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LONGEVITY STUDIES OF THE LARVAL STAGES OF A PHYTO-PARASITIC NEMATODE, DITYLENCHUS TRIFORMIS, USING A MODIFIED LIFE TABLE TECHNIQUE

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LONGEVITY STUDIES OF THE LARVAL STAGES OF A PHYTO-PARASITIC NEMATODE, DITYLENCHUS TRIFORMIS, USING A MODIFIED LIFE TABLE TECHNIQUE

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CHAPTER I

INTRODUCTION AND STATEMENT OF THE PROBLEM

Life Tables

The life table is probably the most comprehensive technique in which the facts concerning the duration of life of any living organism can be presented. The characteristics shared by various populations of living organisms which make the life table method applicable to them are: an aggregate of living things which are similar but need not be identical, which come into being at a particular time, exist for a while, and after an interval, varying from one organism to another within certain limits, die.

Essentially, the life table technique is a method of summarizing age-specific death rates for a chosen time unit, which in the case of man is taken as one year, and in other living organisms may range from hours to days, or weeks, depending upon the life span of the organism. From the age-specific death rates, the expectation of life, or mean

lifetime, can be derived by a simple mathematical procedure (Deevey, 1947).

In any study involving the measurement of the duration of life of an organism, two different longevity measures should be distinguished, viz., the life span, and the expectation of life or mean lifetime.

The people of ancient Rome knew more about the nature of these two measures than might be supposed, as shown by their superstition that: "Ten times twelve solar years were the term fixed for the life of man, beyond which the gods themselves had no power to prolong it; that the fates had narrowed the span to thrice thirty years, and that fortune abridged even this period by a variety of chances, against which the protection of the gods was implored." (Deevey, 1947).

The life span of an organism is not an exact figure; it is the extreme limit of life, beyond which, even under the most favorable conditions, life cannot extend. There is no evidence that man's maximum life span has changed materially since antiquity. Since few men live beyond one century, it is, therefore, generally assumed that the human life span is approximately one hundred years, well over the Biblical "threescore years and ten."

Celebrated accounts of longevity such as the 900-year life span of Methuselah are purely legendary. However, authenticated cases of extreme longevity such as Christen Jacobson Drakenberg (1626-1772) have been cited in the literature (Dublin, Lotka, and Spiegelman, 1949). The life span, although not a known exact figure, remains constant for a given organism.

The mean lifetime is the average number of years lived by all

organisms born at a given period. It is a constantly changing figure, since an average, or mean, can be increased by increasing the proportion of larger items that enter into the average. By eliminating unfavorable factors, either biological or environmental, it is possible to increase the mean lifetime of an organism while its life span remains unchanged.

A life table includes for every interval of age, the number of deaths, the survivors remaining, the rate of mortality (expressed as the probability of dying within stated age intervals), and the expectation of further life or mean lifetime.

Life tables for human populations are calculated by indirect methods. Census data show the number of persons alive by single years of age living in various geographic areas. Published vital statistics give the number of deaths in the same areas each year and the ages of those dying. By combining the two sets of figures, it is possible to arrive at estimates of the rate of mortality suffered by persons of a given age at the time of the census. The life table is then constructed by applying these age-specific death rates to a fixed group of individuals called a cohort. A life table so derived exposes a purely hypothetical population based on the following assumptions: (a) that a standard cohort is born alive uniformly throughout the year of the census; (b) that its members will be exposed throughout their lives to given set mortality rates; and (c) that there is no immigration or emigration. The first life table, produced from human mortality records, was constructed for Breslau in Silesia by the English astronomer, Halley, for the period 1687-1691 (Dublin, Lotka, and Spiegelman, 1949). For over two centuries

vital statisticians and actuaries have been utilizing the life table procedure to obtain an adequate scientific measure of longevity for human populations.

Life tables for animal populations are derived from data obtained directly from the population in question. The experimental zoologist allows a cohort of statistically suitable magnitude to be born at the same time, keeping its members under observation and recording deaths as they occur. Mortality rates are then easily obtained as the ratio of the dying to the living at any age. From these mortality rates, all other life table functions can be calculated.

The first life tables derived from comprehensive observations on an organism other than man were those for the adult fruit fly Drosophila melanogaster (Pearl and Parker, 1921). The list of other non-human forms for which life tables are available is short and includes: the saturniid moth Telea polyphemus (Pearl and Parker, 1924) the rotifer Proales decipiens (Pearl and Doering, 1923); the common Hydra fusca, the roach Blatta orientalis, the slug Agriolimax agrestis (Pearl and Miner, 1935); the flour beetle Tribolium confusum (Pearl, Park and Miner, 1941); the snails Bulinus truncatus and Planorbis boissyi (Barlow and Muench, 1951); and the black widow spider Latrodectus mactans (Deevey and Deevey, 1945). All the survivorship data collected for the aforementioned animals have been from laboratory populations.

Data about duration of life are available for several natural populations of animals. An attempt had been made to condense the information into life tables even though serious deficiencies were apparent in

the data (Deevey, 1947). The field zoologist seldom knows anything of the age structure of a natural population. Growth rings on horns, or the number of corpora lutea in the ovaries of certain mammals, make age determination possible, but for the majority of animals, it is only possible to determine ages of individuals by marking them in some way. However, the source of greatest error in obtaining accurate vital statistics of a natural population lies in the impracticability of keeping its members under continuous observation. Taking into consideration the gaps in the ecological information, Deevey nevertheless managed to construct life tables for mountain sheep Ovis d. dalli, a sessile rotifer Roscularia conifera, the herring gull Larus argentatus, the American robin Turdus m. migratorius, and a barnacle Balanus balanoides. These ecological life tables are an important tool in understanding the survival of animals in nature.

Recently, a life table has been constructed for a parasitic form of life (Fisher, 1958). The test organism chosen by Fisher was the oxyurid Aspiculuris tetraptera, the mouse pinworm. Aspiculuris is an endoparasite which inhabits the colon of its host, and is wholly parasitic throughout its life cycle. An endoparasite poses an unusual problem in the gathering of age-specific mortality data. It depends upon a specific host for survival and one cannot observe the parasitic cohort without sacrificing the host, thereby artificially terminating the survival potential of the parasites. Fisher rationalized the problem in the following manner:

> If one were to start with a standardized universe of infective eggs from which random samples of fixed size were withdrawn and introduced into uniform

> host animals then, as the hosts were sacrificed in chronological sequence, the mortality data on the parasites would also follow in sequence. Survivors could be counted at each periodic interval, and the difference in survivors between two successive intervals would equal the number dying in that interval.

Accepting this rationalization, Fisher was the first to demonstrate that a life table could be formulated for a parasite.

That the life table can serve as a compact vehicle for certain vital statistics for a variety of populations has been amply demonstrated. Its practical value in measuring survivorship following treatment in the field of medicine (Berkson and Gage, 1952) and experimental stress in animal populations is equally great. The use of the life table as a measuring device for stress in an animal population is almost entirely the result of the labor of Pearl and his associates. Life tables have been published for Drosophila melanogaster showing the effect of: temperature (Alpatov and Pearl, 1929), successive etherizations (Pearl and Parker, 1922), density of population (Pearl and Parker, 1922), and mutant genes (Gonzales, 1923). Although a great deal of literature exists on experimental stress situations relating to the duration of life, it is mainly of a general and non-quantitative character, and not applicable to analysis via the life table procedure.

Selection of Test Organism

The primary purpose of this study was to secure survival data on the larval stages of a parasitic form of life which could be analyzed by the life table technique. Because the life cycle of an endoparasite prohibits the direct observation of all its larval stages, an ectoparasite was the logical choice of test organism.

A number of phyto-parasitic nematodes, considered obligate parasites, migrate through the soil and feed intermittently on plant roots and fungi. Among these phyto-ectoparasites is the tylenchoid nematode, Ditylenchus triformis.

