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INSECTICIDAL TESTS ON IMMATURE HEAD LICE, *PEDICULUS CAPITIS* (ANOPLURA)—A NEW TECHNIQUE

Abstract. Methods for rearing nymphs of *Pediculus capitis* for insecticidal tests are described. Results of tests with lindane, dieldrin, malathion, and propoxur are presented.

The recrudescence of head louse infestation in various European countries and in North America has prompted more interest in insecticidal tests on this ectoparasite. Current methods of testing (Maunder, 1971, Med. Off. **125:** 27–29; WHO, 1977, Communication WHO/VBC 75.585 rev. 1, World Health Organization, Geneva, mimeographed; Blommers & van Lennep, 1978, Entomol. Exp. Appl. **23:** 243–51) are entirely restricted to adult lice. However, it appears from results of the study described below that 1st-instar nymphs are much more suitable than adults for evaluating insecticides.

Field-collected lice were kept in gauze-covered pill boxes together with a few strands of human hair (Buxton, 1947, *The louse,* p. 142, E. Arnold, London). Hairs with nits were harvested daily and replaced by fresh strands. The nits were incubated at 30 °C and 75% RH maintained by water-saturated sodium chloride (Winston & Bates, 1960, Ecology **41:** 232–37). A lower temperature (26 °C) was used to delay emergence, for example, until after the weekend. After emergence, nymphs were harvested twice daily and placed on the dorsal side of the hand or arm, enabling them to take a blood meal.

Observations made during several rearings yielded the following data relevant to the production of 1st-instar nymphs in the laboratory. Young adult female head lice kept in a pill box strapped permanently to the leg of a volunteer laid as many as 7 eggs per day per female, but field-collected specimens usually produced fewer than 5 eggs. Nits produced in the pill box and harvested every 24 h started hatching after 6.5 days incubation at 30 ± 1 °C. After 7.25 days, 50% of the nymphs had emerged. Hatching began after 11 days at 26 °C and reached a peak 1 day later. The percentage of eggs hatching normally exceeded 90%, but was usually lower in nits deposited by females the first few days after being collected in the field. Incubation at temperatures below 26 °C was also detrimental to hatching. Continuous incubation at 22 °C prevented hatching completely. TABLE 1 provides an example of the daily production of nits using a batch of lice collected from school children on day 0. The subsequent production of 1st-instar nymphs is presented in the same table. Field-collected adults and the nymphs were kept in different pill boxes.

TABLE 1. Daily production of eggs and 1st-instar nymphs by adult head lice collected from school children on day 0.*

	DAY			
	1	2	3	4
♀	21	24	24	39
Eggs	77	86	141	212
Eggs/♀	3.7	3.6	5.9	5.4
Eggs hatched (%)	69	76	85	96
Nymphs/♀	2.5	2.7	5.6	5.3

* Adults and older nymphs were kept in separate pill boxes strapped permanently to a man's leg. Newly emerged adults were added to the box with adults daily, except on day 2. Nits were incubated at 30 ± 1 °C and 75% RH.

Adults reared from nymphs were transferred to the box containing adults on each day except day 2.

The increase in both egg production per female and percentage hatch was apparently caused by the addition of young, more productive females to the reproductive population. The average production of nymphs grew from 2.5 to over 5 after the 3rd day. These figures seem to be representative for other samples.

Hatchlings survived at least 14 h without feeding at 30 °C; dead nymphs were observed when more than 18 h had elapsed since the last harvest. Hence, the emerged nymphs were harvested twice daily. When placed on bare human skin, these nymphs start probing and feeding almost immediately. Once satiated, they withdraw their mouthparts and often start wandering; they are, then, ready for testing.

A preliminary experiment showed that only very few nymphs reared in this way fail to survive the 24-h starvation at 26 °C and 65% RH required for the insecticidal tests: 4% of 160 nymphs died within 24 h, 20% after 40 h, and 84% after 48 h.

Lice for insecticidal testing originated from a few children in 1 school in either The Hague (Hague strain) or Utrecht (Utrecht strain). The former strain was maintained for 3 generations; the active stages were reared in pill boxes strapped to a human leg and the nits were kept in a cabinet. Surplus 1st-instar nymphs and those (more than 95%) surviving control exposure in the insecticidal tests were used for propagation. Of the Utrecht strain, only nymphs of the 1st generation were tested.

The testing procedure was similar to that used for adults. Batches of nymphs were placed on filter paper impregnated with various concentrations of insecticide following Maunder (1971, loc. cit.) and incubated in the dark at 26 ± 1 °C and 60–70% RH for 24 h. Standard WHO paper was used for dieldrin. For each replicate, 16–20 nymphs were used; 3 replicates were tested per concentration. The following concentrations were tested: 0.0046%, 0.01%, 0.022%, 0.046%, 0.1% propoxur; 0.02%, 0.03%, 0.045%, 0.1%, 0.2%, 0.4% malathion; 0.1%, 1.6%, 4.0% dieldrin; and 0.2%, 0.8% and 3.2%

TABLE 2. Susceptibility of 1st-instar nymphs of the head louse (Utrecht strain) to malathion and propoxur.

