | 2.1 | 3.2 | |
| Mar. 1965 | .. | 71 (85.9) | 49 (53.1) | 48 (8.3) | 75 (80.0) | 71 (23.4) | 67 (6.0) | 2.0 | 2.8 | |

transferred to the holding tubes of the standard WHO adult mosquito test kit, and supplied with 10 percent sugar solution. They were held at laboratory temperatures (about 17°–18° C.) for ½-hour, after which moribund and damaged individuals were removed and the remainder used in the tests. The recommended procedure of WHO (1960) was adhered to, and although initially a few adults were given a 3-hour exposure, most were exposed for 1–2 hours. Constant temperatures and humidities could not be maintained, but temperatures during the exposure periods were kept between 18°–19.5° C. and during the holding periods between 17.5°–19° C. Relative humidities fluctuated from about 65–80 percent.

Few adults had entered hibernation in September, and in January and February the hibernating population of *C. p. pipiens* was greatly diminished, consequently only relatively few adults were available for testing during these months. The slight increase in the numbers tested in March resulted from the discovery of a new shelter which still contained a substantial number of individuals. Although a few hibernating adults were found in early April, their numbers were insufficient for any tests to be made.

No organized insecticidal spraying has been undertaken in the area, and no insecticides have been used on Brownsea Island.

RESULTS WITH DDT. A few adults were exposed to 4 percent DDT for 3 hours, but as nearly all died no further tests were made with this exposure time (see Table 1). Except for the last two months, mortalities of only 50 percent or less were obtained with a 1-hour exposure to 4 percent papers, therefore both 1- and 2-hour exposure periods were used. Because of the small number (10) of adults tested in January to 1 percent papers for 2 hours, little significance can be attached to the unexpectedly high mortality of 20 percent. The low mortality (11.1%) recorded in this month in adults exposed to 2 percent DDT for 1 hour remains unexplained, but because of the larger number tested (72) it is less likely that a sampling error was introduced. Although relatively few adults were tested in September they appeared to exhibit a considerable tolerance to DDT. In subsequent months there was a general increase in mortality; this is most evident in adults exposed to 2 percent for 2 hours and to 4 percent DDT for 1 hour.

The LC 50 is usually used as a useful comparative figure in insecticidal experi-

ments, but Davidson (1964) has stressed its limitations unless the sampled population is homogeneous for either susceptibility or resistance. No such information is available concerning the present population of *C. p. pipiens*, but to afford some comparison with results given by other workers, a crude estimate of this value was obtained by visually plotting the dosage-mortality regression line. The very low mortalities which resulted from 1-hour exposure periods made it necessary to extrapolate to obtain the LC 50. For a 2-hour exposure period the LC 50 varied from 2.0 percent at the end, to 2.8 percent at the beginning, of hibernation, and for a 1-hour exposure from 2.8-10.5 percent.

RESULTS WITH DIELDRIN. As in the trials with DDT a few adults were exposed to the highest concentration (4 percent) for 3 hours, but as complete mortality occurred no further tests were made with this exposure period. One hour exposure to 4 percent papers produced relatively low mortalities (54.3-69.9 percent), consequently tests with 1- and 2-hour exposure periods were made. The small number of adults available in January and February resulted in very few mosquitoes being tested for 2 hours against 1.6 and 4 percent papers, and none against 0.8 percent. Similarly, only 21 *C. p. pipiens* were available for testing in January with 0.8 percent dieldrin for 1 hour, therefore little significance can be attributed to the observed absence of mortality in this test.

In September a 1-hour exposure with 4 percent dieldrin produced only 54.3 percent mortality, and although a slight increase in mortality (64.2-69.9 percent) occurred in later months, this was less marked in adults which were subjected to lower concentrations of insecticide (*vide* Table 2). However, the general increase in susceptibility at the end of hibernation is shown by adults given a 2-hour exposure to 1.6 percent dieldrin. The approximate value of the LC 50 after 2 hours' exposure varied from 1.1

percent at the end, to 2.0 percent at the beginning, of hibernation, and after 1 hour from 2.5-3.7 percent.