The Parasite

Ditylenchus triformis is a relatively recently identified nematode species belonging to the order Tylenchida. It was isolated from soil collected from a gladiolus field in New Hanover County, North Carolina (Hirschmann and Sasser, 1955). At the time it was reported, both a dioecious and intersexual line were described. Intersexual individuals have been reported to occur only one other time in the order Tylenchida. An intersex in nematology is defined as either a modified male or female which functions in one or neither sex; but unlike a hermaphrodite, it never functions as both a male and female in the same animal. Ditylenchus intersexes resemble the female in body shape, they function as normal females, but have male secondary characters (spicules, gubernaculum and bursa).

The life cycle of Ditylenchus triformis was investigated and described by Hirschmann (1962) as follows: Eggs are laid singly and at the time of oviposition are unembryonated. Embryonic development begins after deposition of the egg and is completed within three to five days.

There are four ecdyses in the life cycle, the first taking place within the egg. The second stage larvae hatch within four to six days, are fully developed, and resemble the adults except for body size, reproductive organs and accessory reproductive structures. By the ninth

day, the majority of the larvae undergo the fourth molt; and by the thirteenth day, the larvae have completed molting and have developed into adults. The various larval stages may be distinguished on the basis of body size and development of the reproductive system. From the second molt to the fourth molt, the larvae double in size (346 microns to 688 microns). The adult female measures 867 microns; the male, being somewhat smaller, measures 707 microns.

Mating having occurred, the two-day-old adult female is capable of laying eggs. Thus, the life cycle of Ditylenchus triformis from egg to egg is completed within 16 to 21 days. During her lifetime, the female lays an average of 79 eggs (maximum 168); however she must be inseminated repeatedly to continue egg production. The longevity of adult males averages 74 days, that of females slightly shorter, 63 days. Adults remain sexually functional for three-fourths of their lifetime.

In tylenchoids, all stages of the life cycle, except the first stage larva within the egg, are armed with a protrusible spear or stylet used to pierce plants. The spear is hollow and forms the path of food intake and is formed by the coming together of the sclerotizations of the buccal cavity (Hyman, 1951). The feeding of Ditylenchus dipsaci on fungal hyphae was described by Linford (1937) (Ditylenchus dipsaci is a tylenchoid nematode with a buccal stylet similar to D. triformis). As it moves through the agar of a fungus culture, prior to feeding, it irregularly jerks its stylet slightly forward. When contact with the fungal mycelium occurs, the worm presses its head firmly against a cell, thrusting its stylet repeatedly until it penetrates the cell; or if penetration does not occur, the worm moves away. If penetration is accomplished, the stylet

is thrust forward into the cell, pulsation of the esophageal bulb begins, and the cell contents are sucked up.

Since Ditylenchus triformis was isolated from a gladiolus field, Hirschmann conducted experiments to ascertain whether or not it was pathogenic on gladiolus. The results of her experiments indicated that D. triformis did not reproduce or feed on this plant. However, the nematode proved to be polyphagous when successful propagation occurred in fungus cultures of Fusarium, Trichoderma, and Rhizopus species. Although D. triformis was able to parasitize the aforementioned hosts, reproduction was slow.

The Host

Hirschmann (1962) searched for a suitable host on which the parasite would grow, and eventually, a fungus was found on which the nematode flourished. All attempts to induce the fungus to sporulate failed, therefore it remained unidentified but was suspected to be a species of Fusarium.

Radiation

Tyzzer and Honeij (1926), using radiations from radium, were the first to observe the deleterious effect of ionizing radiation on the larvae of parasitic nematodes. Their work on Trichinella spiralis and later studies on the same parasite demonstrated that X-rays would inhibit its normal development. Although the amount of radiation necessary to produce death in Trichinella larvae was determined to be three-quarters of a million roentgens, inhibition of maturation of larvae to adult forms could be accomplished by an exposure to 5,000 or 6,000 r

(Gould, Van Dyke, and Gomberg, 1953). Exposure to 2,000-3,000 r greatly reduces the reproductivity of the larvae and a dose of 4,000 r and over produces sexual sterilization (Gould, et al., 1957). Levine and Evans (1942) observed formation of an acquired immunity in rats following infections with irradiated larvae which grew to maturity in the intestine but produced no offspring. It has since been shown that the pre-adult stage alone (normal infection stage) can stimulate a demonstrable immunity in mice which is associated with the presence of serum antibodies and a characteristic inflammatory response in the small intestine (Larsh, Race, and Goulson, 1959). These demonstrations of the use of X-irradiated Trichinella larvae as immunizing agents stimulated investigations of several other host-parasite systems. Vaccination using infective larvae attenuated by X-rays has been successfully employed against Dictyocaulus viviparus in calves (Jarrett et al., 1959a), Haemonchus contortus in sheep (Jarrett et al., 1959b), Uncinara stenocephala in dogs (Dow et al., 1959), and Ancylostoma caninum in dogs (Miller, 1964). In each vaccine, the dosage used to render the larvae incapable of normal development in their host was 40,000 r. Trichostrongylus axei required an exposure of 90,000 r before complete inhibition of maturation occurred in a rabbit host (Ciordia and Bizzell, 1960). A dose of 100,000 r to Ascaris lumbricoides infective eggs (whose albuminoid coating had been removed) was effective in preventing larvae from developing in the lungs of guinea pigs (Villella, Gould, and Gomberg, 1958).

Fassuliotis and Sparrow (1955) were the first to study the effects

of X-irradiation on a plant parasitic nematode. Cysts containing larvae of the golden nematode of potatoes, Heterodera rostochiensis, were treated with dosages ranging from 2,500 r to 80,000 r. Larvae from cysts treated at 2,500 and 5,000 r seemed to develop normally; cysts which developed from larvae exposed to 10,000 r contained eggs of which approximately one-half were dead; cysts of 20,000 r treatment contained only dead eggs and those of the 40,000 r dose were completely devoid of eggs. Populations of 11 species of plant parasitic nematodes in soil and chopped roots were irradiated using gamma-radiation from a cobalt-60-source by Myers and Dropkin (1959). Reproduction was completely stopped with doses of 40,000 r in only one species; for the other ten species, doses above 160,000 r were required for complete sterilization. Gamma-ray radiation (Co^{60}) was also used against Rhabditis species and Ditylenchus destructor present in compost from infested mushroom beds. The sterilization dose for both nematodes appeared to lie between 48,000 and 96,000 rep (roentgen equivalent physical) (Wood and Goodey, 1957).

Several workers have investigated the effect of ionizing radiation on the cercarial larva of the schistosomes of man. Hsu and associates (1963) immunized albino mice and rhesus monkeys against Schistosoma japonicum using X-irradiated cercariae as an attenuated living vaccine. Before an infection of S. japonicum can be established in a host, the migrating schistosomula have to pass three major lines of defense: skin, lungs, and liver. The fate of the migrating schistosomula varied with the irradiation dose given to the cercariae: 1,700 r - 6,000 r (schisto-

somula destroyed in the liver), 12,000 r (schistosomula destroyed in skin, seldom in the lungs, and never in the liver), 48,000 r (all schistosomula are destroyed in the skin). Using cobalt-60-irradiation, Radke and Sadun (1963) completely inhibited the development of schistosomula by exposing cercariae of S. mansoni to 4,000 rep. Since the widest dissemination of schistosomula would result in the greatest antigenic stimulation and a relatively less heavy concentration of parasites in a single organ, 4,000 rep would probably be an ideal dosage for a successful vaccine.

Cestodes do not differ from other helminths in the respect that radiations interfere with their normal physiological processes. A dose of 18,000 rep (Co^{60}) prevents maturation of the cysticercoid larvae of Hymenolepis diminuta to adult tapeworms (Villella, Gould, and Gomberg, 1960). An interesting application of ionizing radiation was made by Schiller (1959) who used X-irradiation as a mechanism for facilitating the study of morphological variation in Hymenolepis nana. He showed that the frequency with which certain variants occur could be increased by exposing the eggs to X-irradiation and that the rate of increase is proportional to the amount of radiation employed. Thus, by measuring the effects of different doses and extrapolating the results, a reasonably accurate estimate can be obtained of the frequency with which the variant may be expected to occur in a natural population. The taxonomist can therefore use X-irradiation as a tool to obtain reliable data on the relative stability of the morphological characters he uses for taxonomic purposes.

