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Notes on the Developmental Pattern of Leg Paralysis Induced by Risella 17 oil on *Lucilia sericata* (Meigen)[1]

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Key words: **Risella 17 oil; blowfly; *Lucilia sericata* (Meig.); leg paralysis developmental pattern.**

RINGKASAN

Corak pembentukan lumpuh kaki pada Lucilia sericata *(Meig.) yang disebabkan oleh 0.3 µl minyak Risella 17 (yang digunakan secara topikal) didapati berbeza menurut 'appendages' samaada yang terdapat pada kaki hadapan, tengah atau belakang.*

Biasanya kaki hadapan adalah paling kurang kelemahannya dan jumlah kaki yang lumpuh makin bertambah dengan masa. Tiga hari selepas rawatan didapati kebanyakkan lalat-lalat yang menerima rawatan mempunyai 3-4 kaki yang lumpuh. Hubungan yang kuat di antara jenis tanda-tanda lumpuh dan jenis-jenis "appendages' juga diperhatikan.

SUMMARY

The developmental pattern of leg paralysis in Lucilia sericata *(Meig.) induced by 0.3 µl Risella 17 oil (applied topically) was observed to differ according to whether the appendages belong to the fore-, mid-, or hindlegs. In general the forelegs were least susceptible and the number of paralytic legs increased with time. By 3 days after treatment most of the affected flies had 3-4 legs paralysed. Also evident is a strong association between the type of paralytic symptoms and the kind of appendages.*

INTRODUCTION

Among the various kinds of oils, Risella 17 oil is commonly used in insecticide formulations. On several occasions, it has been used alone or with insecticides in blowfly studies (Bard, 1961; Lewis, 1962, 1963; Busvine, 1962, 1971). These treatments included varying dosages applied topically, by injections, or as surface residues. Although no inducement of leg paralysis was noted in these earlier investigations, Risella 17 oil was found to induce leg paralysis in a later study (Lim, 1972, 1976) with the blowfly, *Lucilia sericata* (Meig.) Following this, studies were conducted to investigate further into the effect of the oil, particularly on the pattern of paralytic development. It is hoped that these investigations would provide not only a deeper insight into their nature and relationships, but also a better understanding on some aspects of the mortality process since the condition of paralysis is noted to be an extended state of moribund condition (Lim, 1972, 1976).

MATERIAL AND METHODS

Throughout the studies, the temperature was maintained at 26 ± 1°C and the relative humidity 65 ± 5%. All the flies used were unmated and of the same age (4 days old) and brood. Within 24 hours of emergence, the males were segregated on the basis of the distance between the eyes, which in the female is approximately more than one-third the total width of the head (Aubertin, 1933). This early separation enabled the flies to remain unmated and their age known throughout the studies.

[1]Taken in part from a thesis for the D.I.C. and M.Sc. degree of the University of London. The study was conducted at the Field Station, Silwood Park (Berkshire), Imperial College of Science and Technology, England.

During treatment, the flies were temporarily immobilized by chilling. Application was made with a foot-operated Burkard micro-applicator fitted with an ordinary 1 ml glass syringe that carried a bent needle with a blunt tip. Each fly was topically applied with a nominal dose of 0.3 µl as delivered by the applicator. The site of application was on the anterior margin of the second last abdominal segment and on the ventral surface.

After treatment, the flies were kept in plastic containers (10 cm diameter x 3.7 cm high) in groups of 10 flies/container. Water and food in the form of granulated sugar were provided. At regular intervals, the flies were observed for symptoms of paralysis; each fly being considered paralytic when it had at least two legs paralysed.

As a control check, a parallel set of untreated flies was used.

RESULTS AND DISCUSSION

Paralytic legs induced by Risella 17 oil were characteristics; and had distinctive positions. Altogether, four major types of effects have been identified (Lim 1972, 1976), these being: Type I, paralytic legs pointing backward; Type II, paralytic legs pointing sideways; Type III, affected legs folded; and Type IV, paralytic legs breaking away. The developmental pattern of these forms is observed to differ according to whether the legs affected belong to the fore-, mid-, or hindlegs.

Forelegs

The forelegs were observed to be the least susceptible to paralysis under the conditions studied. At 1 week after treatment, not more than 12% of the treated flies exhibited paralysis in the forelegs *(Fig. 1)*. Among these, no individual had both the forelegs affected.