DISCUSSION. In Germany most of the tests on the susceptibility of *C. p. pipiens* to DDT and dieldrin were performed at 25° C., but a few were also made at 19°-20° C. (Kuhlow and Garms, 1964). Their value of the LC 50 to DDT (2.0-2.8 percent) after 2-hour exposure periods at this lower temperature is in very good agreement with the present results, but the LC 50 obtained after 1 hour exposure at 25° C. is much lower than that obtained in the present trials. They considered that the value of the LC 50 for 1 hour exposure was about twice that for a 2-hour exposure period, but no such simple correlation was apparent in the present investigations. With dieldrin they obtained 90 percent mortality after 1 hour's exposure to 4 percent papers at 25° C. In the present trials only 54.3-69.9 percent mortality occurred, and, apart from the complete kill of the very few adults tested in January and February, 2 hours' exposure still only resulted in 76.3-87.7 percent mortality. A comparison of the values of the LC 50 (1.3-1.6 percent) obtained by Kuhlow and Garms (1964) for a 1 hour exposure at 20° C. to those (2.6-3.7 percent) found in the Brownsea population shows that the adults on Brownsea are considerably less susceptible to dieldrin than those tested in Germany. The largest increase in mortality from one month to the next occurred, as with DDT, in September-October (*vide* Tables 1 and 2).

In France, mortalities of only 3 and 12 percent resulted when hibernating *C. p. pipiens* from a village which had been regularly sprayed with DDT and BHC for 5 years were exposed for 1 hour to 1.6 and 4 percent dieldrin and DDT, respectively (Hamon *et al.*, 1959). Although the criteria of Davidson (1964) for the detection of resistance in the *C. pipiens* complex to DDT and dieldrin cannot be applied to these results, there is little doubt that the population tested

TABLE 2.—Numbers tested and percentages corrected mortalities (in parentheses) of adult *C. p. pipiens* exposed to various dosages of dieldrin.

| Months | 3 Hour exposure | 2 Hour exposure | | | 1 Hour exposure | | | | LC 50 | |
|------------|-----------------|-----------------|--------------|--------------|-----------------|--------------|--------------|-------------|--------|-------|
| | 4% | 4% | 1.6% | 0.8% | 4% | 1.6% | 0.8% | 0.4% | 2 hrs. | 1 hr. |
| Sept. 1964 | 40 (100) | 76 (76.3) | 45 (40.0) | 42 (19.0) | 46 (54.3) | 46 (17.4) | 51 (7.8) | 50 (2.0) | 2.0 | 3.7 |
| Oct. 1964 | 30 (100) | 65 (87.7) | 44 (50.0) | 44 (22.7) | 83 (69.9) | 79 (20.3) | 44 (9.1) | 47 (6.4) | 1.6 | 2.8 |
| Nov. 1964 | .. | 72 (84.7) | 49 (46.9) | 36 (19.4) | 53 (64.2) | 55 (16.4) | 42 (9.5) | 45 (6.7) | 1.5 | 2.6 |
| Dec. 1964 | .. | 67 (86.6) | 52 (48.1) | 43 (23.3) | 52 (67.3) | 61 (18.0) | 47 (8.5) | 49 (4.1) | 1.5 | 2.8 |
| Jan. 1965 | .. | 18 (100) | 23 (52.1) | .. | 40 (65.0) | 43 (23.3) | 21 (0) | 35 (5.7) | 1.6 | 2.6 |
| Feb. 1965 | .. | 17 (100) | 17 (64.7) | .. | 45 (66.7) | 39 (20.5) | 42 (9.5) | 44 (6.8) | 1.2 | 2.5 |
| Mar. 1965 | .. | 43 (83.7) | 47 (70.2) | 45 (33.3) | 49 (67.3) | 47 (23.4) | 47 (12.8) | 49 (6.1) | 1.1 | 2.6 |

is resistant to dieldrin, but not DDT. This is substantiated by the greatly increased resistance to dieldrin shown by larvae of *C. p. pipiens* collected from this village.