Statement of The Problem

Because of the paucity of data near the beginning of life in mortality studies of animal populations, the almost universal neglect in utilizing the life table as a tool in measuring stress in lower animals, and the existence of only a single life table study for a parasitic form of life, the following experimental objectives were set up:

a) To devise a method whereby an ectoparasitic form of life, namely D. triformis could be propagated in great numbers and could be kept under continuous observation during its larval stages in order to collect the quantitative data needed to formulate a life table.

b) To utilize the e_x^o (mean lifetime) function of the life table to study the effect of (1) total-body irradiation with X-rays - single doses, (2) total-body irradiation with smaller regulated dosages until the equivalent of a specific large dose is reached, and (3) exposure to the carcinogenic hydrocarbon, 3:4 benzpyrene.

CHAPTER II

MATERIALS AND METHODS

Materials

Parasite

The organism, Ditylenchus triformis, was selected as a suitable test organism for the following reasons: (1) It is capable of growing and propagating by deriving nourishment from a fungus host, thereby creating a rather uncomplicated food chain (agar → fungus → parasite) which can be easily and inexpensively maintained in the laboratory; (2) it has a relatively short life span, thus making the collection of quantitative data feasible; and finally, (3) it satisfies an essential prerequisite of a life table study, namely, a one to one relationship between egg and adult (the absence of larval propagative stages).

Host

The fungus displayed two distinct macroscopic cultural characteristics: (1) It grew rapidly covering over one-half of a 100x15 mm Petri plate within seven days; and (2) it produced two distinct surface pigments, the first bright red in the early stages of colonial growth and the second, a dark green to black-green as the colony grew older.

Microscopically, the fungus appears sterile. No reproductive structures were seen when stained with Lacto-Phenol-Cotton Blue. Not once during the entire course of this study, whether grown in the presence or absence of light, did the fungus exhibit sporulation. The hyphae were of a hyaline nature and septate.

Chemical Carcinogen: (Benzpyrene)

The deleterious effect of the polycyclic hydrocarbon benzpyrene on laboratory animals has been well established. Its recent implication in bronchogenic carcinoma (Public Health Service Publication No. 1103) and its probable harmful effect on the embryonic development of rats (Rigdon and Rennels, 1964) make it a suitable stress agent for duration of life studies. The benzpyrene used was obtained from Hoffman-LaRoche and dissolved in a saturated ethyl-alcohol solution.

Methods

Host

The fungus, probably a Fusarium, was grown on potato-dextrose-agar (PDA). Mycelial fragmentation was employed to maintain a constant supply of fungus: periodically a small disc of PDA containing mycelium was transferred to fresh media. To insure against bacterial contamination, chloramphenicol (50 ppm) was added to the potato-dextrose-agar prior to pouring.

Stock cultures of the fungus were preserved using the method recommended by the Mycology Unit of the Communicable Disease Center, Atlanta, Georgia (Bailey and Scott, 1962). Sterile mineral oil

(autoclaved at 121°C for 45 minutes) was poured into test tubes until it completely covered actively-growing fungus cultures on PDA (Figure 1). The test tubes were sealed and stored upright at room temperature. The oiled cultures remained viable for well over a year.

Parasite

In the present study the bisexual population of Ditylenchus triformis used was propagated in cultures of a fusarium-like fungus. Several nematodes, male and female, were added to seven-day-old Petri plate cultures of the fungus. All experiments were conducted at 25-30°C, the optimal temperature range for development and reproduction of both the fungus and the nematode. Large numbers of nematodes in all stages of development were present four to five weeks after the cultures were initiated. The nematodes were harvested in a Baermann apparatus. For this, the agar was cut into small pieces and placed on metal screening which had been lined with two layers of surgical gauze. The screening was suspended in the top of a small glass funnel, and to the stem of the funnel was attached a three-inch length of rubber tubing closed with a Mohr's pinch clamp. A small metal screen was fastened to the end of the rubber tubing to entrap any fungal debris that might filter through the first screening material. The funnel was filled with warm tap water until the pieces of agar were submerged. Within one to one-and-one-half hours, most of the nematodes had settled down into the stem of the funnel, where they could be drawn off through the rubber tubing. This procedure gave a concentrated suspension of nematodes relatively free from contaminating material.

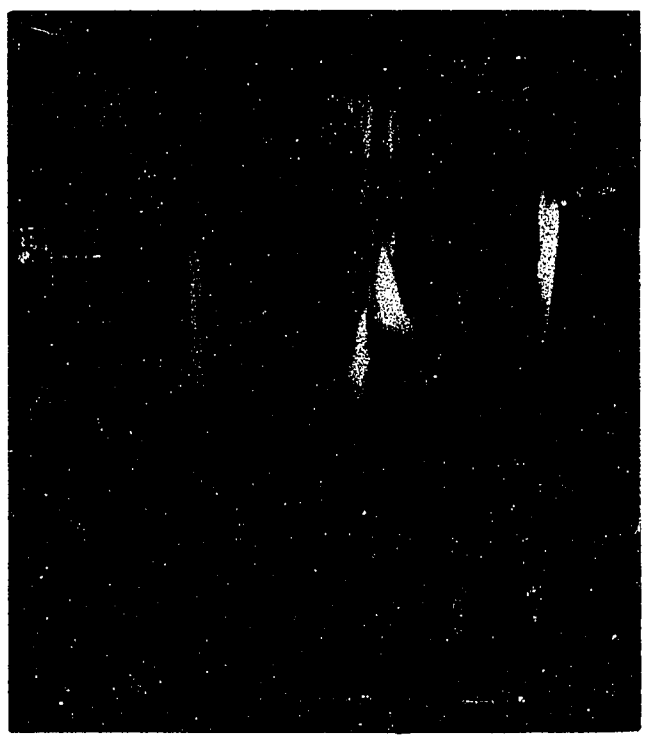

Figure 1. Oiled stock cultures of fungus grown on potato-dextrose-agar.

Since a life table begins with a group of individuals known to have been born at a particular time, it was necessary to develop a method for collecting large numbers of eggs. The Baermann apparatus method previously described for extracting nematodes from cultures proved to be inadequate for the following reasons: (1) not being motile, the majority of eggs could not escape from the agar in the top of the funnel, and (2) the suspension containing nematodes and eggs that were washed free from the agar was so viscous that removal of the eggs was extremely laborious and unrewarding.

In recent years mycologists have used cellophane (dialyzer tubing) as a surface atop solid media on which to culture fungi (Kondo, Graham, and Shaw, 1959). Experiments were undertaken to ascertain whether this cellophane culture technique could successfully be used to propagate the host fungus of Ditylenchus triformis. Dialyzer tubing (three inches flat width) permeable to water and permitting the passage of low molecular weight compounds in aqueous solution was obtained from the Arthur H. Thomas Company. The tubing withstands steam sterilization but should not be allowed to dry, since cellophane wrinkles badly unless completely saturated with water. The wrinkling problem was overcome by the following procedure: Cellophane discs (90 mm diameter) were placed in Petri plates filled with water and autoclaved (15 lbs./ 20 min.). Using sterile technique, the sterile cellophane discs were arranged on the surfaces of poured plates of potato-dextrose-agar. If bubbles occurred, the edge of the cellophane disc was raised and the bubbles were forced out by slowly rolling the disc back to the agar surface. The plates were incubated for 48 hours (25-30°C) not only to test

for contamination, but to provide time for excess moisture to be absorbed by the medium.

After ascertaining the sterility of the plates, they were inoculated with biscuits of mycelium and incubated for seven days. The fungus grew well (Figure 2) and displayed no evidence of cellulase activity — colony growth was restricted to the surface of the cellophane disc. Experiments were conducted to compare the rates of growth with and without a cellophane disc between the inoculum and the medium. The tests demonstrated no apparent critical difference between colonies in relative rates of growth (surface parameters), colony surface characteristics, and pigment development. The fungus colonies on cellophane were found to be very satisfactory for colonization of Ditylenchus triformis.