Paralysis in the forelegs may be of Type II, Type III or Type IV. All these effects occurred in approximately equal proportion.

Midlegs

Unlike the case in the forelegs, paralysis in the midlegs was readily induced by Risella 17. Even at 2 hours after treatment, 20% of the treated flies had already developed paralysis in at least one of the midlegs. This condition rapidly increased with time, more than 90% being affected from 2 days after treatment *(Fig. 1)*.

Initially, most of the affected flies had only one of the midlegs affected. However, by 2 days after treatment, the majority had developed paralysis in both legs.

In the affected midlegs, all the three main types of paralytic symptoms were observed. The most common was Type III; more than 50% of the flies exhibiting it by 2 days after treatment. Type I symptom was relatively less frequent, being not more than 22% throughout the seven-day observational period, while the frequency of Type II

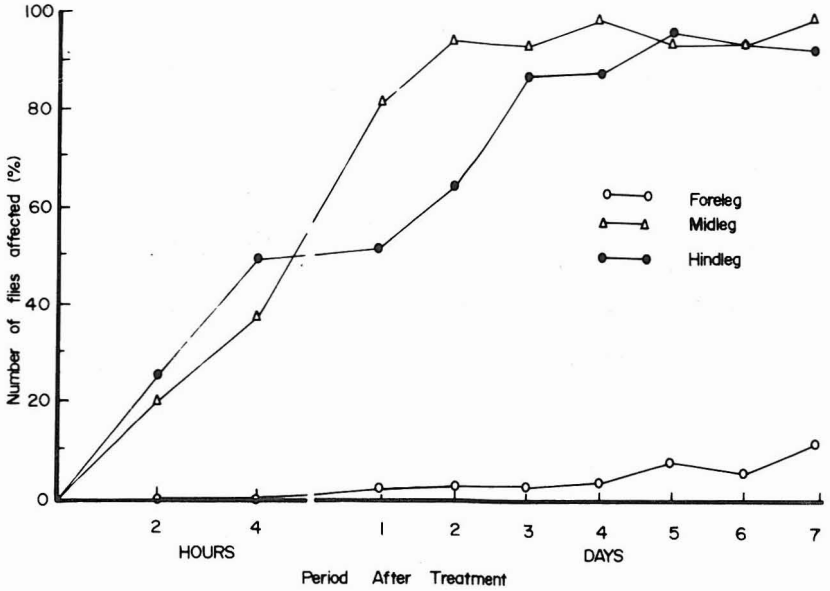

Fig. 1. Development of leg paralysis on male Lucilia sericata *(Meig.) at varying periods after treatment with Risella 17 oil, applied topically at 0.3 µl.*

symptom was intermediate. The latter was exhibited by 27-32% of the treated flies in 1-3 days after treatment. However, all the main symptoms were obvious, with significant numbers possessing them by 1 day after treatment.

Type IV symptom was also a common feature. The number suffering this effect increased with time from 23% to 71% at, respectively, 1 and 7 days after treatment.

Hindlegs

As in the midlegs, paralysis inducement by Risella 17 in the hindlegs was rapid and common. A similar pattern of development was observed i.e. the numbers affected increased rapidly with time *(Fig. 1)*. For instance 26%, 50%, 88%, 91% and 94% of the treated flies showed hindleg paralysis at 2 hrs., 4 hrs., 3 days, 5 days and 7 days after treatment, respectively.

The major paralytic symptom exhibited by the hindlegs was Type I. This symptom developed rapidly, increasing from 26% at 2 hrs. after treatment to more than 70% from 3 days onwards after treatment. None of the other conditions (i.e. Type II, Type III, and Type IV) appeared before 2 days after treatment. Comparatively, very few flies suffered these effects. At 7 days after treatment, only 24% developed Type II symptom while less than 6% exhibited Type III and Type IV symptoms.

All The Legs Considered Together

Number of legs affected: The total number of legs affected in a particular fly was observed to vary with the time interval from treatment *(Fig. 2)*. The longer the interval period, the greater was the number of legs affected. For example, at 2 hrs. after treatment, the percentage number of flies having one, two, and three affected legs was very low (8% – 14%). By the fourth hour, 20% – 24% of the flies had two and three legs affected. The latter increased to about 32% at 1 and 2 days after treatment.