	MALATHION	PROPOXUR
LD_{50}	0.032	0.0076
LD_{95}	0.069	0.0436

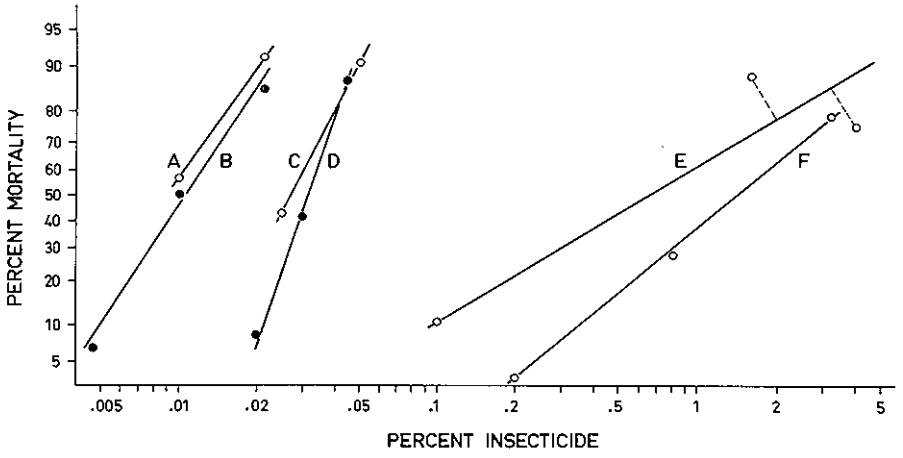

FIG. 1. Susceptibility of 1st-instar nymphs of the head louse (Hague strain) to 4 insecticides. A–B. propoxur; C–D, malathion; E, dieldrin; F, lindane. Open circles = nymphs of 2nd generation, closed circles = 3rd generation.

lindane. Details are given by Blommers & van Lennep (1978, loc. cit.). Abbott's formula was applied to correct the observed mortality percentages.

Results of experiments with 4 insecticides on the Hague strain are presented in FIG. 1. Susceptibility to malathion was tested twice (2nd- and 3rd-generation nymphs). The results of both experiments (C and D) were similar. The LD_{50} was 0.031% and LD_{95} 0.054% for malathion. The results of 2 tests with propoxur (2nd and 3rd generation, A and B, respectively) were also similar. Average LD_{50} was 0.010% and LD_{95} 0.026% for propoxur. Exposures to 0.1%, 0.2% and 0.4% malathion and to 0.046% and 0.1% propoxur yielded 100% mortality. Susceptibility of nymphs to both lindane and dieldrin was extremely low. The LD_{50}'s were 1.4% for lindane and ca 0.7% for dieldrin.

Results with the Utrecht strain revealed susceptibilities not much different from those of the Hague strain for both malathion and propoxur (TABLE 2). The slope of the log-probit regression line is less steep, most notably for propoxur. Resistance to dieldrin was also very high in this strain. Exposure to 1.6% killed only 24%; exposure to 4% killed 32% of the nymphs

Control mortalities did not exceed 10% (mean 4.4%) in any experiment.

The results presented demonstrate that 1st-instar nymphs that are fed once after emergence are well suited for use in insecticidal testing of head louse populations. In contrast, similar tests with adults have met with various difficulties (Maunder, 1971, loc. cit.; Anon., 1976, p. 45, World Health Organ. Tech. Rep. Ser. 585; Blommers & van Lennep, 1978, loc. cit.). Infested persons normally have only a few lice (Buxton, 1947, loc. cit.). Consequently, it is difficult to collect a sufficiently large number for proper testing. Moreover, field-collected lice vary widely in age and sex. As the adult life span is about 10 days (Busvine, 1948, Parasitology **39:** 1–16), natural mortality amounts to 10% per day. Adult head lice are also rather sensitive to starvation; hence, control mortalities are often more than 20% in tests with field specimens. Rearing adults from eggs or nymphs does not substantially improve this control mortality (Blommers & van Lennep, 1978, loc. cit.). The low control mortalities observed by Maunder (1971, loc. cit.) in a few tests are apparently related to his use of bottomless pill boxes, a method rather irritating to the volunteer and not suited to rearing large numbers.

None of these problems are encountered when 1st-instar nymphs are used. Sufficient numbers can be reared easily. Even small, localized populations might be tested with this new method. Young lice are quite homogeneous and, more important, they endure starvation better than adults.

Unfortunately, comparative tests using adults of the same strains as the nymphs failed because of extreme control mortalities (50% and more). A comparison with previous observations (Blommers & van Lennep, 1978, loc. cit.) indicates that nymphs are more susceptible than adults to malathion. The average LD_{50} was 0.1% for adults from 12 different towns against 0.03% in the 2 strains of nymphs used in these experiments.

Maunder (1971, loc. cit.) determined an LD_{50} of approximately 0.04% propoxur for susceptible adult lice from London. My observations suggest a similar level of susceptibility in Dutch adult lice. The data presented here show that nymphs are also more susceptible than adults to propoxur. Since malathion has been used as a pediculicide in Holland only since 1977, and propoxur has not been used for this purpose, the log-probit lines determined for both these insecticides represent most probably the primary susceptibility level for both compounds.

The results with lindane and dieldrin concur with results of earlier studies noting resistance of head lice to these 2 compounds in Holland (Blommers & van Lennep, 1978, loc. cit.).

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