The following method yielded a suspension containing appreciable numbers of nematodes and eggs: Each cellophane disc was removed from the agar, cut into several pieces, and placed in a small glass vial. The vial was filled with lactated Ringer's solution (Lilly) until the pieces of cellophane were completely covered and then placed in a shaking machine for five minutes. Water was originally tried as the eluant, but the eggs became so fragile that they lysed on the slightest manipulation. The contents of the vial were filtered through a single layer of surgical gauze and the eluting procedure repeated at least twice to ensure maximum recovery of nematodes and eggs.

Recovery of eggs. Although the cellophane culture technique made possible the recovery of an adequate number of eggs in a non-viscous suspension, the problem remained of how to separate the eggs from the numerous nematodes also present in the suspension. It was neces-

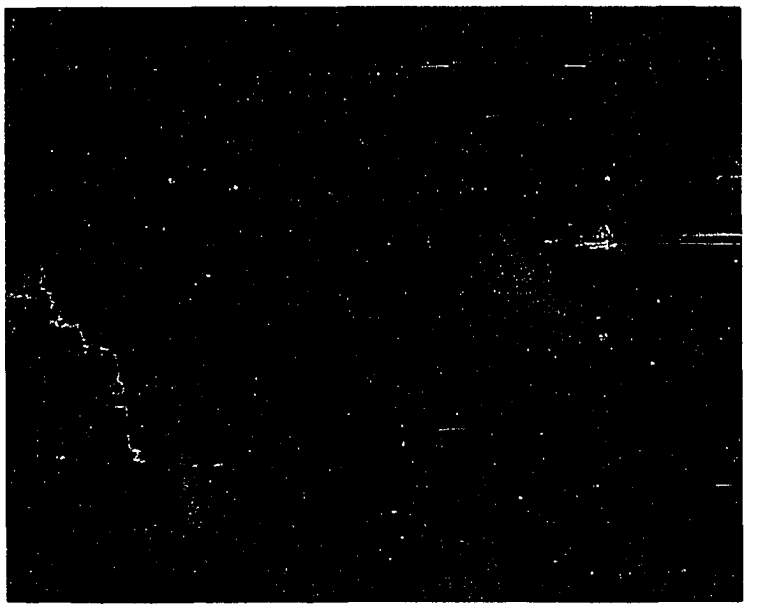

Figure 2. Culture of host fungus of Ditylenchus triformis on cellophane disc.

sary that the method devised for recovery of eggs be one that would introduce the least amount of stress; and for this reason, differential centrifugation was not considered. Separation by the modified centrifugal-flotation technique of Caveness and Jansen (1955) was not attempted since Dropkin and colleagues (1958) demonstrated that dextrose inhibited the hatching of phyto-parasite eggs.

A "differential" Baermann apparatus method was attempted, the rationale being that gravity and nematode motility would accomplish a relatively clean separation of nematodes and eggs. At various time intervals, samples were taken and examined. Although the larger worms came down first, they brought with them a substantial number of eggs. This, in addition to later samples showing no definite separation of the larval stages of the nematodes and eggs, made the technique impractical.

A second experiment was conducted based on motility of the nematodes. Two burets were set up with boiling chips packed in one and glass beads in the other. It was anticipated that the sessile eggs would remain in the burets, but after a period of time the majority of motile nematodes would move around the chips or beads, and settle to the bottom of the buret, where they could be drawn off by opening the stopcock. The results, however, were similar to those obtained with the Baermann apparatus technique. This method also failed to separate the eggs from the larval stages and the lethargic adult nematodes.

Evidence for the orientation of nematodes to temperature and light has been confined largely to zooparasitic species. Positive phototaxis has been reported for rhabditid nematodes (Wallace, 1961a).

Wallace (1961b), investigating the orientation of Ditylenchus dipsaci, found that light had no influence on the nematode's movement, but there was some evidence of a positive thermotaxis. On the supposition that a positive taxis could be used to separate nematodes from eggs, an apparatus was constructed to determine the influence of temperature and light on D. triformis. The study revealed no evidence of either temperature or light influencing the orientation of the nematode.

The method finally employed for recovery of eggs from the nematode-egg suspension was tedious but made possible the cleanest separation with the least amount of stress. Under the dissecting microscope (26x magnification), the majority of larger worms were removed by an orally controlled micropipette. Using fine needles (insect mounting pins), the eggs were gathered into groups and transferred by micropipette into small Petri plates containing fresh lactated Ringer's solution. The plates were then meticulously examined microscopically (60x magnification) to be sure that they contained only eggs.

Estimation of eggs needed for cohort. Eggs at all stages of development were collected at random. Hatching of second-stage larvae could therefore be expected to occur anytime within a six-day period. Thus, it was necessary to conduct experiments to estimate the hatch rate of randomly selected eggs. Several groups, each containing 100 to 1,000 eggs, were observed for various periods of time and the number of larvae which hatched was noted. For a 24-hour period, the hatch rate ranged from 16-25 percent, and at 48 hours, from 30-50 percent. Because of difficulty in obtaining large numbers of eggs (the nematode lays

eggs singly and must be inseminated repeatedly), larvae that hatched during the forty-eight hour period were used to establish a cohort. The second-stage larvae were selected at random and considered to be on the average, 24 hours old. Ten thousand eggs were collected for each life table experiment.

Observation of cohort. Several procedures were used in an attempt to observe the nematode cohort in order to secure age-specific death rates. In each case the heavy fungal growth interfered with accurate counting. During the hatch rate experiments, it was observed that the second-stage larvae were able to survive in lactated Ringer's solution but did not molt. It was assumed that a liquid medium which would support limited fungal growth would also be suitable for the growth and observation of the individuals in the nematode cohort. A potato dextrose solution was prepared in the following manner: Two-hundred grams of sliced peeled potatoes were boiled for one hour in 1,000 ml of distilled water, strained through a double thickness of clean toweling, and restored to its original volume. Glucose (two percent) was added to the potato infusion and the mixture autoclaved (American Public Health Association, 1941).

Nematodes, a small inoculum of fungus, and five ml of potato dextrose solution were added to Petri plates (60x15 mm). Three days after inoculation, fungal growth was diffuse and the liquid medium so murky that observation of nematodes was impossible.

In order to eliminate interference from the fungal growth, an attempt was made to maintain the nematodes in an axenic, undefined,

medium. Twenty grams of glass beads, 30 ml lactated Ringer's solution, and 1.25 grams of fungal mycelium were placed in a semi-micro water jacketed Monel blender. At two-minute intervals, samples were taken to determine the percentage of broken hyphae; after eight minutes, it was estimated that 90 percent of the hyphae had been broken. The cell-free extract was then centrifuged at 12,000 rpm for 20 minutes, and sterilized by filtration through a Seitz filter. Five ml of a 1:1, 1:2, and 1:10 dilution was added to small Petri plates. Five ml of a 1:1 dilution was also added as an overlay to a test tube containing a PDA butt. Five hundred second-stage larvae were added to each of the Petri plates and to the test tube. None of the aforementioned dilutions was able to successfully maintain the nematode.

The following method made possible the successful observation of the nematode cohort. Under sterile conditions, disposable Petri plates (60x15 mm) containing five ml PDA were completely lined with cellophane. The plate was then inoculated with fungus and allowed to incubate for three days (Figure 3) at which time the nematode cohort was added. At appropriate intervals the nematodes were observed and transferred to fresh cohort chambers. (Details of the transfer will be discussed later.)