As more of the legs in a fly became paralytic an obvious change in the number of flies having a few affected legs (one – three) to that having four – five affected legs was noticed. Thus, the former condition gradually decreased with time. Among the latter, flies with four affected legs were predominant (53%) at 3 days after treatment and this condition, with slight variation, was maintained thereafter throughout the observational period.

Flies having five affected legs were noted only 5 days and after from treatment. However, only a few showed this condition; there being 5% – 12%.

Throughout the study no fly was observed to have all its six legs affected.

Symptom development with time: Initially, up to about 4 days after treatment, all the three major paralytic symptoms developed progressively with time. However, except for Type I and Type II paralysis which remained more or less constant,

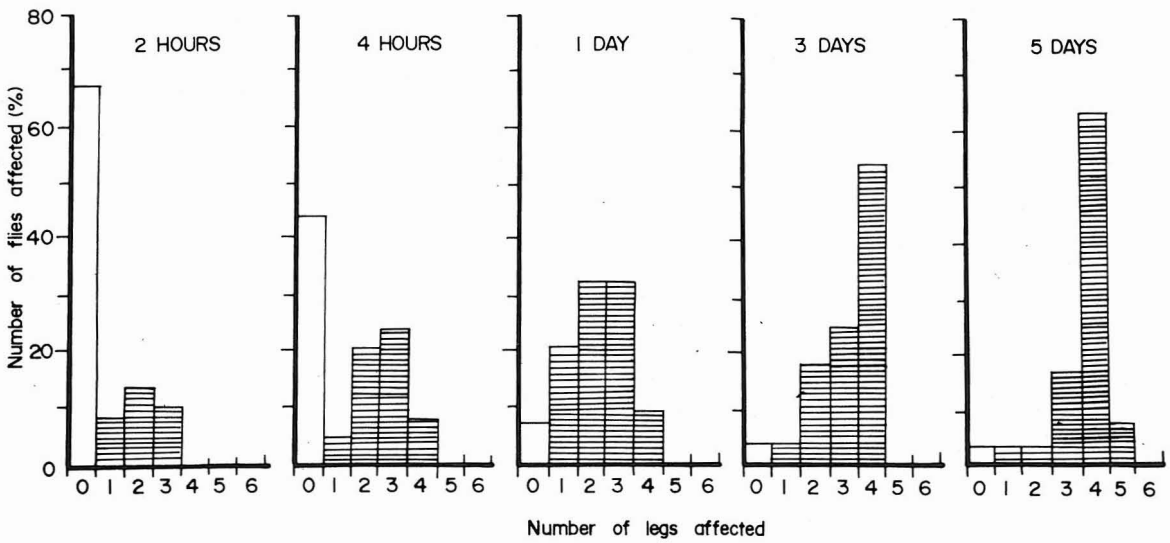

Fig. 2. Paralytic effect of Risella 17 oil (0.3 µl/fly) in relation to the number of legs affected in male Lucilia sericata *(Meig.) at varying period after treatment.*

Type III paralysis showed a sharp decline, falling from 68% at 4 days after treatment to 35% at 7 days after treatment *(Fig. 3)*. As recovery of Type III symptom was not observed, the decline was probably due to the increasing loss of the legs affected in this manner. This appeared to be so as Type III and Type IV (legs breaking away) were both common features of the midlegs.

then indicated the maximum effect induced by the oil. This was based on the findings that the limiting effect was reached by the third day from the oil application.

Due to the large number of zero and small frequencies obtained (Table 1a), the data had to be combined as a 2 x 2 contingency table (Table