There are several records of resistance to DDT and dieldrin by other members of the *C. pipiens* complex. For example, resistance to both these compounds has appeared in *C. p. var. pallens* in certain suburbs of Tokyo, Japan, which have been sprayed with organochlorine insecticides (Yasutomi, 1962); similarly this species has developed resistance to DDT and BHC in Central and West China after 5 years' spraying (Yu, Hsu and Lu, 1963). Although DDT resistance has been reported in *C. p. var. molestus* from Rumania (Ungureanu, Teodorescu and Georgiu, 1964) adults tested in Tokyo were susceptible, but they had developed resistance to dieldrin (Yasutomi, 1962).

The appearance of dieldrin resistance in the *C. pipiens* complex in areas where either dieldrin or BHC has been regularly used is not unexpected. However, the survival of a substantial number of adults from Brownsea Island after 1 hour's exposure to 4 percent dieldrin and the presence of a few survivors even after 2 hours' exposure, is unusual, because it demon-

strates the presence of a high degree of dieldrin resistance from an unsprayed area. Also, since a few adults survived an exposure of 1 hour to 4 percent papers in Germany (Kuhlow and Garms, 1964), a low incidence of dieldrin resistance appears to be also present in this unsprayed area. However, the presence of dieldrin resistance in mosquitoes in the absence of any insecticidal pressure has already been reported (Service and Davidson, 1964).

The most obvious difference between hibernating and non-hibernating *C. p. pipiens* is the development of the fat body with the onset of winter hibernation. It was thought that the gradual decrease in tolerance of adults to DDT and dieldrin towards the end of hibernation might be correlated to a reduction in the size of the fat reserves. Chemical estimations of the fat could not be made, but from October–February random samples of 40, and in March and April samples of 32 and 27, adults were collected, and after anaesthetisation, individually weighed. The mean weights in milligrams, with the range in parentheses, are as follows:—October, 3.0 ± 0.5 (1.9–4.0); November, 2.9 ± 0.6 (1.7–4.1); December, 3.0 ± 0.6 (1.9–4.4); January, 2.9 ± 0.5 (1.9–3.7); February, 2.9 ± 0.4 (2.0–3.7); March,

2.5 ± 0.4 (1.7-3.3) and April, 2.1 ± 0.4 (1.4-2.8). There is no significant difference between the mean weights of adults during October-February, but those collected in March and April weighed less ($>3 \times$ S.E.), and adults caught in April weighed less than those caught in March ($>3 \times$ S.E.). The increase in susceptibility of *C. p. pipiens* to both insecticides which occurred from October-February cannot be correlated with any decrease in the amount of fat reserves during these months.

SUMMARY. Changes in the susceptibility of hibernating adults of *Culex pipiens pipiens* L. collected from Brownsea Island, an unsprayed area in southern England, were investigated. Adults were collected each month from September 1964 to March 1965, from their winter hibernating sites, and tested at 18° - 19.5° C. by the standard WHO method for their susceptibility to DDT and dieldrin. A general increase in mortality of *C. p. pipiens* to both insecticides was noted towards the end of hibernation. The value of the LC 50 for 1 hour's exposure varied from 2.8-10.5 percent DDT and 2.5-3.7 percent dieldrin, the LC 50 for 2 hours' exposure was 2.0-2.8 percent DDT and 1.1-2.0 percent dieldrin. There was no evidence of DDT resistance, but although the population had not been subjected to insecticidal pressure, a high degree of dieldrin resistance was present.

No correlation could be found between the decrease in weight of fat reserves and the increase in mortality, factors which were associated with the end of hibernation.

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