Radiation

A great deal of variation exists among living organisms as to the amount of radiation necessary to cause death or modify growth. A dose as low as 0.01 r is sufficient to modify the growth of the fungus, Phycomyces blakesleeanus, while the lethal dose for various ciliates

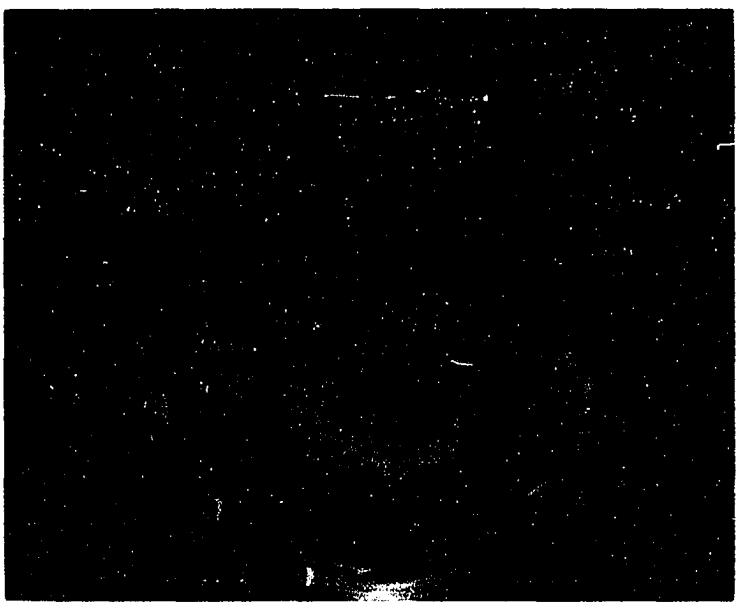

Figure 3. Cohort chamber with three-day-old fungus growth.

often exceeds $3x10^5$ r. Because of this great range of radiosensitivity among living organisms, a review of the literature was necessary to aid in the selection of suitable dosages of radiation to be used in this study.

Although the literature review showed that helminth larvae are very variable as regards radiosensitivity, all effective irradiations were in the kiloroentgen range. Since in no case did a dose of 2,400 r prove fatal to a helminth larva, nor did a dose in the range of 16,000 r kill during irradiation, these two dosages were chosen as the minimum and maximum radiation for this study.

CHAPTER III

EXPERIMENTAL PROCEDURE AND RESULTS

Establishing Initial Cohort

The methods of quantitatively estimating nematode populations for use in sundry parasitological investigations are not adaptable for life table studies. The crucial precept of a life table study is knowing the exact number of individuals that constitute a cohort. In order to obtain an absolute count, a direct counting procedure had to be employed. Using fine needles under direct observation with the dissecting microscope, groups of second-stage larvae were gathered, counted, and transferred to a cohort chamber by an orally-controlled micropipette. The micropipette was rinsed with approximately 0.1 ml of lactated Ringer's solution and the rinse was also added to the cohort chamber. The pipette was then carefully examined microscopically to detect any adhering larvae; if any were present, the count was appropriately adjusted. Before transferring another group of larvae to the cohort chamber, the pipette was thoroughly rinsed with both distilled water and Ringer's solution. In order to avoid injuring the larvae, they were gathered into groups by agitating the solution around them in such a way that the resulting current would propel them to the desired area. Each cohort chamber contained 500 larvae.

Transfer Procedure

At suitable intervals the cellophane containing the nematode cohort and fungus was assiduously removed from the Petri plate and placed in a small glass vial. The vial was filled with Ringer's solution until the cellophane was entirely covered and it was then placed in a Dubnoff Metabolic Shaking Incubator and shook for two minutes at motor speed 3.5. The contents were emptied into the bottom of a Petri plate marked for counting. After the above eluting process was repeated twice, the cellophane containing the fungus was removed from the vial and spread out in the top of a Petri plate. The vial was rinsed twice, and both rinses emptied into plates marked for counting. After an intensive search throughout the counting plates, the larvae were gathered, counted, and transferred to fresh cohort chambers utilizing the same direct counting procedure described for establishing the initial cohort.

The following three criteria were used to determine whether larvae were alive: (1) motion, (2) flexible rather than rigid appearance (All rigid appearing larvae were further examined for life because nematodes approaching a molt become sluggish and finally lie motionless during molting. Using a fine needle the tail portions of such larvae were gently prodded. If these larvae were alive, the head portion would respond to the tactile stimulus by a slight jerk.), and (3) movement of body content.

The vial, and complete cohort chamber, including agar, were all inspected microscopically for the presence of larvae. The top of the Petri plate containing the cellophane fungus culture was also microscop-

ically examined. Both the cellophane around the fungus colony and the colony itself were inspected, the latter being teased apart, until it was made certain that all larvae were accounted for. Larvae other than those recovered from counting plates were considered to be withdrawals from the study because of the impracticality of collecting them. To these withdrawals were added those larvae which were accidently destroyed during the transferring process. The withdrawals (losses) were taken into consideration in the calculation of the actuarial estimate of the probability of survival.

To test the reproducibility of the transferring and counting procedures, duplicate cohort chambers were run for all cohorts. In none of the experiments did an apparent difference occur between duplicate runs. Consequently, to reduce sampling variation the results of the duplicate runs were pooled so that the radix of each life table was 1,000.

The larvae were necessarily exposed to light while being transferred; but during the remainder of the experiment, they were kept in darkness because of the following phenomena:

(1) Photoreactivation — "The restoration of ultraviolet radiation lesions in a biological system with light of wave length longer than that of the damaging radiation" (Jagger, 1958). Evidence indicates that damage produced by ionization is not photoreactivable; however, there does exist a slight photoreactivation after X-rays, probably being caused by reversal of damage caused by excitation only.

(2) Photochemical Degradation of Polycyclic

Hydrocarbon — Kuratsune and Hirohata (1962) conducted experiments showing that prolonged exposure to illumination from commercial fluorescent lamps and to sunlight coming in through glass windows is capable of causing decomposition of benzpyrene. The study emphasized that transient exposure to these artificial and natural illuminations probably does not cause degradation of the hydrocarbon.

Irradiation Procedure

The larvae were irradiated in lactated Ringer's solution suspensions 15 mm deep and 60 mm in diameter (the vessels containing the suspensions were the bottoms of glass Petri plates). The X-irradiation was delivered by a Kelehet X-ray machine. Radiation factors were 250 Kv., 15 ma with an open filter. The distance from the inside plate of the X-ray machine to the surface of the suspensions was 10 cm, which yielded an exposure dose rate of 800 r/minute. The irradiations were of two kinds, namely, single doses (2,400 r and 16,000 r) administered at the beginning of the first interval of the life table, and a dose of 4,000 r administered at the beginning of the first four intervals of the life table.

Application of Benzpyrene

Before undertaking studies using benzpyrene as a stress agent, it was necessary to determine whether the hydrocarbon would penetrate the larvae. The method used is based on studies by Richter and Saini (1960) on the in vitro penetration and intracellular accumulation of the

hydrocarbon in diverse living cell types.

A suspension containing nematodes and eggs at all levels of development was placed on slides on which a field of microcrystalline benzpyrene had been deposited, coversliped, and examined under the ultraviolet fluorescence microscope. Benzpyrene when activated by ultraviolet light emits a blue fluorescence which can be detected visually by ultraviolet fluorescence microscopy in dilutions of 1:40 million to 1:1 billion. Both eggs and nematodes showed accumulation of fluorescing benzpyrene (Figure 4, 5, and 6). Benzpyrene crystals were then deposited on the cellophane in a cohort chamber; the chamber was inoculated with fungus, and incubated for seven days. At the end of the incubation period, the fungus was examined by u-v fluorescence microscopy, and it also demonstrated an accumulation of fluorescing benzpyrene (Figure 7). Thus, it was established that the larvae could be exposed to benzpyrene via their food source as well as through their environment.

The u-v fluorescence microscopic apparatus was identical with that used by Richter and Saini and included: (1) quartz substage optics, (2) American Optical ordinary and reflecting objectives, (3) u-v absorption filters of two types: the Corning no. 3389 filter, and five percent aqueous sodium-nitrite, (4) infrared absorption filters of two types: the Corning no. 3966 filter, and aqueous copper-sulfate, (5) the Corning u-v transmitting filter (300-425 mu) no. 5840, and (6) the Osram HBO-200 mercury arc lamp.