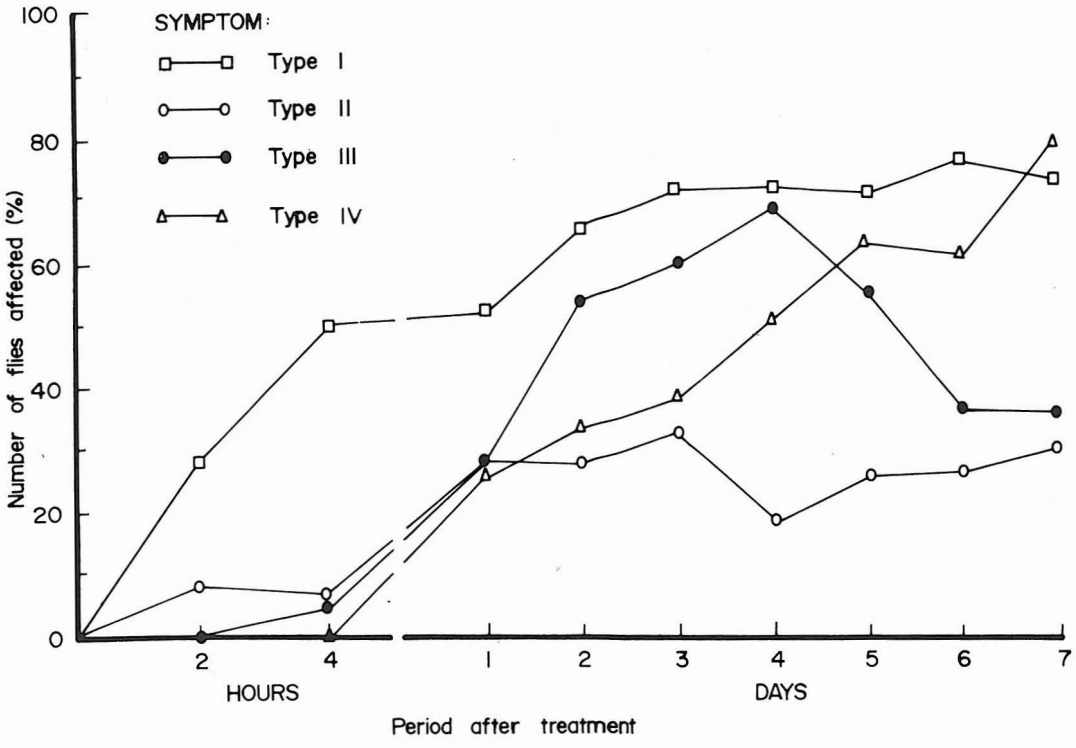

Fig. 3. Development of different types of leg paralysis in male Lucilia sericata *(Meig.) induced by Risella 17 oil, applied topically at 0.3 µl/fly.*

Association of leg position and types of symptom: It was noticed that certain paralytic symptoms normally developed only on certain pair of legs in the affected fly. For example, Type I symptom was most common on the hindlegs but comparatively rare on the midlegs, while Type III was predominantly on the midlegs. In the midlegs, symptoms of Type II, Type III and Type IV were normally observed, with Type III being the most common. These suggest that there may be an association between certain symptoms and the types of legs in an affected individual. This is clearly evident from Table 1 which shows the frequencies of the types of legs and symptoms at 3 days after treatment. The data at 3 days after treatment are used as the symptoms exhibited

1b) and analysed by the Fisher exact probability test (Siegel, 1956). In the analysis, it was found that $P = 0.467 \times 10^{-13}$ which shows conclusively that the types of paralytic symptoms developed in affected individual are highly dependent on the types of legs affected.

In general, it is noted that there is a strong association between certain symptoms and the types of legs in an affected individual. Also, each of the major symptoms rarely occurred by itself. More often, an affected fly would exhibit combination of the different symptoms; many combinations being possible and these varied with different individuals.

TABLE I

Number of *Lucilia sericata* (Meig.) exhibiting the different types of paralytic sypmtoms in relation to the different types of legs. The paralytic symptoms were induced by Risella 17 oil applied topically at 0.3 μl on the male flies.

a. Uncombined values recorded.

Paralytic symptoms	Forelegs	Midlegs	Hindlegs
Type I	0	0	24
Type II	0	11	1
Type III	0	20	4
Type IV	1	11	1

b. Data from "a" combined as 2 x 2 contingency table.

Paralytic symptoms	Forelegs + Midlegs	Hindlegs	Total
Type II + Type III + Type IV	43	6	49
Type I	0	24	24
Total	43	30	73

Some of the symptoms appeared to be interchangeable, at least for short period, e.g. Type II to Type III symptoms and *vice versa*. This was more common in flies in the initial period of symptom development, suggesting that the different symptoms observed were probably due to different degrees in the effect of the oil at varying intervals from treatment. Also, once paralytic symptoms have developed there is generally no recovery, i.e. the paralytic effects are permanent in the affected flies.

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