Prior to fungus inoculation, the cellophane bases of the cohort chambers were covered by benzpyrene crystals which had been deposited

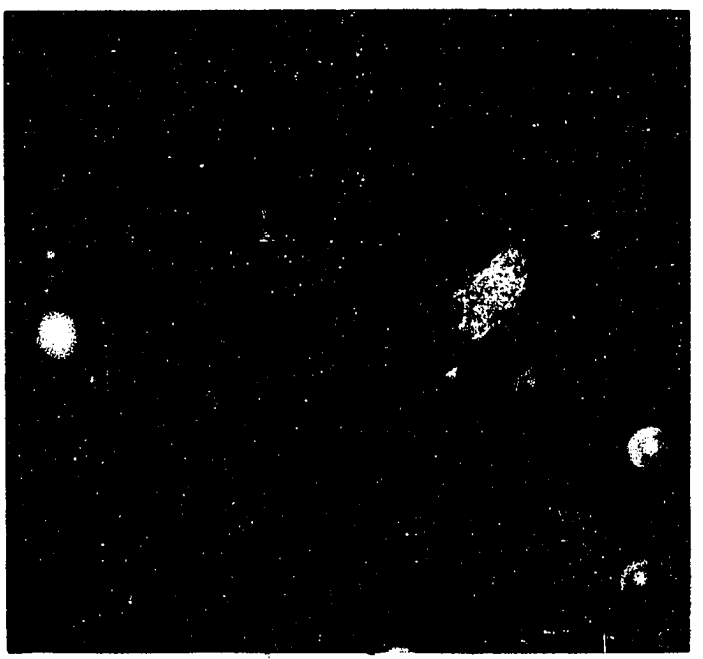

Figure 4. Unembryonated egg of D. triformis showing accumulation of fluorescing benzpyrene; ultraviolet photomicrograph, x 715.

Figure 5. Embryonated egg showing fluorescing benzpyrene (the slight fluorescence of the egg shell is not the characteristic blue fluorescence of benzpyrene, but is an orange fluorescence inherent to the shell); ultraviolet photomicrograph, x 1317.

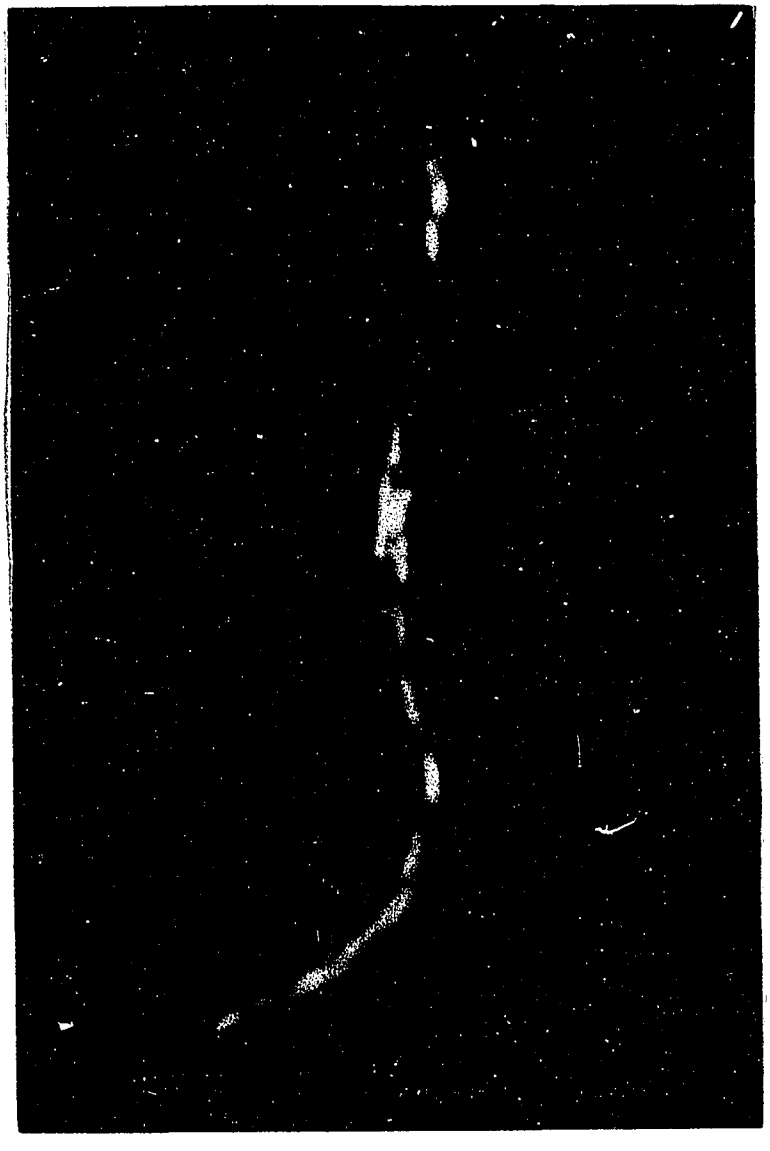

Figure 6. Larva of D. triformis showing high accumulation of fluorescing benzpyrene (the photograph is slightly out of focus due to the difficulty in photographing moving objects); ultraviolet photomicrograph, x 715.

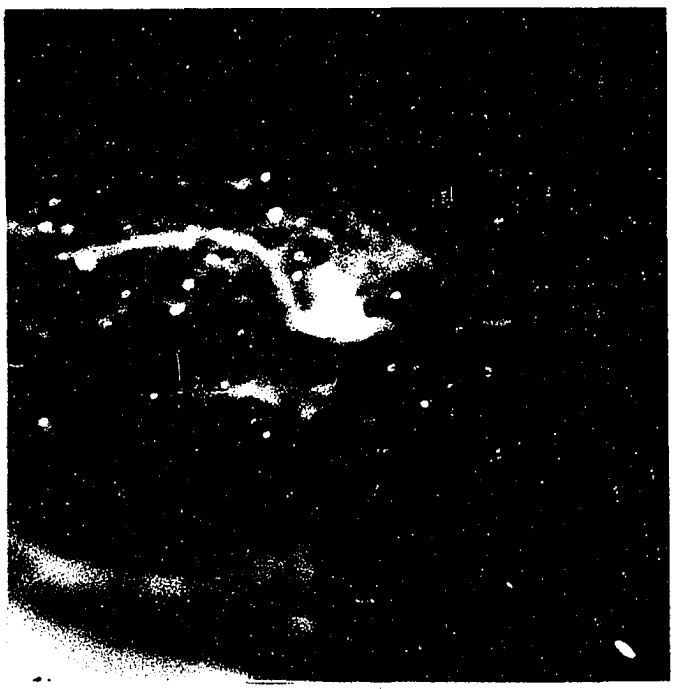

Figure 7. Mycelium of host-fungus of D. triformis showing fluorescing benzpyrene; ultraviolet photomicrograph, x 177.

by evaporation from a saturated ethyl-alcohol solution. The larvae of D. triformis were exposed to the benzpyrene crystals for the entire life table experiment.

Results

The losses encountered during this study (accidental or controlled) were assumed to be analagous to losses in a follow-up (prospective) study following a specific treatment in medical research. In order to take these losses into consideration, it was decided to use a modified life table technique. The technique employed was derived by Kaplan and Meier (1958).

Life Table Structure

The life table functions used in this study are defined as follows:

l_x = number entering interval

d_x = number dying within interval

s_x = number surviving interval

w_x = number of losses during interval

$\hat{p}_x$ = conditional probability of surviving through interval, given survival through the previous interval

$\hat{P}(t)$ = unconditional probability of surviving to the end of the interval

Equations used to calculate probabilities, mean lifetime, variance, and standard deviations:

(1) $\hat{p}_x = \dfrac{s_x - w_x/2}{l_x - w_x/2}$ (actuarial estimate)

(2) $\hat{P}(t) = \prod_{x=1}^{t} \hat{p}_x$

(3) $\hat{e}_x^o$ = mean lifetime $= \int_0^\infty \hat{P}(t)dt$

(4) $\hat{V}(\hat{e}_x^o)$ = variance of mean lifetime $(\hat{e}_x^o) = \int_0^\infty \frac{A^2(t)}{l(t)P(t)} \left| dP(t) \right|$

where $A(t) = \int_t^\infty P(t)dt$

(5) $\hat{\sigma}(\hat{e}_x^o)$ = standard deviation of mean lifetime $= \sqrt{\hat{V}(\hat{e}_x^o)}$

All integrals were evaluated by plotting a P(t) vs t curve and using the trapezoidal rule.

The results of the life table studies are summarized in Tables 1-11. An inspection of the $\hat{P}(t)$ columns of the control life tables (Tables 1, 5, and 9) indicates a positively skewed survivorship curve. The last figure in column $\hat{P}(t)$ of all life tables except Table 10 represents the probability of a larva (under the conditions of the experiment) surviving from an average age of one day (represented by zero time) to adulthood. A single dose of 16,000 r (Tables 3 and 7) completely inhibited maturation as did exposure to four 4,000 r doses (Table 6). Exposure to benzpyrene crystals inhibited maturation beyond the second larval stage (none of the larvae in column s_x of Table 10 were developed beyond second-stage larvae).

Interpretation of Statistical Summaries

If confidence intervals do not overlap, the interpretation is that the mean lifetimes differ at the 5% significance level. If overlapping occurs, this indicates that there is not sufficient evidence to reject the hypothesis of equality of mean lifetimes.

TABLE 1

LIFE TABLE FOR DITYLENCHUS TRIFORMIS LARVAE EXPOSED TO NORMAL LABORATORY CONDITIONS (CONTROL)

Interval in Days	l_x	d_x	s_x	w_x	$\hat{p}_x$	$\hat{P}(t)$
0-1	1,000	618	382	29	0.373	0.373
1-2	353	203	150	31	0.399	0.149
2-4	119	51	68	6	0.560	0.083
4-6	62	23	39	5	0.613	0.051
6-9	34	15	19	3	0.538	0.027
9-12	16	7	9	0	0.563	0.015

Symbols used in column headings are described on page 36.

TABLE 2

LIFE TABLE FOR DITYLENCHUS TRIFORMIS LARVAE EXPOSED TO 2,400 r AT THE BEGINNING OF THE FIRST INTERVAL

Interval in Days	l_x	d_x	s_x	w_x	$\hat{p}_x$	$\hat{P}(t)$
0-1	1,000	520	480	67	0.452	0.452
1-2	413	203	210	25	0.492	0.222
2-4	185	116	69	12	0.351	0.078
4-6	57	24	33	5	0.560	0.043
6-9	28	20	8	0	0.286	0.013
9-12	8	3	5	0	0.625	0.008

Symbols used in column headings are described on page 36.

TABLE 3

LIFE TABLE FOR DITYLENCHUS TRIFORMIS LARVAE EXPOSED TO 16,000 r AT THE BEGINNING OF THE FIRST INTERVAL

Interval in Days	l_x	d_x	s_x	w_x	$\hat{p}_x$	$\hat{P}(t)$
0-1	1,000	725	275	46	0.259	0.259
1-2	229	134	95	5	0.409	0.106
2-4	90	68	28	0	0.311	0.033
4-6	28	8	20	1	0.710	0.023
6-9	19	17	2	0	0.105	0.002
9-12	2	2	0	0	0.000	0.000

Symbols used in column headings are described on page 36.

TABLE 4

STATISTICAL SUMMARY FOR LARVAE EXPOSED TO SINGLE DOSES OF X-IRRADIATION

Treatment	Mean Lifetime	Standard Deviation of Mean Lifetime	95% Confidence Limits on Mean Lifetime
Control	1.49	0.09	1.31 to 1.67
2,400 r	1.57	0.07	1.43 to 1.71
16,000 r	1.04	0.06	0.92 to 1.16

TABLE 5

LIFE TABLE FOR DITYLENCHUS TRIFORMIS LARVAE EXPOSED TO NORMAL LABORATORY CONDITIONS (CONTROL)

Interval in Days	l_x	d_x	s_x	w_x	$\hat{p}_x$	$\hat{P}(t)$
0-1	1,000	566	434	40	0.422	0.422
1-2	394	246	148	20	0.359	0.151
2-4	128	52	76	10	0.577	0.087
4-6	66	27	39	6	0.571	0.050
6-9	33	16	17	1	0.508	0.025
9-12	16	6	10	0	0.625	0.016

Symbols used in column headings are described on page 36.

TABLE 6

LIFE TABLE FOR DITYLENCHUS TRIFORMIS LARVAE EXPOSED TO 4,000 r AT THE BEGINNING OF EACH OF THE FIRST FOUR INTERVALS

Interval in Days	l_x	d_x	s_x	w_x	$\hat{p}_x$	$\hat{P}(t)$
0-1	1,000	600	400	39	0.388	0.388
1-2	361	220	141	20	0.373	0.145
2-4	121	51	70	8	0.564	0.082
4-6	62	43	19	0	0.306	0.025
6-9	19	15	4	0	0.211	0.005
9-12	4	4	0	0	0.000	0.000

Symbols used in column headings are described on page 36.

TABLE 7

LIFE TABLE FOR DITYLENCHUS TRIFORMIS LARVAE EXPOSED TO 16,000 r AT THE BEGINNING OF THE FIRST INTERVAL

Interval in Days	l_x	d_x	s_x	w_x	$\hat{p}_x$	$\hat{P}(t)$
0-1	1,000	802	298	25	0.289	0.289
1-2	273	174	99	9	0.352	0.102
2-4	90	53	37	5	0.394	0.040
4-6	32	15	17	1	0.524	0.021
6-9	16	14	2	0	0.125	0.003
9-12	2	2	0	0	0.000	0.000

Symbols used in column headings are described on page 36.

TABLE 8

STATISTICAL SUMMARY FOR LARVAE EXPOSED TO 16,000 r
SINGLE DOSE AS COMPARED WITH FOUR 4,000 r DOSES

Treatment	Mean Lifetime	Standard Deviation of Mean Lifetime	95% Confidence Limits on Mean Lifetime
Control	1.55	0.07	1.41 to 1.69
4,000 r	1.35	0.05	1.25 to 1.45
16,000 r	1.08	0.04	1.00 to 1.16

TABLE 9

LIFE TABLE FOR DITYLENCHUS TRIFORMIS LARVAE EXPOSED TO NORMAL LABORATORY CONDITIONS (CONTROL)

Interval in Days	l_x	d_x	s_x	w_x	$\hat{p}_x$	$\hat{P}(t)$
0-1	1,000	488	512	52	0.499	0.499
1-2	460	188	272	17	0.582	0.290
2-4	255	103	152	7	0.590	0.171
4-6	145	73	72	5	0.488	0.083
6-9	67	29	38	2	0.561	0.047
9-12	36	22	14	1	0.380	0.018

Symbols used in column headings are described on page 36.

TABLE 10

LIFE TABLE FOR DITYLENCHUS TRIFORMIS LARVAE EXPOSED TO BENZPYRENE CRYSTALS

Interval in Days	l_x	d_x	s_x	w_x	$\hat{p}_x$	$\hat{P}(t)$
0-1	1,000	468	532	54	0.519	0.519
1-2	478	248	230	26	0.467	0.242
2-4	204	98	106	10	0.508	0.123
4-6	96	45	51	4	0.521	0.064
6-9	47	27	20	2	0.413	0.026
9-12	18	17	1	0	0.056	0.001

Symbols used in column headings are described on page 36.

TABLE 11

STATISTICAL SUMMARY FOR LARVAE EXPOSED TO BENZPYRENE CRYSTALS

Treatment	Mean Lifetime	Standard Deviation of Mean Lifetime	95% Confidence Limits on Mean Lifetime
Control	2.15	0.09	1.97 to 2.33
Benzpyrene	1.87	0.07	1.73 to 2.01

A single dose of 16,000 r decreases the mean lifetime as compared to the control by a significant amount. Exposure to a single 2,400 r dose apparently does not effect mean lifetime as compared to the control as they are not significantly different statistically. The difference between four single doses of 4,000 r each and the control appears to be equivocal because of a very small overlap of confidence intervals; the former compared to a single dose of 16,000 r does show a significant difference. There appears to be a significant difference between the mean lifetime of the control and the mean lifetime of larvae exposed to benzpyrene crystals as indicated by the confidence intervals for these two groups.

CHAPTER IV

DISCUSSION

Pearl and Miner (1935) studying the duration of life of lower organisms described three possible types of distribution of survivorship with respect to age. Type I, the negatively skew rectangular, where individuals of a cohort born at the same time die more or less simultaneously after a life span characteristic of the species. Type II is diagonal and implies a constant death rate at all ages. Type III, the positively skew rectangular, shows a heavy mortality early in life; however, the few individuals surviving enjoy a relatively high expectation of further life. Under the previously-described laboratory conditions, life tables calculated for Ditylenchus triformis in fungus cultures show heavy larval mortality.

Hirschmann (1962) reported the life span of adult males of D. triformis to range between 33-145 days and adult females 31-124 days. Observations on the adults obtained from the life table study summarized in Table 9 tend to confirm Hirschmann's observations. The survivorship curve obtained from the larval life table studies and the adult longevity studies of Hirschmann indicate a Type III survivorship curve for Ditylenchus triformis. Although complete data is not available, there is evidence that the life curves of other organisms mentioned be-

low approach Pearl's Type III distribution.

Population studies have shown high mortality rates for the larval and post-larval stages of the mackerel. The total mortality from the beginning of development to when the young fish (50 mm stage) gather into groups and school like the adults is 99.9996 percent. Among terrestrial vertebrates, survival data for the song sparrow (calculated from the egg stage) also approaches the positively skew rectangular type survivorship curve. Deevey (1947) notes that if the barnacle and rotifer life tables were complete, their life curves might also exhibit a "Type III" distribution. Evidence of extremely high death rates in larval stages of nematodes other than D. triformis has been reported. For example, only one out of 1,000 Trichostrongylus axei eggs reach the third larval stage (Taylor, 1957).

Because of biological variation within the same species, comparisons of mean lifetimes when measuring stress were made only between those cohorts obtained from the same group of second-stage larvae.

The 16,000 r dose which inhibited maturation in this study was closer to the radiation effect on zooparasitic nematodes than to the results obtained by Fassuliotes and Sparrow (1955) or Myers and Dropkin (1959) on phytoparasites. This could probably be explained by the lack of standardization of irradiation conditions in different laboratories. Some possible factors, other than the total dose, which could influence irradiation damage are:

(1) Dose Rate — In the absence of oxygen or at reduced oxygen tension, the effects of X-rays are diminished. In a larval suspension, depletion of the avail-

able oxygen is constantly taking place; therefore at a low dose rate, a given dose of X-rays is delivered when the larvae are in a solution of low oxygen tension.

(2) Quality of Radiation — The quality of X-rays is determined by the degree of filtration and by the voltage at which the tube is operated.

(3) Contamination — Certain chemical substances are capable of protecting biological systems against X-rays.

Results of different authors are difficult to compare, not only because physical conditions differ in various laboratories, but also because variations of radiosensitivity probably exist even within the same species. On the basis of the hypothesis made by Bird (1957) that the nematode cuticle is a living structure and in a state of constant metabolic activity, and that during molting there is increased activity and complex structural changes occurring, it was anticipated that consecutive small dose radiations during the first four intervals of the life table would have a more deleterious effect on the larvae than a larger single dose administered at the beginning of the first interval. However, the results showed that the cumulative effect of chronic irradiations was not equivalent to that caused by a single dosage of the same total amount. The mean lifetime of larvae receiving a single dose of 16,000 r was significantly lower than those exposed to four 4,000 r doses.

An interesting phenomenon concerning duration of life for various organisms exposed to X-irradiation has been reported in the literature. Small dosages of X-rays were reported to stimulate paramecia

to a more rapid rate of division for a short time, after which a depressed rate was observed (Hance and Clark, 1926). Sullivan and Grosch (1953) described a significant increase in the length of life of a parasitic wasp, Habrobracon, after irradiation with doses up to 180,000 r. The life span of the flour beetle, Tribolium confusum, was extended by several percent by irradiation with gamma-radiation (3,000 r) (Cork, 1957). Rhabditis pellio larvae exposed to 5,000 r just prior to maturity lived as much as 12 days longer than controls. It appears that the death of the female of R. pellio is hastened by the development of the larvae which apparently destroy the internal organs of the mother. When offspring are destroyed by radiation, the internal organs of the female remain intact and the nematode continues to live for several days after the controls have become mere shells containing growing larvae (Evans, Levin, and Sulkin, 1941). However, the dosages of X-rays used in this study produced no statistically significant increase in the mean lifetime of D. triformis larvae.

The refractive shell of nematode eggs is a chitinoid and is permeable to water; inside the egg shell there is a well-developed vitelline membrane which is similar to, if not identical with, the major ingredient of beeswax, myricyl palmitate.

Evidence indicates that the exterior layer of the cuticle of Ditylenchus species is a thin lipid membrane. Furthermore, it has been demonstrated that fatty acids are the chief products of glycogen metabolism in nematodes (Chitwood, 1938, 1952). The above facts on nemic physiology plus the relatively high solubility of benzpyrene in lipids probably explain the penetration and accumulation of the carcinogenic

hydrocarbon within the Ditylenchus triformis larvae and eggs. Not so easily explained is its mode of action on the nematode. Exposure to the hydrocarbon not only significantly reduced the mean lifetime, but appeared to inhibit the maturation of the larvae beyond the second stage. Heidelberger (1959) suggests that carcinogenic hydrocarbons bind to proteins and irreversibly inactivate them. The hypothesis is put forward, without any direct evidence, that the carcinogenic hydrocarbon binds to an exsheathing enzyme thereby inhibiting the molting process.

CHAPTER V

SUMMARY

A cellophane culture technique used by mycologists for preparation of permanent mounts of fungus colonies was successfully adapted for the colonization of the phytoparasitic nematode, Ditylenchus triformis. On the other hand a cell-free extract of the host fungus failed to maintain the nematode.

In conjunction with attempts to separate eggs from a nematode-egg suspension, a study was made on the influence of temperature and light on D. triformis. The study revealed no evidence of either temperature or light influencing the orientation of the nematode. The cellophane culture technique significantly increased the recovery of nematode eggs as compared with the Baermann apparatus, and other methods tried, and made possible the establishment of cohorts of statistically respectable magnitude. Further modification of the technique made possible the collection of the quantitative data needed to formulate a life table for the larval stages of the nematode. Because of the nature of the data, a modified life table technique was employed. The life table showed for each age interval the number of deaths, the number of survivors, the number of losses, and conditional and unconditional probability of surviving to the end of the interval for an initial cohort of 1,000 nematodes.

From these life table functions, the mean lifetime (e_x^o) was calculated and used to measure the effect of stress (X-irradiation and the carcinogenic hydrocarbon, benzpyrene) on the larval stages of the nematode.

Larval survivorship and observations on longevity of adults indicated a positively skewed J-shaped survivorship curve.

Exposure to a single 2,400 r dose did not effect the mean lifetime of the larvae; 16,000 r significantly decreased the mean lifetime and completely inhibited maturation to the adult.

It has been reported that the nematode cuticle is a living structure, and that during molting there is increased activity and complex structural changes occurring; it was, therefore, anticipated that a series of consecutive small doses of X-ray would have a more deleterious effect on larvae than a large single dose. However, the nematode larvae responded the same as other biological systems, the more significant effect occurring with the large single dose.

The carcinogenic hydrocarbon not only significantly reduced the mean lifetime of the larvae, but appeared to inhibit their maturation beyond the second stage.

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